广东茂名农林科技职业学院2023年10项代表性学术成果

序号	类型	成果名称	影响力
1	省级科学技术成果	《牛羊布氏杆菌和结核快速筛查技术研发和应用》	获得河北省科学技术成果国际先进的评价。
2	学术论文	《Efect of Echinacea on gut microbiota of immunosuppressed ducks》，本论文于2023年1月在《Frontiers in Microbiology》发表，被SCI收录	IF：2区5.2
3	学术论文	《Implementation of Intelligent Potted Plant Management System Based on Internet of Things》，本论文于2023年《Advances in Artificial Intelligence, Big Data and Algorithms》被EI收录	IF：1.557
4	学术论文	《副鸡禽杆菌毒力质粒缺失株的构建及其致病性的研究》，本论文于2023年3月发表于北大中文核心期刊《中国预防兽医学报》	IF：1.191
5	著作	《兽医外科学实验指导》，2023年9月在中国农业出版社出版	中国农业出版社动物医学类专业“十四五”规划教材
6	实用新型专利	一种鱼料投喂器；专利号：CN202223102159.3，于2023年5月26日获得权利授权；	实用新型专利
7	发明授权专利	一种荔枝酒的制备方法；专利号：CN202210394955.4；于2023年3月10日获得权利授权；	发明授权专利
8	应用软件	智能花盆物联网养花系统，登记号：2024SR0072052；完成日期：2023-7-20	
9	应用软件	猪解剖三维数字仿真教学软件，登记号：2023SR0979731；发证日期：2023-8-29	
10	标准	化橘红嫁接育苗技术规程（DB4409T36-2023）	

目录

1、省级科学技术成果：《牛羊布氏杆菌和结核快速筛查技术研发和应用》

河北省科学技术成果

证 书

成果名称：牛羊布鲁氏菌和结核杆菌快速筛查技术研发与应用

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河北省科学技术厅

二〇二三年九月

河北省科学技术厅 科技成果登记专用章

2、学术论文：《Efect of Echinacea on gut microbiota of immunosuppressed ducks》

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Effect of Echinacea on gut microbiota of immunosuppressed ducks

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Abstract

Introduction: Immunosuppression puts animals in a susceptible state and disrupts the balance of intestinal flora, which can increase the risk of disease and cause serious harm to the farm. Echinacea can exert its immunomodulatory effect in various ways, but its influence on intestinal flora is unclear.

Methods: Therefore, we investigated the effect of Echinacea extract (EE) on gut microbiota in immunosuppressed ducks by 16s-RNA sequencing in this experiment.

Results: The results showed that EE significantly improved the weight gain of immunosuppressed ducks ($p<0.001$). It also increased the immune organ index ($p<0.01$) and upregulated the levels of TNF-α and IFN-γ ($p<0.05$) as well as IL-2 in the serum. The lesions of the bursa were evident compared to the spleen and thymus. After treatment in the EE group, the lymphocyte count of the bursa returned to healthy levels and the lesions were significantly improved. The diversity analysis showed that neither of the alpha-diversity indices showed a significant difference ($p>0.05$). However, the EE group had a trend closer to the healthy group compared to the M group. β-diversity analysis revealed a high degree of sample separation between the healthy and immunosuppressed groups. The sequencing result showed a significantly higher relative abundance of *Prevotella* and *Prevotella_UCG_001* in the dexamethasone-treated group, which could be potential biomarkers of dexamethasone-induced immunosuppression. EE increased the relative abundance of *Akkermansia*, *Bacteroides*, and *Alistipes* and significantly decreased the relative abundance of *Megamonas*, *Streptococcus*, and *Enterococcus* ($p<0.05$).

Conclusion: The results showed that Echinacea extract improves the development of immunosuppressed ducks and modulates intestinal immune function by increasing the abundance of beneficial bacterial genera in the intestine.

Keywords: Echinacea extract; Prevotella; duck; gut microbiota; immunosuppression.

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Effect of Echinacea on gut microbiota of immunosuppressed ducks

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KEYWORDS

gut microbiota, immunosuppression, Echinacea extract, duck, *Prevotella*

1. Introduction

China is the world's largest producer and consumer of waterfowl, including the meat duck, egg duck, and meat goose industries. The total value of waterfowl production has exceeded $100 billion, with duck farming accounting for 74.3% of world production and goose farming for 93.3%. They can provide large quantities of high-quality meat and down. In recent years, diseases caused by immunosuppression have become more and more prevalent in large intensive farms, and the direct or indirect losses and hazards caused by them are quite huge. Immunosuppression can lead to retarded weight gain, decreased egg production in laying hens, and decreased litter size in breeding pigs by affecting animal intake and reducing feed conversion ratios. Meanwhile, the animals are vulnerable to infection, erosion by pathogenic microorganisms, and secondary diseases, which can be fatal in serious cases. However, there is still a gap in studies related to immunosuppression in waterfowl compared to reports in chickens, pigs, and rats. The factors leading to immunosuppression are mainly divided into disease factors, human factors, and feeding environment factors. Most of the factors causing immunosuppression in ducks are viral diseases, such as duck circovirus (DCV) (Hong et al., 2018), duck euthero virus (Wang et al., 2020), duck influenza virus, duck herpesvirus type 2, duck distemper virus (DPV) (Dhama et al., 2017), etc. These diseases are characterized by damage to the immune organs and hinder the process of the humoral immune response.

Dexamethasone can cause an immunosuppressive state in animals, and it was selected as an immunosuppressive drug in this test. In experiments studying animal models of dexamethasone-induced immunosuppression pathology, more attention has been paid to changes in leukocytes and immune cells, and a lack of focus on clinical signs such as body weight (Lo et al., 2005; Harada et al., 2011; Hundakova et al., 2022). Immunosuppression led to atrophy of the thymus and the bursin, organ function was affected, and organ indexes showed a significant decrease after modeling, whereas the spleen showed no difference. It was found that dexamethasone-induced immunosuppression significantly reduced splenic lymphoid follicles in the spleen of house sparrows. But did not affect their CD3 immune effect and had a minimal effect on splenic lymphocytes in mice (Jeklova et al., 2008; Crouch et al., 2022).

Research on natural herbal medicines is critical to reducing the risk of drug resistance on farms. Echinacea, as a natural herb, possesses a wide range of medicinal effects, and it contains a great potential medical value that is worth exploring. Echinacea was already used to treat traumatic injuries, septicemia, and toothache by Indians in the 18th century. Nowadays, it is more commonly used to treat skin diseases and to combat respiratory diseases such as influenza and asthma in Western countries (Aarland et al., 2017). A large number of studies have also reported that EE can exert immunomodulatory effects by affecting immune system mechanisms in different ways (Block and Mead, 2003; Randolph et al., 2003; Sharifi-Rad et al., 2018), such as activating immune cells and promoting the secretion of interferon-α (Zhai et al., 2007). However, the effects of its interaction with the intestinal flora on the immune system are still inconclusive.

It has been found that the immune regulation of the body is inseparably related to gut microbiota (Hansen et al., 2010). The gut microbiota is a system composed of a large variety of bacteria, including beneficial, harmful, and neutral bacteria. These microbiotas play a key role in digestion and absorption, growth and development, immune regulation, and physiological and structural changes in the intestine (Liu et al., 2009; Quinteiro-Filho et al., 2012). The immune function of the host is closely linked to the dynamic balance of the gut microbiota (Yamashiro, 2017; Liu et al., 2021). Normal flora has an important role in promoting the maturation of immune cells and tissues, while the presence of imbalances in the flora, is associated with the development of infectious and inflammatory diseases such as bacterial vaginosis, inflammatory bowel disease, and rheumatoid arthritis (Srinivasan et al., 2012; Ferreira et al., 2014; Trompette et al., 2014; Wagenaar et al., 2021). The gut microbiota can affect the host's immune system in direct or indirect ways. The flora directly eradicates pathogenic competitors by competing for nutrients and ecological niches, acting as a biological barrier together with the intestinal mucosa; or indirectly influencing the host's immune system through flora metabolites, enhancing its defense mechanisms (Kamada et al., 2013). For example, SCFAs are common metabolites of the flora, mainly produced by Firmicutes and Bacteroidota. They provide energy to intestinal epithelial cells, maintain the integrity of the intestinal mucosa, balance the pH of the intestinal microenvironment, have a positive regulatory effect on intestinal immune cells, and exert an inhibitory effect on intestinal inflammation (Correa-Oliveira et al., 2016; Parada et al., 2019; Blaak et al., 2020).

Abnormalities in the species, ratio, and the number of gut microbiota could occur due to medical origin, drug abuse, and other problems. The immune regulation and metabolic function of gut microbiota will be affected as the homeostasis of the microbial population are out of order. As a result, changes in the intestinal flora may lead to disruption of the normal immune response process and even immunosuppression. It may also lead to changes in the microenvironment in the intestinal tract and abnormalities in the digestive and absorption functions of the animal. This effect can affect the increase in body weight, decrease in meat yield, increase in feed weight ratio, etc., causing economic losses to the farm (Choi et al., 2014).

In this experiment, we analyzed the effect of EE on the treatment of the dexamethasone immunosuppressed duck model by the 16 s-RNA intestinal flora sequencing method and explored the relationship between the immunomodulatory effect of EE and intestinal flora.

2. Materials and methods

2.1. Animals and treatment

The protocol was performed after the approval of the Institutional Animal Welfare and Research Ethics Committee of

South China Agricultural University, Guangzhou, China, and every effort was made to minimize animals suffering during the experiments. A total of 60 healthy 7-day-old Pekin ducks (purchased from Foshan Guiliu Poultry Co., Ltd.) were randomly divided into three groups of 20 ducks each. They were divided into a blank group (K), a model control group (M) and an Echinacea extract treatment group (EE). In the M and EE groups, dexamethasone (purchased from Chongqing Buur Animal Pharmaceutical Co., Ltd.) was injected intramuscularly at a dose of 3.5 mg/kg for 7 days to construct an immunosuppressed animal model, with no dexamethasone injection in group K. After the animal model was established, the EE group added 0.6 g/kg of Echinacea purpurea extract powder (purchased from Sichuan Hengrui Tongda Veterinary Medicine Co., Ltd.) to the basic diet, while the K and M groups had no addition to the basic diet. During the experiment, all three groups were fed and watered *ad libitum*.

2.2. Body weight, immune organ index, and serum cytokines

We randomly selected six ducks from each group and sampled them at 0, 7, and 14 days after EE administration. The ducks were euthanized. And the weight of the body, spleen, thymus, and bursa of each duck was measured and recorded.

The immune organ index is calculated as follows. Immune organ index = immune organ weight (mg)/body weight (g). Their blood was obtained from the jugular vein, centrifuged at 3000 rpm/min for 10 min, and the serum was collected to detect the TNF-α, IFN-γ, and IL-2 levels in it by Elisa.

2.3. Pathological histological sections

After modeling, the spleen, thymus, and bursa of ducks in the healthy and immunosuppressed groups were randomly dissected and placed in 10% neutral formalin fixation, paraffin-embedded and HE stained to observe histopathology. According to the pathological changes, test ducks were randomly selected for dissection at 7 and 14 days of treatment and immune organs with lesions were obtained for HE staining to observe the pathological changes.

2.4. Study on the diversity of cecum contents microbiota

After dissection of 5 randomly selected test ducks in each group at 14 d after the administration, 2 g of cecum contents were placed in lyophilized tubes and stored at −80°C for the study of intestinal contents flora diversity. The total genomic DNA of the samples was extracted by CTAB/SDS method, and the DNA concentration and purity were detected on 1% agarose gel. Depending on the concentration, DNA was diluted to 1 ng/μL with sterile water, and the 16S rRNA genes of different regions were amplified with specific primers and barcodes. Equal amounts of 1X loading buffer (containing SYB green) were mixed with PCR products, DNA detection was performed on a 2% agarose gel, and the mixed PCR products were purified using Qiagen Gel Extraction Kit. Sequencing libraries were generated using the NEBNext® Ultra™ IIDNA Library Prep Kit (Cat No. E7645). Library quality was assessed by Qubit@2.0 fluorometer (Thermo Science) and Agilent Bioanalyzer 2,100 system. Finally, the library was sequenced on the Illumina NovaSeq platform and a 250 bp paired-end read was generated.

To continue expanding the sequencing volume, the sample size was first predicted and measured by plotting sparsity and species accumulation curves. Based on the results of species annotation, the top 10 species with maximum abundance in each group from taxonomic levels of phylum and genus were selected to generate cumulative bar charts of species relative abundance to visualize species with greater relative abundance at different taxonomic levels and their proportions. Alpha diversity reflects the richness of the sample communities through Chao1, Dominance, Observed_otus, Pielou_e, Shannon, and Simpson. Beta diversity was analyzed by PCA for similarity and similarity in the community structure of different samples. The top 35 genera in terms of abundance were selected and clustered at both species and sample levels based on species annotation and abundance information and plotted as a heat map to facilitate the discovery of the high and low aggregation content of species in each sample. Species abundance data between groups were hypothesis tested using the MetaStat method to obtain *p*-values, species with significant differences between groups were screened based on p-values, and histograms of differential species between groups were plotted. To discover and interpret high-dimensional biomarkers (genes, pathways, and taxonomic units), comparisons were performed using the LEfSe (LDA Effect Size) analysis tool (Segata et al., 2011) to find statistically different Biomarkers between groups based on statistical significance and biological relevance. In addition, KO database-based metabolic function prediction of the colony was performed by PICRUSt2 based on 16S sequencing data.

2.5. Data statistical analysis

The raw data of each group was collected during the experiment and analyzed by IBM SPSS Statistics 26 statistical analysis software. The values were analyzed with One-way ANOVA, LSD, and Kruskal-Wallis tests and converted to graphs by GraphPad Prism 8. The analysis results are expressed as "mean ± standard error."

3. Results

3.1. Effect of Echinacea on growth performance and immune enhancement

The results showed that Echinacea extract significantly improved the slow body weight gain and decreased immune organ index levels

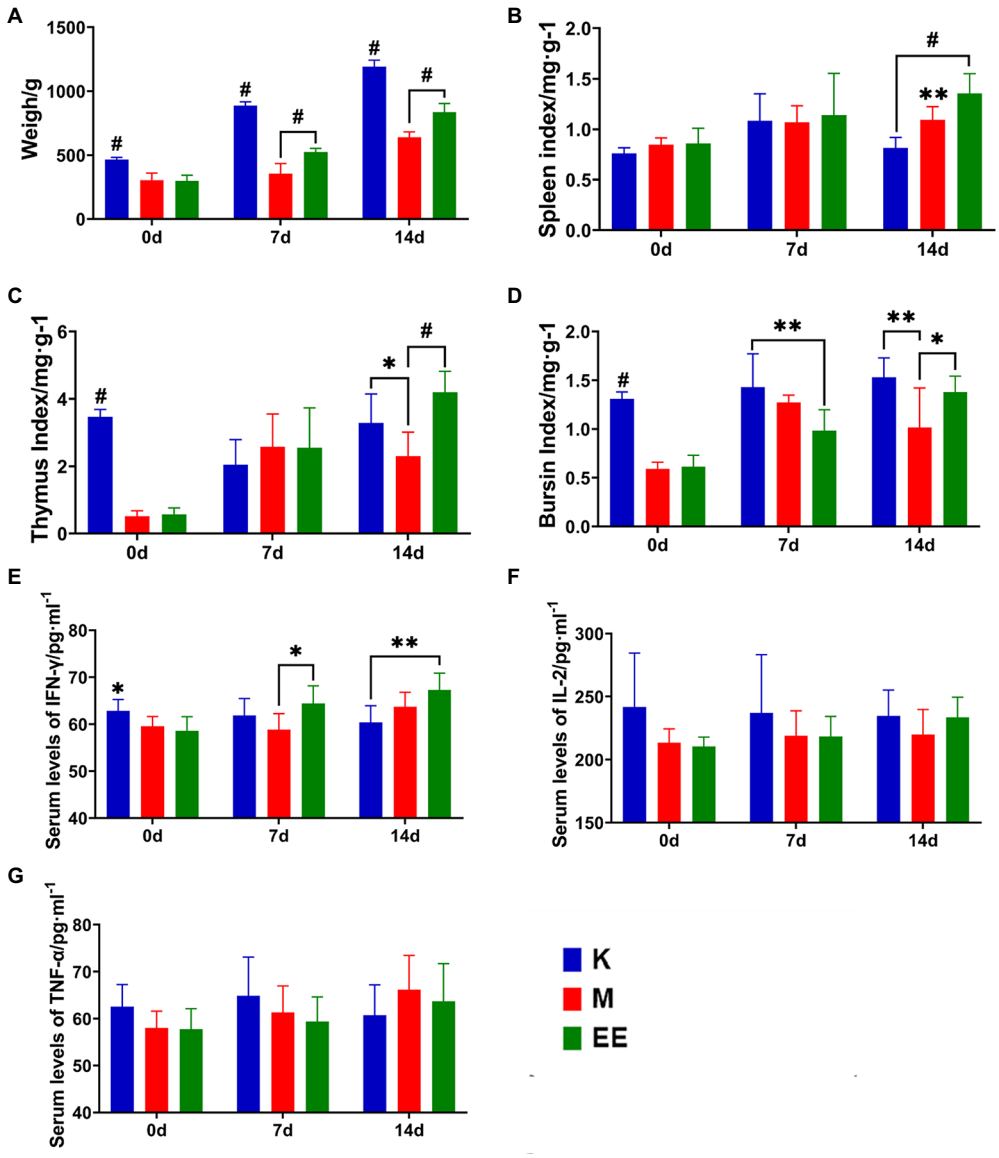

FIGURE 1
Effect of Echinacea on growth performance and immune enhancement. **(A)** Body weight; **(B–D)** Immune organ indices; **(E–G)** Levels of cytokine content in serum. *N*=6, $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (#).

caused by immunosuppression, and increased the levels of IFN-γ, TNF-α, and IL-2 in the serum of immunosuppressed ducks. The immunosuppressed animal model was established after 7 days of continuous dexamethasone injection. The body weight of animals in the immunosuppressed group was significantly lower compared to the K group ($p<0.001$). Echinacea extract was started in the EE and M groups. At 7 and 14 days, the body weight of ducks in the EE group was significantly higher than in the M group ($p<0.001$). However, there was still a significant difference compared to the K group ($p<0.001$) (Figure 1A). In the comparison of immune organ indices between the groups, the spleen index showed a significant difference between the EE and M groups only at 14 days of the administration, with the EE group being significantly higher than the M group ($p<0.01$) (Figure 1B). While before treatment with Echinacea extract, the thymic and bursal indices showed significant differences between the healthy control group and the immunosuppressed group, immunosuppression significantly reduced the levels of both of these immune organ indices ($p<0.001$). At 14 days of the administration, the EE group showed a significant recovery in the thymus ($p<0.001$) and bursal ($p<0.05$) organ index levels, both higher than the M group (Figures 1C,D). The levels of IFN-γ, TNF-α, and IL-2 in the serum of the EE group showed a tendency to increase during drug administration (Figures 1E–G). IFN-γ showed a significant decrease ($p<0.05$) after immunosuppression. But at 7 days of drug administration, it was significantly higher ($p<0.05$) in the EE group compared with the M group (Figure 1E).

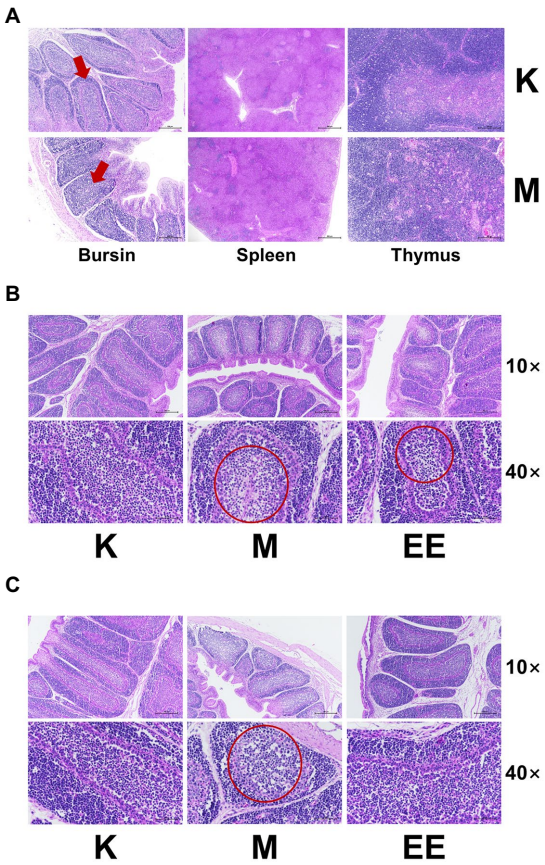

FIGURE 2
Effect of Echinacea on histopathology of immune organs (HE staining). **(A)** Pathological changes of immune organs after 7days of immunosuppression. The area indicated by the red arrow shows a poorly demarcated bursal skin medulla; **(B)** Pathological changes of bursa of each group after 7days of treatment; **(C)** Pathological changes of bursa of each group after 14days of treatment. In the area circled by the red circle, the cells are less aggregated and sparser and the number of lymphocytes is reduced. (*N*=6).

3.2. Effect of Echinacea on histopathology of immune organs

After modeling, the cortical and medullary boundaries of the lymph nodules of the bursa phalloides in the model group were indistinct, the epithelial reticular cell layer disappeared, and the cortical and medullary lymphocytes were significantly reduced. In contrast, the spleen and thymus showed no significant abnormalities (Figure 2A). Therefore, after the administration, the bursa was taken for staining at 7 and 14 days, respectively. In the bursa of group K, the lymph nodes were demarcated between the cortex and the medulla, separated by epithelial reticular cells, and there were a large number of lymphocytes in the cortex and medulla. In contrast, lymphocytes were significantly reduced in the M group and slightly reduced in the EE group after 7 days of treatment (Figure 2B). After 14 days, lymphocytes in the cortex and medulla of the bursa of the M group decreased significantly, medullary lymphocytes showed vacuolar degeneration, while the number of lymphocytes in the EE group recovered to healthy levels (Figure 2C).

3.3. Regulation of gut flora abundance in immunosuppressed ducks by Echinacea purpurea

The number of species that could be observed leveled off when the sample size reached 19–20, showing that the depth and richness of this sequencing test could already indicate the diversity of species in the sample community. The sequencing results are reliable and can be used for subsequent data analysis.

Among the components of the gut microbial community at the phylum taxonomic level in each group of ducks, *Bacteroidota*, *Firmicutes*, *Desulfobacterota*, *Actinobacteriota*, and *Verrucomicrobiota* were the main dominant microbiotas. The species composition of the K and EE groups was similar, with *Bacteroidota*, *Firmicutes*, and *Verrucomicrobiota* as the main dominant microbiotas. The relative abundance of *Bacteroidota* increased to 49.86% in the EE group, which was markedly higher compared to the K (43.14%) and M (44.46%) groups. The relative abundance of Firmicutes was significantly lower in the EE group (40.68%) compared to the K group (47.19%) and the M group (50.58%). The relative abundance of *Verrucomicrobiota* in the EE group reached 4.55%, more than that of the K group (2.59%) and the M group (0.67%) (Figure 3A). At the genus classification level, *Bacteroides*, *Butyricicoccus*, *Akkermansia*, *Megamonas*, and *Streptococcus* are the main dominant microbiotas. The relative abundance of *Bacteroides* in the EE group (29.88%) is more than that of the K (27.62%) and M (25.51%) groups. The relative abundance of *Megamonas* markedly increased in the M group (16.51%) compared to the K (6.36%) and EE (6.68%) groups. The relative abundance of *Akkermansia* in the EE group reached 4.55%, more than that of the K (2.59%) and M (0.67%) groups. Remarkably, the relative abundance of *Prevotellaceae_UCG-001* in the M group was up to 5.34%, while that of the K group was only 0.25%, and the EE group was 1.36% (Figure 3B).

3.4. Effect of Echinacea on the diversity of intestinal flora

None of the α-diversity indices showed significant differences ($p > 0.05$). But the EE group showed a trend of recovery in all indexes compared to the M group. The indices of Chao1, Dominance, and Observed_otus in the M group were lower than those of the K group. In contrast, the indices of the EE group were closer to the K group than the M group. The Shannon, Simpson, and Pielou_e indices increased in group M compared to group K, but those in group EE decreased to a similar level to group K compared to group M (Figure 3C). Analysis of β-diversity using PCA revealed a significant degree of sample separation between the healthy and immunosuppressed groups and a marked effect of

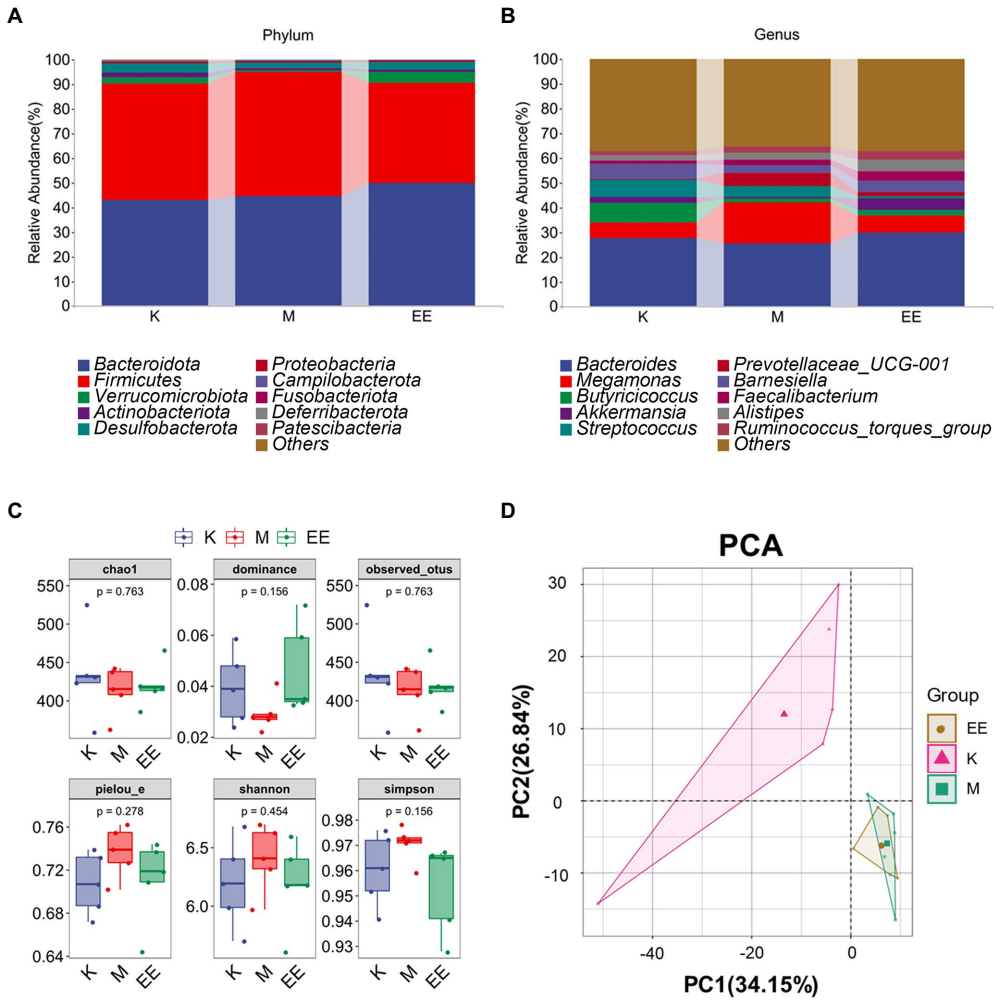

FIGURE 3
Regulation of gut flora abundance in immunosuppressed ducks by Echinacea purpurea. **(A)** Cumulative histogram of the top 10 species in relative abundance at the phylum taxonomic level; **(B)** Cumulative histogram of the top 10 species in relative abundance at the genus taxonomic level; **(C)** Box plot of the alpha diversity index; **(D)** PCA analysis of beta diversity. (*N*=5).

immunosuppression on the gut microbiota. The EE group was more similar to the M group, indicating that no significant changes in the diversity of the gut microbial community were produced after the administration (Figure 3D).

3.5. Clustering of the main intestinal flora affected by Echinacea

The top 35 genera in terms of abundance were selected and clustered at both species and sample levels. There were 19 genera belonging to *Firmicutes* and seven genera belonging to *Bacteroidota*. The genera that showed differences in variation due to immunosuppressive effects were mainly in these two groups. It can also be found that the abundance of some genera in the EE group is more convergent to the healthy group compared to the M group. *Enterococcus*, *Megamonas*, and *Fusobacterium* were more abundantly aggregated in the M group, while the genera with higher abundance aggregation in the EE group included *Akkermansia*, *Bacteroides*, and *Alistipes* (Figure 4A).

3.6. Analysis of species differences between groups

After 14 days of treatment, it was found by Metastat analysis that *Megamonas* ($p < 0.05$), *Prevotellaceae_UCG_001* ($p < 0.05$), *Ruminococcus_torques_group* ($p < 0.05$), and *Prevotella* ($p < 0.001$) all showed a significant increase in relative abundance, while *Collinsella* ($p < 0.01$), *Muribaculaceae* ($p < 0.05$) showed a significant decrease. The relative abundance of Megamonas, Streptococcus, and Enterococcus was significantly decreased in the EE group compared with the M group ($p < 0.05$). The EE group, in comparison with the K group, significantly increased the

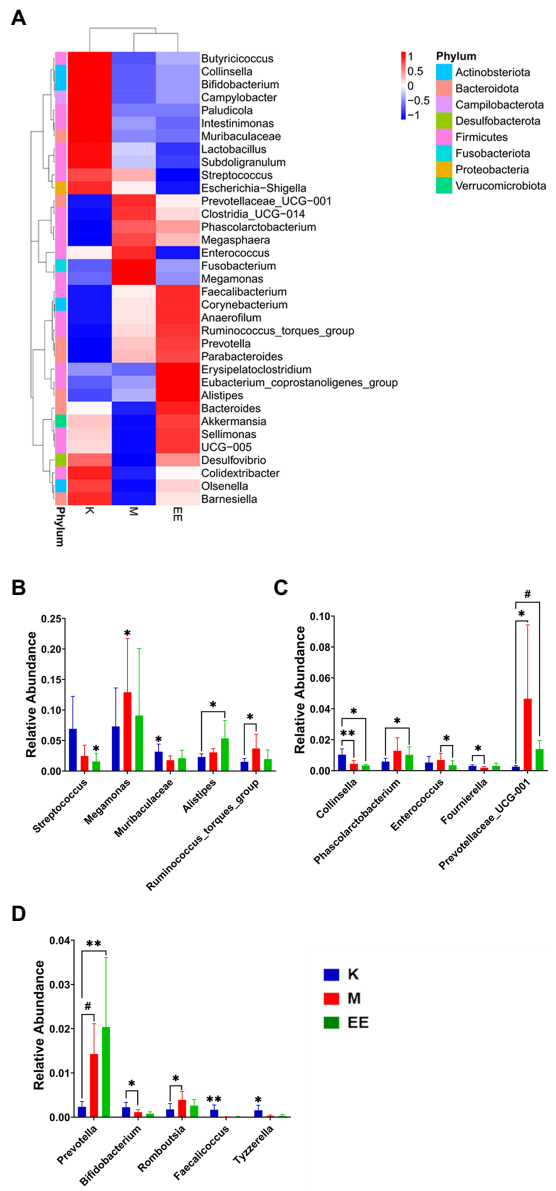

FIGURE 4
Species abundance clustering analysis and analysis of species differences between groups. **(A)** Clusters of species abundance in the top 35 relative abundances at the genus level; **(B–D)** Histograms of species abundance with significant differences between groups (top 15). *N*=5, $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (#).

relative abundance of *Alistipes* ($p<0.05$), *Prevotellaceae_UCG_001* ($p<0.001$), and *Prevotella* ($p<0.01$). Instead, decreased *Streptococcus*, *Collinsella*, and *Muribaculaceae* in relative abundance ($p<0.05$) (Figures 4B–D). In the Lefse analysis, it was found that the dominant microbiota in the M group was *Prevotellaceae*, *Prevotellaceae_UCG_001*; the K group mainly had *Streptococcaceae*, *Lactobacilliales*, *Butyricoccus*, *Bacilli* as the dominant microbiota; and the dominant microbiota in the EE group was *Mogibacterium_sp_*, *Prevotella* (Figure 5).

3.7. Predicting the metabolic function of microbiota affected by Echinacea

Functional predictions based on the KO database showed that among the top 35 metabolic pathways of relevance, the M group had a significantly higher abundance of flora associated with six of these pathways than the K and EE groups, including *K1091*, *K07024*, *K07482*, *K07491*, *K07496*, and *K08303*. Meanwhile the abundance with 15 of these pathways was significantly lower than the other two groups. On the other hand, the EE group had a significantly higher abundance associated with seven of these pathways than the K and M groups, including *K01915*, *K05349*, *K03530*, *K01897*, *K03100*, *K01190*, and *K03169* (Figure 6A). According to the KO database classification of these metabolic pathways, 27.3% of them are related to metabolism, 15.2% to genetic information processing, 12.1% to cellular processes, while organismal systems, human diseases and unclassified each account for 12.1% and environmental information processing for only 9.1% (Figure 6B).

4. Discussion

Dexamethasone-induced immunosuppression significantly inhibited the growth performance of ducks. It included a significant slowing of body weight gain, and a marked reduction in the thymus and bursal index ($p<0.001$). In the trial, immunosuppression damaged the normal structure of the bursa of Fasciola and reduced the number of lymphocytes. And this damage was significantly relieved by the administration of Echinacea extract and restored the number of lymphocytes to a healthy level. The above results indicated that Echinacea extract could effectively repair the damage of the bursa of Fasciola, promote lymphocyte proliferation and improve the immune organ index. It was reported in several studies that the immune-enhancing effects of the polysaccharide components of herbal medicine were mainly achieved by significantly increasing the levels of TNF-α, IFN-γ, and IL-2 in serum (Fan et al., 2013; Zhou et al., 2018; Liu et al., 2022; Nam et al., 2022), and Echinacea extract also increased the levels of these three cytokines in the serum in this trial. It is worth considering that there is a link between changes in serum levels of immune-related cytokines and changes in the intestinal flora. Several studies have reported that the flora is involved in host immunity mainly through their metabolites as signaling factors, acting on immune cells and regulating the expression as well as the release of anti-inflammatory or pro-inflammatory factors. For example, butyric acid in SCFAs can inhibit the proliferation of Th1 cells (Guilloteau et al., 2010), the main cytokines secreted by Th1 cells are TNF-α, IFN-γ, and IL-2, so butyric acid can inhibit the secretion of pro-inflammatory factors and play an immunomodulatory role; or lipopolysaccharide in the flora can promote the secretion of pro-inflammatory factors and induce chronic systemic inflammation (Nicholson et al., 2012).

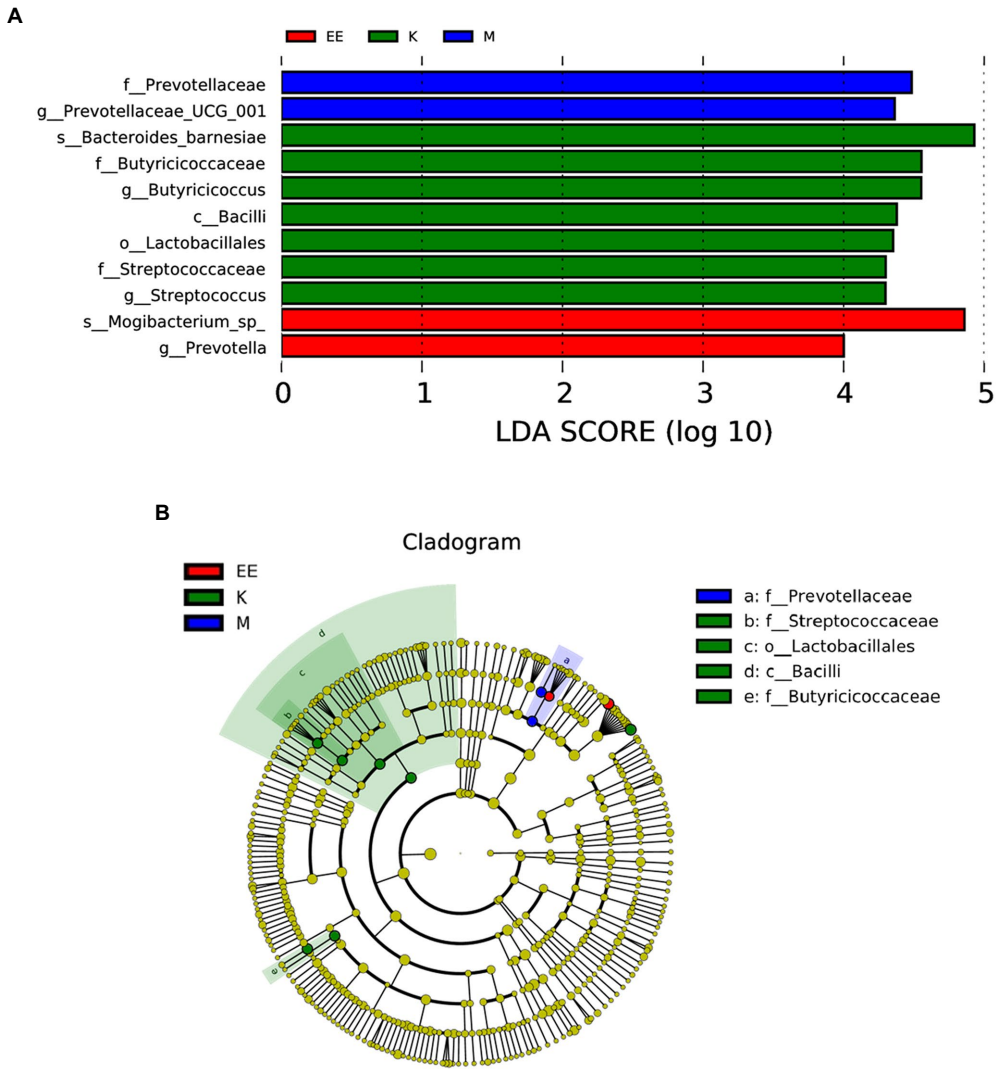

FIGURE 5
Analysis of species differences between groups. **(A)** Histogram of the distribution of LDA values; **(B)** Evolutionary branching plots, *N*=5. Species with LDA scores greater than the set value (set to 4 by default), biomarker with statistical differences between groups, are shown in the histogram of the LDA value distribution. In the evolutionary branching diagram, the circles radiating from inside to outside represent the taxonomic rank from phylum to genus (or species). Each small circle of a different taxonomic level represents a taxon of that level, and the size of the diameter of the small circle is proportional to the relative abundance size.

The intestinal flora, as another organ of the animal body, is not only involved in the digestion and absorption process but also influences the immune process of the animal body by metabolizing and synthesizing essential nutrients needed by the body. The intestinal flora has a crucial role in the development and maturation of the immune system. Lack of colonization of the intestinal flora reduces metabolites associated with the development of the body's immune organs and tissues, thereby inhibiting the development of the body's immune function, which may be defective, as is common in germ-free and neonatal animals (Ennamorati et al., 2020; Yang and Cong, 2021). During colonization, infection training enhances the resistance of the microbiota to infection, while stimulating the host immune system to respond, which can promote the development and maturation of the immune system (Butel et al., 2018; Stacy et al., 2021). The absence of specific intestinal flora may affect the maturation and differentiation of immune cells, such as CD4+ T cells in the spleen (Ostman et al., 2006) and Th17 cells in intestinal lymphoid tissue (Ivanov et al., 2009). Conversely, deletion of immune organs can likewise affect the stability of the gut microbiota, as splenectomy can result in abnormal intestinal flora composition in mice (Wei et al., 2021). Immunosuppression can lead to changes in the intestinal flora, which in turn can cause many problems. In this study, Echinacea purpurea was found to regulate the changes in flora caused by immunosuppression.

The sequencing results revealed a recovery trend in the EE group. Although no significant differences were seen in the alpha-diversity indices ($p > 0.05$), the EE group showed an opposite trend in each index compared to the M group, gradually returning to healthy levels. There were similar reports in the intestine of immunosuppressed mice (Fang et al., 2019; Li et al., 2021).

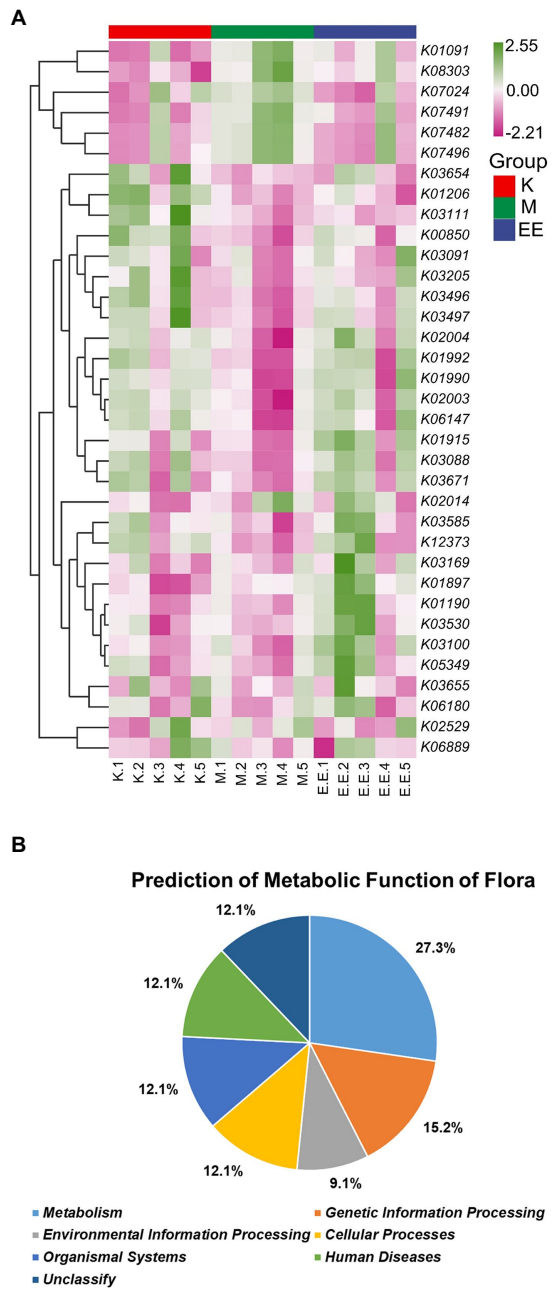

FIGURE 6
Prediction of metabolic function of the microbiota. **(A)** Heat map of the predicted metabolic functions of the flora based on the KO database (top 35). The horizontal coordinate is the sample name and the vertical coordinate is the associated metabolic pathway number, *N*=5; **(B)** Plot of the predicted metabolic functions of the flora as a percentage.

Remarkably, immunosuppression significantly increased the abundance of *Megamonas* and *Prevotellaceae_UCG_001*, while *Akkermansia*, *Alistipes*, and *Butyricicoccus* were significantly reduced. In contrast, Echinacea extract is effective in alleviating these changes in flora and may even increase the abundance of beneficial bacteria to improve the immune deficiency of the body. The relative abundance of *Prevotella*、*Prevotellaceae_UCG_001* was significantly higher in the immunosuppressed group. Both could be potential biomarkers in dexamethasone-induced immunosuppression observed in the Lefse analysis. *Prevotella* is strongly associated with systemic and chronic inflammation. *Prevotella copri* may increase the probability of developing colitis by affecting the structure of the flora when colonized in the mouse intestine (Scher et al., 2013). *Prevotella intestinalis* colonization may affect the metabolic processes of the intestinal flora, exacerbating intestinal inflammation and potentially systemic autoimmunity (Iljazovic et al., 2021). Some studies have reported a positive association between *Prevotella* and HIV-induced intestinal inflammation (Dillon et al., 2016). However, further research is needed to uncover the relationship between *Prevotella* and immunosuppression.

Megamonas, together with *Bifidobacterium*, can act as beneficial bacteria to regulate the composition of the gut microbiota to promote the synthesis and secretion of SCFAs (Dillon et al., 2016; Wu et al., 2022). In the immunosuppression model group, its elevated abundance may be more associated with the positive aspects. The decrease in its relative abundance correlates with the activation of abnormal immune responses, such as in the intestine of patients with Crohn's disease (Maldonado-Contreras et al., 2020), immune thrombocytopenia (Yu et al., 2022), or IgA nephropathy (Dong et al., 2020), where its abundance is significantly reduced. It suggests that the relative abundance of *Megamonas* is related to the immune status of the organism. Its abundance increases when the immunity declines, while it decreases significantly with abnormal activation in the immune response.

Akkermansia is a genus of beneficial bacteria that has received recent attention in research reports. Its *Akkermansia muciniphila* could enhance the activity of immune cells by being injected intravenously into mice to reduce the tumor burden in mice (Dong et al., 2020; Luo et al., 2021). Its colonization of the intestine increases the expression of genes involved in the immune response, producing IL-8 to participate in the host's mucosal immune regulation. It also produces mucins that positively works on intestinal epithelial cells to maintain the integrity of the intestinal epithelial mucosa (Derrien et al., 2011; Reunanen et al., 2015). Immunosuppression significantly reduced the relative abundance of *Akkermansia* in the gut to only 0.67% in the immunosuppressed model group. Its low abundance may lead to the absence of the functions described above and put the already immune dysregulated hosts at increased risk of disease infection. However, its relative abundance was significantly higher in the EE group supplemented with Echinacea extract, enhancing the protective effect on the intestine and modulating mucosal immune function. It also displays significant anti-inflammatory properties in the intestine, effectively relieving DSS-induced acute colitis (Qu et al., 2021). Echinacea extract may improve intestinal mucosal immune function and restore host immunity by increasing the abundance of *Akkermansia* in the gut of immunosuppressed ducks. It also enhances the immunity by increasing the abundance of *Alistipes*. Because *Alistipes* could bind to *TLR4* and activate the expression of TNF to enhance the immune clearance of tumor cells (Iida et al., 2013). However, there is no definitive evidence for the main components of Echinacea extract that act with the flora.

The gut microbiota interacts with the host primarily through metabolites produced during the metabolism of the flora. The prediction of the metabolic function of the flora revealed that immunosuppression had a significant effect on the metabolism of the flora and involved metabolic pathways associated with some human diseases and organism systems. While Echinacea extract antagonized the effect of immunosuppression on the mycota and increased the abundance of mycota associated with metabolic functions of human diseases and organic systems. The classification based on the KO database revealed that *KO3671* is associated with the immune system. It has a regulatory role not only in plant immune responses (Mata-Perez and Spoel, 2019) but also in mammals，playing a role in the regulation of immune signal release (Kim et al., 2008; Mougiakakos et al., 2011). It mainly through its protection of cells against oxidation and thus reducing immune cell apoptosis positively affects the immune system (Lu and Holmgren, 2012). *Akkermansia*, *Alistipes*, *Butyricoccus*, and *Bacteroides*, whose relative abundance increased in the EE group, were found to have genes corresponding to *KO3671* in the functional prediction. We speculate that the increased abundance of the genus mentioned above may have increased the *Trx* content in the intestine, exerting its enhancing and modulating effects on the immune system. It could be one of the pathways of immune function modulation by Echinacea extract, but more evidence is needed to prove it.

5. Conclusion

To sum up, Echinacea extract can significantly alleviate the immunosuppressive effect of dexamethasone on ducks. It mainly contributes by improving the growth performance of immunosuppressed ducks, restoring the function of immune organs, and regulating the level of immune-related cytokines in the serum. 16 s-rRNA sequencing identified *Prevotella* as a potential biomarker for dexamethasone-induced immunosuppression. Echinacea extract may modulate intestinal immune function by increasing the abundance of beneficial bacterial genera such as *Akkermansia* and *Alistipes* in the intestine. The trial provides a possibility for the application of Echinacea in waterfowl and enriches the research on immunosuppression in waterfowl.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/, PRJNA895924.

Ethics statement

The animal study was reviewed and approved by the Institutional Animal Welfare and Research Ethics Committee of South China Agricultural University, Guangzhou, China.

Author contributions

RL, CZ, YS, JC, DG, and SL: were responsible for study conception and design. DS: revised the manuscript. RL, CZ, YS, JC, DG, SL, and DS: were involved in the drafting of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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3、学术论文：Implementation of Intelligent Potted Plant Management System Based on Internet of Things

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Design and Implementation of Intelligent Potted Plant Management System Based on Internet of Things

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Abstract : To solve the problem of difficult cultivation of precious potted plants, this paper designed an intelligent potted plant cabinet which can be monitored remotely. Embedded control system was developed based on Keil environment, Wireless communication and real-time monitoring devices are brought together, IP address is created in Alibaba Cloud server, data center is established, cloud middleware is set up, B/S version and mobile App access system are developed, and automatic plant of plant cabinet and intelligent monitoring of potted growth status are realized through temperature and humidity control device, light intensity control device, watering control device and real-time monitoring device. The growth and flowering period of jasmine was taken as the experimental object to verify the plant effect. The intelligent plant cabinet controlled the temperature deviation within ±0.5 ℃, the air humidity deviation within ±1.5% RH, the light intensity deviation within ±23 Lx, the soil humidity deviation within ±4.1% RH, and the flowering period was prolonged by 4-5 days. The plant cabinet can intelligently control environmental factors according to the growth habits of jasmine, and the system has fast response and stable operation, which can be extended to intelligent cultivation of various potted plants.

Keywords: potted plant cabinet; internet of things; user interaction; intelligent cultivation; remote control

1. Introduction

With the improvement of people's living standards, all kinds of precious potted plants appear in every household, but most of them have harsh growth conditions, which are affected by many factors such as soil, sunshine, water, fertilizer, etc. It is difficult to properly control them by relying solely on manual experience. In addition, due to people's busy work or business trips, they neglect potted plants, resulting in poor growth and even death of precious potted plants. The use of intelligent plant cabinet is an effective method for the healthy growth of potted plants. Its plant characteristics are mainly reflected in remote real-time monitoring and intelligent control of various environmental factors, which can provide the best growth conditions according to the different growth needs of various potted plants [1].

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In recent years, many researchers have put forward a variety of design methods for intelligent plant cabinets. British scholar Natalie King designed an intelligent plant basin that can be called thirst [2]. The plant basin uses microcomputer chips and sensors, which has certain intelligence, but does not apply the Internet of Things to the plant system [3]. The plant basin uses microcomputer chips and sensors, which has certain intelligence, but does not apply the Internet of Things to the plant system. Y. Z. Zheng developed an intelligent flowerpot system that can move autonomously. The flowerpot has automatic watering and charging functions, and realized the use of mobile App control system for intelligent plant [4], but only limited to a small range of Internet of Things systems; The new intelligent flowerpot control system based on Internet of Things technology adds Bluetooth and GMS short message devices on the basis of microchip, and realizes the functions of short-distance Bluetooth communication and long-distance short message notification [5]. The above designs are all based on microchips, adding some communication functions and Bluetooth functions, realizing the functions of environmental factor data collection and remote monitoring, but it is difficult to realize all-round monitoring and intelligent plant of environmental factors and growth information of different kinds of potted plants in different growth periods [6].

2. Overall Composition of the System

The intelligent plant monitoring and management system mainly adopts Alibaba Cloud server and applies the Internet of Things framework, which is divided into sensing layer, transmission layer, support layer and application layer[7]. All kinds of sensors collect data according to the preset frequency, while the embedded control module is mainly responsible for effectively processing the perceived information, sending it to the cloud server by the communication module, establishing a data center in Alibaba Cloud server, designing middleware, and realizing information collection, combing, storage and display [8].

The sensing layer integrates temperature and humidity sensors, soil humidity sensors, cameras, stepping motors, electromagnetic locks, etc. Through data collection and calculation, the information aggregation of environmental factors (temperature, humidity, light intensity, etc.) in the plant cabinet can be realized [9]; In the transmission layer, the plant cabinet has a fixed IP address in Alibaba Cloud, and the embedded control module transmits the status data of the plant cabinet to the IP address through GPRS, and receives the control command from the cloud at the same time. In the support layer, Alibaba Cloud Server provides services such as cloud services, cloud storage and cloud data security library to the intelligent management system of the plant cabinet, meeting the requirements of creating an intelligent plant cabinet cloud data center and installing middleware; In the application layer, according to different application requirements, the mobile App version application system for mobile phones is developed, and users can freely access the server through mobile phones and view the growth status of potted plants in real time [10] .

3. Hardware Design

Embedded control module is the core of this system design, which is mainly

responsible for a great deal of data exchange with temperature and humidity control device, illumination intensity control device, watering control device, cabinet door lock control device, real-time monitoring device and GPRS module.

The microcontroller STM32F103ZET6171 based on ARM Cortex-M kernel is used as the main control chip of this system, which receives and processes data through serial port. The sensors used are GY-30 illumination sensor, FDR soil moisture sensor and 485 temperature and humidity sensor. GY-30 illumination sensor is a digital ambient illumination sensor with I2C interface and built-in standard I2C communication protocol. The controller can directly read the digital amount of current illumination. The clock line SCL of GY30 is connected with PA4 of the controller, and the data line SDA is connected with PA5 of the controller. Both soil moisture sensor and temperature and humidity sensor adopt standard MODBUS_RTU485 communication protocol, which is connected with the sensor through standard 485 interface, and the collected values are obtained according to the protocol. GPRS communication is connected with the controller through SIM 800C module. The sensor information collected by the controller is sent to the GPRS communication module through serial port 3, and then the GPRS module transmits the data to the remote end. The real-time monitoring function is realized by the ALIENTEK OV7725 camera module, which is connected with the interface (P6). The OLED display screen of this system adopts IIC communication interface. The controller PB6 is connected with the clock line SCL of OLED, and the controller PB7 is connected with the data line SDA of OLED. The display screen is driven by time sequence. Atomizer, peristaltic pump, fan and refrigerator are all high-power devices. The controller controls the operation and closing of power devices by controlling the switch of relays. When the controller outputs low level, the relays are pulled in, and when the controller outputs high level, the relays are turned off. The atomizer control interface is connected with the IO port PE1 of the controller, the peristaltic pump control interface is connected with the IO port PE2 of the controller, the fan control interface is connected with the IO port PE3 of the controller, and the refrigerator control interface is connected with the IO port PE4 of the controller.

4. Software Design

System program is mainly local, remote and cloud three levels of programming. The local program is developed based on Keil environment, which mainly completes sensor data collection and real-time display of plant cabinet status, and controls the operation of each device according to cloud-matched plant information. The remote access system is a mobile App, which provides users with user rights management, potted growth data display, remote monitoring and control; Cloud middleware, as a bridge connecting the plant cabinet and the remote access system, sends the real-time status data of the plant cabinet to the data center, and also provides services for users, transmitting user instructions to the plant cabinet. In addition, the cloud middleware also provides the support of the control model and algorithm of double feedback of data, environmental factors and potted growth status for the plant cabinet system.

The communication module of this design adopts Socket communication design program, and transmits the status information of plant cabinet collected by sensors to the cloud for processing. After the initialization of the Socket communication program, it will automatically establish a connection with the port and start monitoring and

blocking, waiting to establish a connection with the port of the plant cabinet. After being connected with the port of the plant cabinet, The plant cabinet sends coded data to the cloud. In order to reduce the power consumption of the plant cabinet system, only the code of updated data is sent. After receiving the data, the cloud decodes and updates the plant cabinet information base and operation log according to the specified format, and then feeds back the response information to the plant cabinet. Users can send control commands to the plant cabinet by delegating middleware through mobile App, and the embedded control module of the plant cabinet receives and executes the commands. When the plant cabinet is started and closed, each component runs according to the instruction in turn. When the plant cabinet is about to reach a preset instruction state, according to the running speed of the plant cabinet, the suspension instruction is sent to the system in advance to reduce the error caused by system delay.

A camera is installed in the plant cabinet, can automatically take pictures of potted plants. Then the image is transmitted to the cloud, and the cloud server automatically identifies the types of potted plants according to the image characteristics, and formulates the plant scheme. Then the system automatically configures environmental factors such as temperature, humidity and light intensity in the plant cabinet, and automatically completes the plant task. At the same time, the potted plant growth data is fed back to the user App for the user to assist in decision-making.

5. Test

In order to verify the effectiveness of this system, jasmine is used as the experimental object, and the plant system test and plant effect test are carried out respectively. Two pots of jasmine plants with similar growth status and potted soil were put into the plant cabinet and named as No.1 plant and No. 2 plant respectively. The plant cabinet system corresponding to No.1 plant operated normally, while the plant cabinet system corresponding to No.2 plant was set to a closed state, that is, No.1 plant was planted with the plant cabinet and No.2 plant was planted normally.

5.1 Experiment of Plant System

Start the plant cabinet for plant, and the sensors of the intelligent plant cabinet collect data twice a day, once at 8: 00 a. m. and once at 8: 00 p. m. In order to ensure the accuracy and stability of the collected data, the data are collected from the third day after being put into the plant cabinet, and the collection days are 10 days. The collected data are shown in Table 1. From the experimental data in Table 1, it can be seen that the temperature average of 20 times of temperature and humidity sensing collection the average value of temperature and humidity sensor collected for 20 times is 29.755 ℃, and the temperature acquisition deviation is controlled within 0.08 ℃; The average value of air humidity collected for 20 times is 70.225 RH, and the deviation of air humidity collection is controlled within 0.115 RH; The average of 20 times of illumination is 9198.31 x, and the deviation of illumination acquisition is controlled within 171x; The average value of soil moisture collected for 20 times is 63.315% RH, and the deviation of soil moisture collection is controlled within 3.6% RH. Therefore, the sensor data acquisition accuracy is high, which shows that the plant cabinet system designed in this paper has good reliability and stability.

In this experiment, by observing the number of petals of jasmine flowers, we can determine whether the flowers fall off. The test time is 15 days, and the number and corresponding time of bud wilting under the two cultivation methods are recorded. The test data are shown in Table 1.

Table 1. System Test Data.

Collect number	Temperature/℃		Air Humidity/% RH		Illumination/lx		Soil Moisture Content/% RH	
	Collection	Deviation	Collection	Deviation	Collection	Deviation	Collection	Deviation
1	28.3	0.5	69.2	-1.4	9225	18.0	59.5	4.5
2	27.6	-0.1	69.0	-1.5	9218	11.0	61.0	2.2
3	28.1	0.5	71.0	0.6	9200	-10.0	62.0	0.9
4	28.4	0.2	70.3	1.3	9175	-15.0	61.0	-1
5	31.6	-0.3	68.2	-1.2	9205	20.0	61.2	-3.8
6	30.0	0.1	70.0	0.5	9231	-17.0	59.9	-1.6
7	31.2	-0.3	71.9	-1.5	9186	30.0	61.8	-1.4
8	28.1	0.4	69.2	1.2	9183	-13.0	61.9	0.9
9	31.3	0.1	70.3	0.9	9210	24.0	62.6	0.2
10	29.5	0.3	69.0	1.5	9215	16.0	64.4	0.5
11	29.2	0.2	71.3	-0.4	9195	-8.0	57.4	-2.3
12	29.6	0	69.1	-1.3	9175	-19.0	57.2	2.4
13	31.9	-0.2	70.0	1.8	9185	19.0	64.9	-1.6
14	30.9	-0.1	70.7	1.9	9195	31.0	62.3	2.9
15	31.7	0.3	71.2	1.2	9200	22.0	58.1	2.1
16	29.8	0	71.1	-0.1	9214	14.0	60.6	0.6
17	28.9	-0.4	69.6	-0.8	9205	37.0	61.9	0.3
18	28.0	0.3	72.3	-0.5	9175	-16.0	61.2	-1.6
19	29.9	0.2	70.1	1.2	9188	13.0	64.3	-1.5
20	31.1	-0.1	71.0	-0.3	9186	-19.0	63.1	1.8

5.2 Cultivation Effect Test

In this experiment, by observing the number of petals of jasmine flowers, we can determine whether the flowers fall off. The test time is 15 days, and the number and corresponding time of bud wilting under the two cultivation methods are recorded. The experimental results show that: The No. 1 plant put in the plant cabinet has a better growth environment, Compared with No. 2 plant, its flowers bloom for a long time, However, due to the lack of a better growth environment for the normally planted No. 2 plant, Its flowers bloom for a relatively short time, Therefore, the flowers of No. 2 plant wither faster than those of No. 1 plant, Therefore, the flowering period of No. 1 plant is relatively prolonged, It can be seen from Table 2 that the flowering period of jasmine flowers cultivated by intelligent potted system is prolonged by about four to five days compared with that corresponding to ordinary cultivation mode, and the total number of buds is also more than that under ordinary cultivation, indicating that various environmental factors provided by the plant cabinet system are beneficial to prolong the blooming time of buds and increase the number of buds, and the plant effect is obvious.

Table 2. Flowering Test Data.

Time (day)		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Number of blight buds	Plant N0.1	0	0	0	1	4	8	13	17	20	25	26	28	32	37	42
	Plant N0.2	0	0	8	14	15	18	19	22	26	27	29	32	35	35	35

6.Conclusions

A kind of intelligent management plant cabinet of Internet of Things suitable for plant potted plants is designed. Using network communication technology, The system is developed on the basis of Alibaba Cloud architecture, and the two-way communication between plant cabinet and Alibaba Cloud server is realized through cloud middleware, and the mobile App is developed for users as the access terminal, which can better guide users to carry out fine management and verify the feasibility of plant potted plants by using the new generation of information technology.

This design solves the problem that potted plants are not well cared for by people. The product realizes intelligent plant through the control unit, combines the basic factors required for plant growth, provides the most suitable growth environment for plants, and effectively improves the survival rate of potted intelligent plant.

The test results show that, The intelligent plant cabinet system developed by this design runs stably, It is safe and reliable. The temperature deviation is controlled within ±0.3 ℃, the air humidity deviation is controlled within ±1.2% RH, the light deviation is controlled within ±171x, and the soil humidity deviation is controlled within 3.6% RH. The plant cabinet can intelligently control the environmental factors according to the growth habits of jasmine flowers, and the plant effect is obvious, which can be extended to the intelligent plant of various potted plants.

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4、学术论文：副鸡禽杆菌毒力质粒缺失株的构建及其致病性的研究

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副鸡禽杆菌毒力质粒缺失株的构建及其致病性的研究

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摘 要：为研究副鸡禽杆菌(APG)质粒与菌株致病力的关系，本研究采用42 ℃诱导的方法缺失APG分离株APG-Hu中的毒力质粒，经PCR鉴定质粒缺失菌株后将其命名为APG-HuΔ。分别采用分光光度计测定OD_{600nm}值法、微量生化鉴定管法、K-B纸片法和雏鸡感染试验测定APG-HuΔ的生长曲线、生化特性、药物敏感性和对雏鸡的致病性。结果显示，在42 ℃诱导条件下，能够有效缺失APG-Hu中的毒力质粒p250和pA14，而质粒pYMH5未被缺失；APG-HuΔ生化特性、生长曲线及耐药性与APG-Hu相比均无明显差异。雏鸡感染试验结果显示，多数APG-HuΔ感染鸡无明显临床症状，总体发病情况明显弱于同等剂量APG-Hu感染的鸡，表明APG-HuΔ毒力显著降低。本研究结果表明，APG毒力与质粒p250和pA14相关，而质粒pYMH5可能增加APG耐受多种抗生素的风险。本研究为APG致病机理及耐药机制研究奠定基础，也为挖掘APG质粒的应用潜力提供一定参考。

关键词：副鸡禽杆菌；质粒p250；质粒pA14；质粒缺失菌株APG-HuΔ；致病性
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Construction and pathogenicity of plasmid deletion strain of *Avibacterium paragallinarum*

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Abstract: In order to study the relationship between the plasmid and the pathogenicity of of *Avian paragallinarum* (APG) strain, the virulence plasmid of APG isolate APG-Hu was deleted by maintain at 42℃. The plasmid-deleted strain was identified by PCR and named APG-HuΔ. The physiological and biochemical characteristics, growth curve, drug sensitivity and pathogenicity of APG-HuΔ were determined by biochemical micro-identification tube method, spectrophotometer at OD_{600nm}, test paper method and chicken infection test respectively. The results showed that plasmids p250 and pA14 were deleted in APG-HuΔ, except for pYMH5. There were no significant difference in the physiological and biochemical characteristics, growth curve, and drug resistance between APG-HuΔ and the parent strain of APG-Hu. The results of chicken infection experiments showed that most of chickens inoculated with APG-HuΔ had no obvious clinical manifestations, which was similar as normal, and the overall incidence was significantly weaker than that of the same dose of APG-Hu infected chickens, indicating the virulence of APG-HuΔ was much

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lower than that of APG-Hu. The results suggested that the virulence of APG was related to plasmid of p250 and pA14, and the potential tolerance to a variety of antibiotics might associated with the presence of pYMH5. This study not only lays a foundation for the study of the pathogenesis and drug resistance mechanism of APG, but also provides some reference for exploring the application potential of APG plasmid.

Key words: *Avibacterium paragallinarum*; plasmid p250; plasmid pA14; plasmid deletion strain APG-HuΔ; pathogenicity

鸡传染性鼻炎(Avian infectious coryza，AIC)是危害养鸡业的一种重要呼吸道疾病[1]，在国内表现出一定的多样性与临床疾病的复杂性[2-3]。不仅由于其病原副鸡禽杆菌(*Avibacterium paragallinarum*，APG)存在多种血清型，不同血清型APG灭活疫苗之间的交叉保护力弱，不同地区流行的血清型也存在一定差异，而且出现了一些新的变异菌株，如多重耐药性[4-5]和烟酰胺腺嘌呤二核苷酸(Nicotinamide adenine dinucleotide，NAD)-非依赖性菌株[6-7]。研究发现，不同血清型的APG毒力也不相同[8]。目前已发现的APG毒力相关抗原有以下几种[9]：血凝素，决定病原菌的免疫原性和致病性；脂多糖，革兰阴性菌特有的内毒素，能引起中毒；荚膜，同时具有抗宿主吞噬和粘附并攻击宿主靶细胞的作用；多糖，其结构和成分常与APG的致病病原性和血清型相关，该菌的多糖往往能引起鸡心包积液和鼻炎等症状[10-11]。目前关于APG抗原毒力因子研究最多的为血细胞凝集蛋白和荚膜。除此之外，细胞外蛋白酶可能在APG所致AIC的过程中起重要作用[12]。另外，有研究表明质粒也与APG的毒力相关。最早报道的质粒来自中国台湾分离株(HP250)的p250[13]，其携带一个血红素产生位点，该位点表达一种能够杀死一系列其他革兰氏阴性菌的蛋白质[14]。最近，又报道2个质粒，分别是pA14和pYMH5，其中pA14含编码与毒力相关的Mg1A蛋白和R1Vase11蛋白[15]，pYMH5编码链霉素、磺胺类、卡那霉素、新霉素抗性基因，与质粒pLS88具有很高同源性[16]。Hsu等对于1999年～2003年收集的18株APG进行血清分型和药物敏感性试验，结果显示所有菌株均有溶血活性，72%耐药菌株包含质粒pYMH5和pA14[15]。推测pA14和p250的血红素活性可能与APG的发病机理有关，但缺乏直接证据。本研究室前期从湖北恩施地区某蛋鸡场疑似AIC的病例中分离获得一株APG (命名为APG-Hu)。该菌不仅能在无NAD的胰酪大豆胨液体培养基(TSB)中较好的生长，而且还携带p250、pA14和pYMH5 3种质粒[17]。为了探究这些质粒与APG的毒力关系，本研究在前期研究基础上，通过42 ℃诱导方法缺失APG中的3种质粒，并利用K-B纸片法和鸡感染模型分别检测质粒缺失菌株的药物敏感性和毒力，为APG的毒力和耐药性的机制研究提供一定参考。

1　材料与方法

1.1　主要实验材料　APG-Hu由华中农业大学动物医学院兽医微生物学与免疫学实验室分离、鉴定并保存；70 d日龄APG抗体阴性的海兰灰蛋鸡由华中农业大学实验鸡场提供；TSB和胰酪大豆胨琼脂培养基(TSA)购自海博生物技术有限公司；NAD购自上海源叶生物科技有限公司；药敏纸片购自常德比克曼生物科技有限公司；DNA提取试剂盒购自天根生化科技(北京)有限公司；2×*Taq* MasterMix、DL2000 Marker 等均购自北京赛百盛基因技术有限公司；GoldenView核酸染料购自北京索莱宝科技有限公司。

1.2　APG-Hu毒力质粒缺失株APG-HuΔ的构建及PCR鉴定　将APG-Hu接种于含5%牛血清和0.01% NAD的TSB中，于42 ℃培养16 h～24 h，取0.1 mL接种于0.9 mL TSB中，在42 ℃培养16 h ～24 h。重复上述步骤培养20代后，取适量培养物划线接种于TSA (含5%牛血清和0.01% NAD)平板上，置于37 ℃ 5% CO_2培养16 h～24 h后。挑取3～5个单菌落利用TSB (含5%牛血清和0.01% NAD)纯培养，取培养物利用质粒DNA提取试剂盒提取质粒，用ND2000 (Thermo NanoDrop 2000微量紫外分光光度计)测定质粒浓度后作为模板。同时提取等量APG-Hu培养物的质粒作为参考。根据文献[16]报道的3种质粒基因设计特异性引物进行PCR检测(表1)。PCR产物经1%琼脂糖凝胶电泳检测。将42 ℃高温培养20代的毒力质粒缺失株命名为APG-HuΔ，并开展后续试验。

表 1 质粒检测用引物

Table 1 Primers used for plasmid detection by PCR

质粒 Plsmid	基因来源 GenBank accession	引物 Primer	起止序列(nt) Size of start and end (nt)	序列(5'-3') Sequence of (5'-3')	产物 bp Product size	Tm ℃
p250	AY300023.1	p250-F	363-382	GAACAAGGGGTTAGGTATGG	590	52
		P250-R	952-933	ATTGTAGCACCTGAGTTTCC		
pA14	EF120635.1	pA14-F	3-24	GCGATTGCTCATCATATTGTCC	386	50
		pA14-R	388-368	AAATCACGATAATTGCCACCC		
pYMH5	EF015636.1	pYMH5-F	107-127	TCGGCATCGTCAACATAACC	612	55
		pYMH5-R	718-737	GCGAAACAGACAGAAGCACC		

1.3 APG-HuΔ的生化鉴定 挑取APG-HuΔ和APG-Hu单菌落分别接种于硫化氢、氧化酶、糖发酵、硝酸盐还原、吲哚等细菌生化微量鉴定管(含终浓度为40 μg/mL的NAD)，置37 ℃ 5% CO_2培养16 h，观察细菌的生化特性，按照细菌生化微量鉴定管使用说明书判定生化鉴定结果。

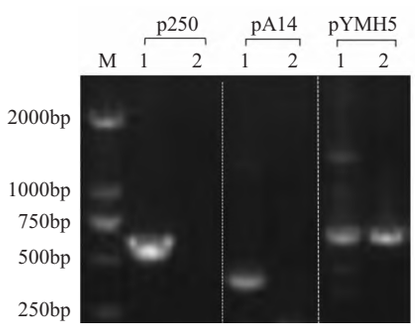
1.4 APG-HuΔ生长曲线的测定 将质粒缺失株APG-HuΔ与亲本株APG-Hu分别接种TSB液体培养基(含5%牛血清和0.01% NAD)，培养至OD_{600nm}值达到1时按1:100的比例接种至含10 mL TSB培养基中，置37 ℃ 200 r/min振荡培养，每隔1 h取出100 μL菌液，利用分光光度计测其OD_{600nm}值，持续至24 h。每种菌3个重复，取平均值作为最终结果，绘制生长曲线。

1.5 APG-HuΔ的药敏试验 将菌株APG-HuΔ接种于TSB(含5%牛血清和0.01% NAD)，37 ℃ 5% CO_2培养16 h，取适量培养物涂布于含0.01% NAD和5%牛血清的TSA平板上，分别将庆大霉素、氧氟沙星、恩诺沙星、阿米卡星、妥布霉素、环丙沙星、头孢噻吩、卡那霉素、氯霉素、红霉素、头孢地儿和氨苄青霉素12种药敏纸片贴附在培养基表面，培养24 h，测量抑菌圈大小，判定结果。抑菌圈直径≥15 mm判为敏感，11 mm～14 mm判为中介，≤10 mm判为耐药。

1.6 APG-HuΔ对鸡的致病性试验 将42只70 d APG抗体为阴性的海兰灰蛋鸡随机均分为7组。除灭菌PBS组6只鸡外，其他组鸡分别眶下窦接种APG-HuΔ和APG-Hu。每株菌分为3组，感染剂量分别为10^4 cfu/只、10^5 cfu/只和10^6 cfu/只。实验鸡分组隔离饲养，逐日观察临床症状，持续5 d。接种后5 d采集鼻拭子进行细菌分离培养及菌株16sRNA PCR鉴定[17]。

2 结 果

2.1 APG-HuΔ的构建及PCR鉴定结果 将42 ℃高温培养20代获得的菌株APG-HuΔ及亲本株接种于TSB液体培养基中，培养后分别提取质粒进行PCR检测，并测定质粒浓度。结果显示，APG-HuΔ培养物能检测到pYMH5质粒，但未能检测到pA14和p250质粒(图1)。APG-Hu和APG-HuΔ提取的质粒浓度分别为108.7±2.72 ng/μL和10.9±2.45 ng/μL。表明，42 ℃条件可以有效地诱导APG-Hu中pA14和p250质粒的缺失，但未能诱导pYMH5质粒的缺失，可能需要优化诱导条件并延长诱导传代次数或尝试其他方法。



M: DL2000 DNA Marker; 1: APG-Hu; 2: APG-HuΔ

图 1 APG-HuΔ 中质粒的 PCR 检测

Fig. 1 Identification of plasmid in APG-HuΔ by PCR

2.2 APG-HuΔ的生化鉴定结果 将APG-HuΔ和APG-Hu分别接种生化微量鉴定管进行生化试验，结果显示，APG-HuΔ对多种糖类，如蔗糖、葡萄糖、麦芽糖等，过氧化氢酶、硝酸盐还原试验均呈阳性；氧化酶和吲哚试验呈阴性。APG-HuΔ与APG-Hu生化特性基本一致，表明质粒缺失并未改变APG的基本生化特性。

2.3 APG-HuΔ生长曲线的测定 利用分光光度计测定OD_{600nm}值的方法绘制APG-HuΔ生长曲线。结果显示，APG-HuΔ在3 h～10 h增殖速度较快，比同一时间APG-Hu的OD_{600nm}值高0.1～0.2，10 h之后二者生长速度无显著差异，二者在16 h左右达到稳定期，OD_{600nm}值约为1.6 (图2)。表明缺失质粒p250和pA14对APG的生长性能无明显影响。

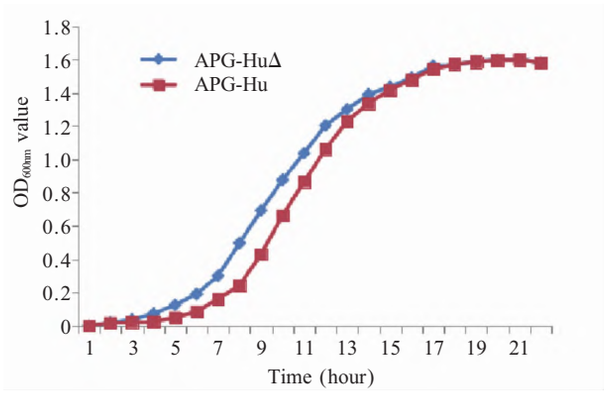


图 2　APG-HuΔ和 APG-Hu 的生长曲线
Fig. 2　The growth curve of APG-HuΔ and APG-Hu

2.4　APG-HuΔ的药敏试验　采用K-B纸片法测定APG-HuΔ对药物的敏感性，结果显示，APG-HuΔ对恩诺沙星、阿米卡星、环丙沙星、卡那霉素、头孢地尔等敏感，对庆大霉素、氧氟沙星、氯霉素、红霉素等中度敏感，对头孢噻吩、氨苄耐药(表1)。与亲本菌株APG-Hu相比，二者对选择的药物敏感性无显著差异，均无明显的耐药性(表1)。这表明，质粒p250和pA14缺失不影响APG耐药性，质粒pYMH5可能未能表现出显性表型，其携带的多种抗性基因暂时处于沉默状态。

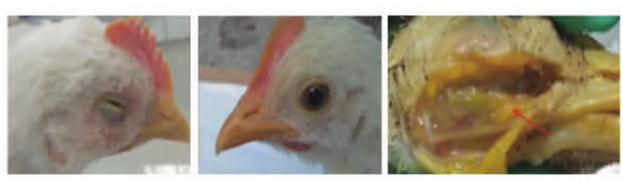
表 2　APG-Hu 和 APG-HuΔ 的药物敏感性检测(抑菌圈直径，mm)
Table 2　Antibiotic susceptibility of APG-Hu and APG-HuΔ (Inhibition zone diameter, mm)

菌株 / 药物 Strain/drug	庆大霉素 Gentamycin	氧氟沙星 Ofloxacin	恩诺沙星 Enrofloxacin	阿米卡星 Amikac	妥布霉素 Nebcin	环丙沙星 Ciprofloxacin	头孢噻吩 Cefalotin	卡那霉素 Kantrex	氯霉素 Chloramphenicol	红霉素 Erythromycin	头孢地尔 Cefalexin	氨苄 Amoxicillin
APG-Hu	2.1	1.7	3.9	2.7	1.8	3.6	0.9	3.1	1.7	2.1	3.5	0.8
APG-HuΔ	2.4	1.7	4.2	2.6	1.8	3.7	0.9	3.2	1.9	2.1	3.5	0.8
	S	I	S	S	I	S	R	S	I	S	S	R

注 Note：S：敏感 Susceptible；I：中介 Intermediate；R：耐受 Resistant

2.5　APG-HuΔ对鸡的致病性试验　将APG-HuΔ与APG-Hu分别经眶下窦接种70日龄健康海兰灰蛋鸡，每天观查鸡的发病情况，连续观察5 d，记录结果，并取鼻拭子进行病原分离。结果显示，APG-HuΔ组鸡临床症状轻微，除10^6 cfu/只组有2只鸡在感染后120 h存在眶下窦肿胀(单侧或双侧)外(图3A)，其余鸡均无明显异常，与PBS对照组基本一致(图3B)。实验鸡未见明显鼻液，多数在用手挤压时流出少量清鼻液，或经12 h～48 h部分发病鸡临床症状减轻至消失。APG-Hu实验组鸡在感染后24 h，超过50%以上的鸡发病，持续至试验结束症状表现明显，具体表现为单侧或双侧眶下窦肿胀，流鼻液、有少数鸡面部水肿，眶下窦解剖有黄色干酪样分泌物(图3C)；剂量越大发病率越高，症状越明显，持续的时间越长；APG-Hu 10^4 cfu/只组鸡的临床表现与APG-HuΔ 10^6 cfu/只组鸡的发病情况类似，但症状更明显。这表明，APG-Hu具有较强的毒力，而APG-HuΔ毒力较弱，这与质粒p250和pA14缺失有关。也表明质粒p250和pA14是毒力因子，与APG的毒力相关。

利用含鸡血清和NAD的TSA培养基，从人工感染鸡，尤其是出现临床症状鸡中重新分离到圆形、灰白色的半透明露滴状小菌落，经16s RNA PCR鉴定为APG。无临床症状感染鸡的APG分离率及菌落数远低于有临床症状的感染鸡。而对照组鸡未出现发病症状(图3B)，也未能分离到APG。表明APG感染鸡的发病情况与鸡鼻部APG的载菌量关系密切。



A：一侧眶下窦肿胀；B：正常；
C：眶下窦有黄色干酪样变化
A: Unilateral infraorbital sinus swelling; B: Normal;
C: Yellow caseous material in infraorbital sinus

图 3　实验鸡分别感染 APG-Hu 和 APG-HuΔ 后的临床表现
Fig. 3　Clinical symptoms of experimental chickens infected with APG-Hu and APG-HuΔ

3　讨　论

AIC是危害世界养鸡业的一种重要呼吸道疾病，潜伏期短，传播速度快。通过流行病学调查发现，该病在我国普遍流行，经常出现灭活疫苗免疫效果不佳或免疫失败。其原因与国内菌株新血清型和多种血清型共存的新流行特点有直接关系[3,11,18-19]，也有可能与其他新型毒力菌株的出现有关，如NAD非依赖APG与质粒携带菌株。目前，世界多个地区如墨西哥[20]、南非[21-22]、秘鲁[23]、韩国[4]、中国大陆[17]报道NAD非依赖APG。NAD非依赖APG的出现增加了APG的多样性与临床疾病的复杂性，增加了疾病的

防控难度。在实验条件下，NAD依赖APG疫苗株免疫的个体对NAD非依赖APG攻击的免疫保护力很低[24-25]。有学者认为，韩国最近之所以有APG疫苗免疫鸡群发生APG感染，很有可能是因为韩国的某些鸡场受到NAD非依赖APG的感染[4]。国内某些鸡场也存在APG免疫失败。李春等从云南省多个APG多价疫苗免疫的鸡场分离获得APG[5]。但这是否存在NAD非依赖菌株的感染情况，以及国内这种情况的严重程度，还有待深入研究。

早期有研究推测，APG的发病机制与质粒pA14和haemocin活性(有些菌株中质粒p250基因的产物具有该活性)有一定的相关性[15]。前期研究发现APG-Hu同时携带pA14、p250和pYMH5，这是国内外首次发现3种质粒同时存在于一株NAD非依赖APG中[17]。为了研究APG质粒的生物学功能，本研究前期通过物理法、化学法或二者结合法尝试消除APG-Hu中的质粒。吖啶橙(AO)是一种化学诱变剂，镶嵌于两个相邻的碱基对之间，这样在APG DNA复制过程中，会使其DNA链增加或缺失一个碱基，造成移码突变，从而导致遗传变异。而42 ℃接近鸡体温，理论上对细菌基因组DNA影响很小。因此，高温结合AO的诱导方法更能有效诱导APG中pA14和p250缺失，但本研究仅采用42 ℃诱导即获得了质粒缺失株APG-HuΔ。本研究结果证实，pA14和p250缺失并不明显影响菌株的生化特性、生长特性及对药物敏感性，但影响菌株的毒力。从感染鸡临床症状和病原分离情况可见，pA14和p250的缺失不仅减弱了APG-Hu的毒力，也影响其在机体内的增殖能力，因为感染组鸡中APG-HuΔ的分离效率低于APG-Hu。由此提示，质粒pA14和haemocin活性与APG毒力及其在体内生存能力密切相关。因此，检测质粒pA14和携带编码haemocin活性的质粒p250可能有助于APG的毒力研究。

令人意外的是，本实验并未成功诱导缺失携带多种抗性基因的质粒pYMH5，因为不仅从APG-HuΔ提取的少量质粒DNA中，而且从其菌体中均能经PCR检测到pYMH5 (结果未展示)。由此推测，pYMH5可能是一种稳定的内源性质粒，可能同时存在于APG的基因组DNA和细胞质中。由于pYMH5含多种抗性基因，且与质粒pLS88同源，因此曾尝试将APG-Hu和APG-HuΔ的质粒提取物转化DH5α感受态细胞，利用卡那霉素能够筛选到相应阳性克隆，并利用PCR检测到pYMH5的特定DNA序列。由此提示，尽管拷贝数很低，pYMH5仍具有作为穿梭载体的潜力。pYMH5的稳定存在可能增加扩散APG多重耐药性的风险。

国内外已经发现APG药物耐受现象严重的事实，但其耐药机制尚不清楚。李春等通过药敏试验发现APG分离菌株对多种常用药物如阿莫西林、链霉素、红霉素、罗红霉素、强力霉素、四环素耐药[5]。Jeong等发现韩国境内的APG菌株存在显著的抗生素抗性差异，而且存在多重耐药现象。而在其他国家也存在类似的情况[4,26]。然而，目前尚无证据证实pYMH5与APG耐药性的关联性。本研究中APG-Hu携带pYMH5，并未表现出明显的耐药性，可能由于选用的抗生素种类有限以及pYMH5携带的抗性基因可能受其他因素调控。尽管未能成功缺失质粒pYMH5，但pYMH5的存在，预示APG-Hu可能存在多种药物抗性及跨种传播的潜力。为揭示pYMH5与APG耐药性的关系，后续研究可以通过测定APG-Hu中pYMH5的DNA序列明确其抗性基因，或构建pYMH5缺失株等方法开展。同时扩大APG分离株数量和来源，深入研究pYMH5在国内的流行情况，从而揭示国内APG多重耐药性与pYMH5存在的关联性。

为了应对集约化养殖中AIC危害日趋复杂的形势，亟需加强APG的病原特性和致病机理的研究，本研究对APG质粒生物学特性的研究将有助于APG致病机理及耐药机制的研究，进一步明确病原的分子特征，为探寻更有效的疫病防控策略奠定基础。

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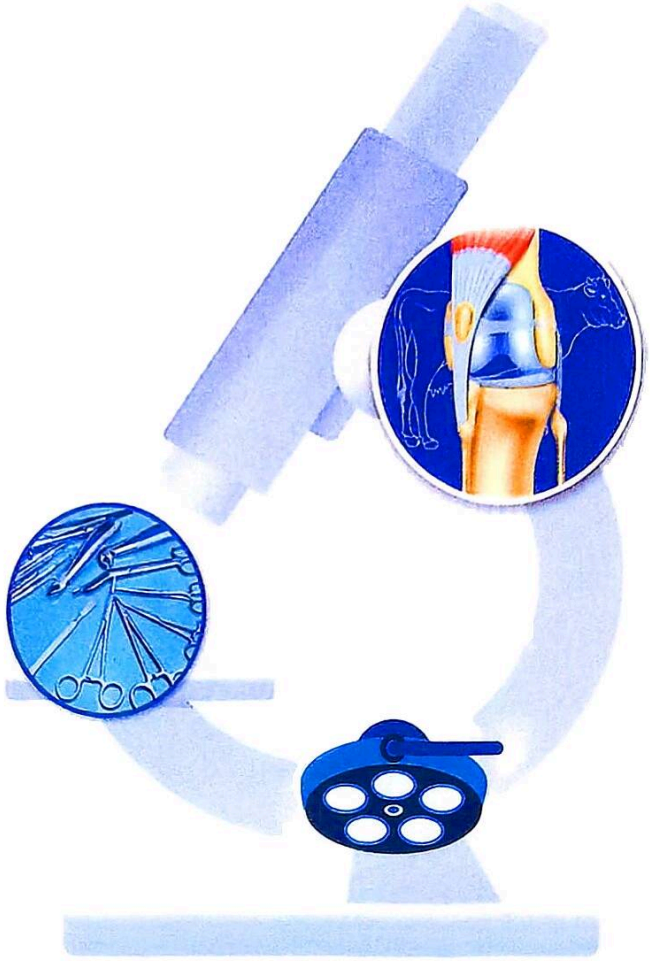

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前　言

兽医外科学是研究动物外科疾病的发生、发展、诊治和预防的一门科学，是高等院校动物医学专业主要专业课之一。兽医外科学实验是在学习兽医外科学基础理论的基础之上，通过动物模型，对常见的动物外科疾病开展临床诊疗的一门课程，也是国家规定动物医学专业本科生必修的一门课程。

兽医外科学是一门实践性十分强的课程，兽医外科学实验对外科学的学习和专业技能的培养十分重要。学习兽医外科学要求对其基本理论有深入了解，不但要掌握外科疾病的发生、发展、病理过程、转归规律，而且要对外科疾病做出正确的诊断，制订合理的治疗措施。为培养学生对外科病例进行合理治疗的基本能力与素质，除了要求学生在课堂上利用实验动物进行诊疗、处置等以练习外科基本功以外，还要人为制作疾病模型以模拟学生平时难以见到的疾病。对临床常见病和多发病，则要在临床兽医院寻找合适的病例，由教师带学生参加临床实践，以提高学生解决实际问题的能力。

兽医临床各学科间有密切联系和相互渗透的关系，如动物的肠梗阻、肠变位、肠结石及皱胃移位，在发病早期药物治疗阶段是内科疗法，当发展到晚期需要手术治疗，便需要外科疗法。外科临床实践中为了识别某一外科疾病和确定病性，必须与其他各临床学科疾病进行鉴别诊断，方能得出正确结论，孤立的外科学观点，缺乏多临床学科的广泛的基本理论、知识和实践技能，既不会学好兽医外科学，也不能成为能够预防、治疗动物疾病的临床兽医。学习掌握兽医外科学理论与实践技能，要求具备雄厚的专业基础知识，在学习兽医外科学实验之前要具备较好的专业理论基础，对解剖学、生理学、病理学、药理学、生物化学等各方面的知识都有较深入的认知。在此基础之上，开展外科手术治疗及术后的护理性治疗。

本书由东北农业大学肖建华担任主编；广东茂名农林科技职业学院丁晓担任副主编。具体撰写分工如下：肖建华负责撰写实验三十八—实验四十三内容（共计约：22.4 万字）；丁晓负责撰写实验一—实验三十七内容（共计约：10.4 万字）。全书统稿工作由肖建华负责完成。限于时间和水平有限，书中难免存在不足或不妥之处，敬请读者批评指正。

目　录

第一篇　兽医外科手术学相关操作

第二篇　兽医外科学相关诊疗技术

附　录

6、专利：一种鱼料投喂器

主分类号： A01K61/80

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主权项：

1.一种鱼料投喂器,包括旋浮力底板(1),其特征在于：所述浮力底板(1)上表面右侧固定安装有机箱(15),所述浮力底板(1)上表面中央设置有储存箱(2),所述储存箱(2)外围表面设置有封闭门(3),所述封闭门(3)表面安装有门把手A(4),所述储存箱(2)顶部连通设置有开口,所述储存箱(2)顶部安装有轴承(5),所述轴承(5)安装在储存箱(2)顶端,所述轴承(5)上安装有连接筒(6),所述连接筒(6)外围表面上端设置有齿环(7),所述储存箱(2)顶部右侧设置有第二电机(11),所述第二电机(11)的传动轴上连接有齿轮(10),所述齿轮(10)与齿环(7)呈齿轮啮合传动,所述连接筒(6)顶部连接有送料筒(9),所述送料筒(9)顶部安装有第一电机(8),所述送料筒(9)外围表面连接有输料管(12),所述输料管(12)另一端连接有出料箱(13)。

摘要：

本实用新型公开了一种鱼料投喂器,包括旋浮力底板,浮力底板上表面右侧固定安装有机箱,浮力底板上表面中央设置有储存箱,储存箱外围表面设置有封闭门,封闭门表面安装有门把手A,储存箱顶部连通设置有开口,储存箱顶部安装有轴承,轴承安装在储存箱顶端,轴承上安装有连接筒,连接筒外围表面上端设置有齿环,储存箱顶部右侧设置有第二电机,第二电机的传动轴上连接有齿轮,齿轮与齿环呈齿轮啮合传动,连接筒顶部连接有送料筒,送料筒顶部安装有第一电机,送料筒外围表面连接有输料管,本一种鱼料投喂器具有多个出料箱进行投喂,可以在水中进行漂浮投喂,可以通过电机进行带动旋转均匀投喂,提高了投喂效率的优点。

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7、专利：一种荔枝酒的制备方法

1.一种荔枝酒的制备方法,其特征在于,包括以下步骤：步骤1.将荔枝冻果依次进行解冻、清洗、去皮去核、低温压榨得到荔枝汁,向荔枝汁中加入果胶酶,静置,过滤,得到清液；步骤2.向所述清液中添加柠檬酸调节酸度、白砂糖调节糖度,进行灭菌处理,之后向清液中接种酵母进行第一次发酵；步骤3.向第一次发酵完成的清液中接种益生菌进行第二次发酵,过滤,得到荔枝酒；步骤2中所述第一次发酵的条件具体为：于14.5±0.5℃发酵7d,在发酵的第2d、第5d分别进行30-35KHz超声处理15min；所述第二次发酵的条件具体为：于14.5±0.5℃发酵24h,在发酵的第2h、第7h分别进行20-25KHz超声处理15min；步骤2中所述酵母具体为葡萄酒酵母、果酒酵母和啤酒酵母；所述酵母接种量为100-150ppm；步骤3中所述益生菌具体为保加利亚乳杆菌、嗜热链球菌、鼠李糖乳杆菌和嗜酸乳杆菌；所述益生菌的接种量为所述清液质量的2-3%。

摘要:

本发明公开了一种荔枝酒的制备方法,涉及酿酒技术领域。本发明方法包括以下步骤：将荔枝冻果依次进行解冻、清洗、去皮去核、低温压榨得到荔枝汁,向荔枝汁中加入果胶酶,静置,过滤,得到清液；向所述清液中添加柠檬酸调节酸度、白砂糖调节糖度,进行灭菌处理,之后向清液中接种酵母进行第一次发酵；向第一次发酵完成的清液中接种益生菌进行第二次发酵,过滤,得到荔枝酒。本发明所制备的荔枝酒极大的保留了荔枝风味物质玫瑰醚的含量,且采用荔枝冻果作为原料,解决了荔枝鲜果不能远距离运输、储藏时间短的难题。

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8、应用软件：智能花盆物联网养花系统

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软 件 名 称： 智能花盆物联网养花系统
V1.0

著 作 权 人： 广东茂名农林科技职业学院;王宇杰;黄浪彬;李健珊

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计算机软件著作权登记专用章

2024年01月10日

9、应用软件：猪解剖三维数字仿真教学软件

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计算机软件著作权登记证书

证书号： 软著登字第[redacted]号

软 件 名 称： 猪解剖三维数字仿真教学软件
V1.0

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2023年08月29日

10、技术服务--标准：《化橘红嫁接育苗技术规程》

ICS 65.020.40
CCS B 64

DB4409

茂 名 市 地 方 标 准

DB4409/T 36—2023

化橘红嫁接育苗技术规程

Technique Rules For Grafted Seedling Production For Citrus Grandis 'Tomentosa'

2023－12－20 发布　　2023－12－25 实施

茂名市市场监督管理局　发布

前　言

本文件按照 GB/T 1.1-2020《标准化工作导则　第1部分：标准化文件的结构和起草规则》的规定起草。

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