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DISSERTATION

Development of technological methods of growing and use the plant extracts to improve the meat quality of broiler chicken

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ANNOTATION

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Poultry products are an indispensable food source in people's daily life and ensuring their greenness, environmental protection and safety is an important part of food safety. However, in recent years, with the increasing intensification of the poultry farming industry, to improve economic benefits, farmers use antibiotics heavily in feed, which has brought great harm to human health and environmental safety. Under the background of comprehensive "antibiotic prohibition", exploring "new, efficient, safe and green" antibiotic substitutes has gradually become a research hotspot in the feed industry. In recent years, many studies have found that plant extracts have strong immune regulation activity, can enhance the immune function of the body, have the advantages of green, environmental protection, safety, and non-toxicity, and can act on immune cells in the body, thereby regulating the immune state of the body. It plays an important role in resisting the invasion of various pathogenic bacteria. *Astragalus* is one of the traditional Chinese medicines. *Astragalus* contains polysaccharides, proteins,

alkaloids, amino acids, flavonoids, and other active substances, which have the functions of regulating animal immune function, anti-oxidation, anti-tumor, and anti-infection. As one of the traditional Chinese herbal medicines, *Glycyrrhiza* contains rich bioactive substances such as triterpenoid saponins, flavonoids, coumarin, alkaloids, volatile oils, organic acids, and sugars. It has been concerned about its biological activity functions such as antioxidant, antibacterial, antiviral, anticancer, anti-inflammatory, immune regulation, hypoglycemic, and other biological activities. In addition, *Glycyrrhiza* also has antitussive, expectorant, antiasthmatic, lung protection, and anti-respiratory pathogenic effects. However, the current research on *Astragalus* extract and *Glycyrrhiza* extract mostly focuses on the effects on animal production performance. There are relatively few studies on intestinal health and respiratory health and their compatibility applications. Therefore, it is of great significance to research the feeding effect and mechanism of *Astragalus* extract and *Glycyrrhiza* extract on broilers and use the *Astragalus* extract and *Glycyrrhiza* extract in poultry production as new antibiotic substitutes.

The first part of this paper reviewed the physiological functions of *Astragalus*, such as anti-inflammatory, immune regulation, regulation of mucosal immune barrier, antioxidant, anti-cancer, and its application in improving poultry production performance, intestinal function, and disease resistance (P31-38). The antiviral function, anti-inflammatory function, antioxidant function, immune regulation function, and improved gut health of *Glycyrrhiza* were also reviewed (P39-43). The physical barrier, chemical barrier, microbial barrier, immune barrier, and the evaluation of the function of

the poultry intestinal barrier were reviewed (P44-49). The structure and function of the mechanical barrier, chemical barrier, microbial barrier, and immune barrier of the respiratory mucosa of poultry were reviewed (P50-52). In addition, the effect of high temperature boiled technology on the nutrient content of chicken was reviewed (P60-61). The purpose of the experiment is to study the feeding effects of *Astragalus* extract and *Glycyrrhiza* extract on broilers and their effects on intestinal health and respiratory health and to explore their potential mechanism of action. This research will supply a new type of antibiotic substitute in broiler feed, and provide a theoretical basis for the application of *Astragalus* extract and *Glycyrrhiza* extract in poultry production. The research content of the experiment includes the following parts: (1) Effects of *Astragalus* extract and *Glycyrrhiza* extract on production performance, nutrient metabolic rate, and meat quality of broilers; (2) Effects of *Astragalus* extract and *Glycyrrhiza* extract on immune function, antioxidant function and inflammatory factors in broilers; (3) Potential mechanisms of the effects of *Astragalus* extract and *Glycyrrhiza* extract on the intestinal barrier and respiratory tract barrier of broilers. (4) The effects of adding *Astragalus* extract and *Glycyrrhiza* extracts to broiler diets on meat quality traits of chicken meat treated by high temperature water boiling technology, such as color, shear strength, nutrient composition and fatty acid content.

In the second part (P64-74), the experimental design is carried out according to the research topic of this paper. 720 1-day-old Arbor Acres broiler chickens were selected and randomly divided into 6 treatments with 6 replicates comprising 20 broilers each. All the animals were managed according to the guidelines for the care and use of

experimental animals approved by The Ethics Committee of Henan Agricultural University (No. HNND2021031503). The control group (CON) was fed the basal diet without antibiotics, and the antibiotic group (ANT) was fed the basal diet supplemented with 500 mg/kg of Terramycin calcium, the *Astragalus* extract group (AE) was fed the basal diet supplemented with 300 mg/kg of *Astragalus* extract, the *Glycyrrhiza* extract group (GE) was fed the basal diet supplemented with *Glycyrrhiza* extract 150 mg/kg, *Astragalus* extract + *Glycyrrhiza* extract Ⅰ group (AE + GE Ⅰ) was fed the basal diet supplemented with *Astragalus* extract 300 mg/kg and *Glycyrrhiza* extract 150 mg/kg, *Astragalus* extract +*Glycyrrhiza* extract II group (AE+GE II) was fed the basal diet supplemented with *Astragalus* extract 150 mg/kg and *Glycyrrhiza* extract 75 mg/kg. The experimental diets were fed for 42 d.

The three part (P76-117): by measuring the production performance such as body weight, average daily gain, average daily feed intake, and feed conversion rate of broilers, the results showed that the groups added with plant extracts improved the average daily weight gain and energy metabolic rate of broilers and decreased the feed-to-weight ratio, which was no different from the effect of adding antibiotic on the performance of broilers. The fatty acid content of the muscle was measured by gas chromatography, and the pH value, shear force, and muscle color of the muscle were also measured. The results showed that the groups added with the plant extract increased the content of polyunsaturated fatty acids in the muscle, and the effect was better than that of antibiotics. Enzyme-linked immunosorbent assay was used to determine the immune indexes, antioxidant indexes, and inflammatory factors in serum. The results

showed that AE+GE Ⅰ group and AE+GE II group increased thymus index and serum IgA content, the GE group and the AE+GE group increased serum IgM content, AE group, GE group, and AE+GE Ⅰ group increased serum IgG content, AE+GE II group increased spleen index. The groups added with plant extracts increased the SOD content of the trachea. In addition, the AE group increased the serum T-AOC content, the GE group increased the serum SOD content, the AE group and the GE group increased the tracheal T-AOC content, The content of MDA in serum was decreased, and the content of GSH-Px in the trachea was increased in GE group and AE+GE II group. Adding *Astragalus* extract and *Glycyrrhiza* extract was more effective than Terramycin calcium in improving the immune function and antioxidant function of broilers. The levels of TNF-α and IL-6 in serum were decreased in each group supplemented with plant extracts, the levels of IL-1β in serum were decreased in the AE group, GE group, and AE+GE II group, and IL-1β and IL-6 content in lungs were decreased in GE group and AE+GE II group. Adding plant extracts was as effective in suppressing inflammation as adding antibiotics. The intestinal slices were made by hematoxylin-eosin staining, and the mucosal morphology of the intestinal tract was observed by electron microscope. The villus height and the ratio of villus height to crypt depth for adding plant extracts groups were higher than that of ANT. The expression of tight junction protein and mucin in intestinal mucosa was determined by microspectrophotometer, and the content of DAO in serum was determined by enzyme-linked immunosorbent assay. NRF2 mRNA expression in duodenum, jejunum, ileum and trachea, SOD1, SOD2, GSH-Px mRNA expression in the duodenum, SOD1, GSH-Px mRNA expression in the jejunum, SOD1,

SOD2, GSH-Px mRNA in ileum were increased in each group supplemented with *Astragalus* extract and *Glycyrrhiza* extract, and the DAO content was decreased. The results showed that adding *Astragalus* extract and *Glycyrrhiza* extract was more effective than antibiotics in improving the intestinal antioxidant function and permeability of broilers. Using high-throughput sequencing to analyze the microbial abundance of cecal contents, the results showed that the addition of *Astragalus* extract and *Glycyrrhiza* extract increased the diversity and richness of intestinal microorganisms, improved the structure of intestinal flora, increased the beneficial bacteria, and decreased the harmful bacteria. The effect is better than antibiotics in improving intestinal flora. Correlation analysis showed that *Astragalus* extract and *Glycyrrhiza* extract had effects on production performance, serum antioxidant function, immune function, inflammatory factors, intestinal mucosal morphology, and intestinal permeability by improving the structure of intestinal flora. The meat quality traits of broilers were determined by the method of high temperature boiled. The results showed that the high temperature boiled technology could improve the meat quality traits of broilers fed with *Astragalus* extract and *Glycyrrhiza* extract.

The fourth and fifth Parts (P119-139): based on the above research, the following conclusions are drawn: the addition of *Astragalus* extract and *Glycyrrhiza* extract in the diet improves the production performance, the apparent metabolic rate of energy, the meat quality, antioxidant and immune function in broilers, and improves the intestinal mucosal morphology, intestinal and respiratory barrier function, inhibits the occurrence of pro-inflammatory factors. In addition, the high temperature boiled technology can

improve the meat quality traits of broilers fed with *Astragalus* extract and *Glycyrrhiza* extract. It is speculated that *Astragalus* extract and *Glycyrrhiza* extract can affect production performance, serum antioxidant performance, immune function, inflammatory factors, intestinal mucosal morphology, and intestinal permeability by improving the structure of intestinal flora. *Astragalus* extract and *Glycyrrhiza* extract can be used in poultry production as an alternative to antibiotics. Among them, the feeding effect of adding 150 mg/kg *Astragalus* extract + 75 mg/kg *Glycyrrhiza* extract group was the best, and the feeding effect of adding *Glycyrrhiza* extract alone was better than that of adding *Astragalus* extract alone. In addition, adding *Glycyrrhiza* extract alone has the best economic benefits.

This paper is the first to systematically study the effects of *Astragalus* extract, and *Glycyrrhiza* extract and their combined use on production performance, meat quality, antioxidant function, immune function, and intestinal barrier function of broilers. For the first time, through the analysis of gut microbial diversity, the possible mechanism was investigated for the effects of *Astragalus* extract and *Glycyrrhiza* extract on broilers by regulating gut microbes. The effect of high temperature boiled technology on meat quality traits of broilers fed with *Astragalus* extract and *Glycyrrhiza* extract was studied for the first time. The results of the study provide a theoretical basis for the replacement of antibiotics with *Astragalus* extract and *Glycyrrhiza* extract and their application in broiler production and provide an effective way for safe broiler food production.

In the future, we will further study the mechanism of *Astragalus* extract and *Glycyrrhiza* extract and their combined use on the respiratory barrier function of broilers, and on the intestinal microbial metabolic pathway of broilers.

***Keywords**: Astragalus* extract, *Glycyrrhiza* extract, broiler, growth performance, slaughter, metabolism, meat quality, productivity, technology, antioxidant, poultry

АНОТАЦІЯ

Цзао Іньінь «Удосконалення технологічних прийомів вирощування та використання рослинних екстрактів для покращення м’ясних якостей курчат-бройлерів». Кваліфікаційна наукова праця на правах рукопису.

Дисертація на здобуття наукового ступеня доктора філософії за спеціальністю 204 – технологія виробництва продуктів тваринництва. – Сумський національний аграрний університет Міністерства освіти і науки України, Суми, 2023.

Продукція птахівництва є незамінним джерелом їжі в повсякденному житті людей, при цьому важливою складовою є забезпечення вимог екологічності та охорони навколишнього середовища як основної складової частини безпеки харчових продуктів. Проте в останні роки, з динамічним розвитком галузі

птахівництва, а також з метою підвищення економічної ефективності фермери досить активно використовують антибіотики в кормах, що завдає великої шкоди здоров'ю людей та екологічній безпеці. На тлі всеосяжної «заборони антибіотиків» дослідження нових, ефективних, безпечних та екологічно чистих замінників антибіотиків поступово стає актуальною тема досліджень у кормовій промисловості. В останні роки багато досліджень довели, що рослинні екстракти мають сильну імунно регулюючу дію і можуть підвищувати імунну функцію організму, тим самим регулюючи імунний стан організму, а також можуть впливати на імунні клітини в організмі. При цьому вони є екологічні, безпечні та нетоксичності, не впливають на навколишнє середовище. Також вони відіграють важливу роль у протистоянні вторгненню різних патогенних бактерій. Astragalus є одним з традиційних китайських трав'яних ліків. Astragalus містить полісахариди, білки, алкалоїди, амінокислоти, флавоноїди та інші активні речовини, які мають функцію регуляції імунної системи тварин, антиокислювальну, протипухлинну та протиінфекційні властивості. Glycyrrhiza також є один із традиційних китайських трав'яних ліків які містить велику кількість біологічно активних речовин, такі як тритерпеноїдні сапоніни, флавоноїди, кумарин, алкалоїди, фітонциди, органічні кислоти та цукор. Вживання якого призводить до змін біологічної активності, антиоксидантні, антибактеріальні, противірусні, протипухлинні, протизапальні та імунну регуляцію, гіпоглікемічну та інші біологічні дії. Крім того, Glycyrrhiza також має протикашльову, відхаркувальну, протиастматичну властивість, позитивно впливає

на легеневий захист та антиреспіраторний патогенний ефект. Проте поточні дослідження екстракту Astragalus та екстракту Glycyrrhiza зосереджені переважно на вивченні збільшення продуктивності тварин, і існує відносно небагато досліджень щодо здоров’я кишківника та дихальних шляхів або застосування їхньої сумісності. Саме тому є актуальним провести дослідження, щодо вивчення ефективності та механізму згодування екстракту Astragalus та екстракту Glycyrrhiza курчат бройлерів, для підтвердження збільшення їх продуктивності та можливості використання екстракту Astragalus та екстракту Glycyrrhiza у птахівництві як нового замінника антибіотиків.

У вступі та першому розділі наших досліджень розглядаються фізіологічні функції Astragalus, такі як імунна регуляція, протизапальна, антиоксидантна, протипухлинна, регуляція імунного бар'єру слизової оболонки, а також його застосування для покращення продуктивності птиці, функції кишківника та стійкості до хвороб (стор.31-38). Також були розглянуті хімічні складові, протипухлинна функція, антиоксидантна функція, функція захисту печінки, функція імунної регуляції, протизапальна функція, противірусна функція, гіпоглікемічна та ліпідна функція крові, захист шкіри та інші фізіологічні функції (Р39-43). Під час досліджень розглянуто фізичний, хімічний, мікробний, імунний бар’єр та зроблена оцінка функції кишкового бар’єру птиці (Р44-49). Досліджено структуру та функції механічного, хімічного, мікробного та імунного бар’єру слизової оболонки дихальних шляхів птиці (Р50-52). Крім того, було розглянуто

вплив технології високо-температурного варіння курятини на вміст поживних речовин (P60-61).

Мета експерименту – вивчити дію екстракту Astragalus та екстракту Glycyrrhiza при годівлі бройлерів та їхній вплив на здоров'я кишечника та дихальних шляхів, а також дослідити їхній потенційний механізм дії. Це дослідження дасть новий тип замінника антибіотиків у кормах для бройлерів та надасть теоретичну основу для застосування екстракту Astragalus та екстракту Glycyrrhiza у птахівництві.

Дослідницький зміст експерименту включає такі частини:

(1) вивчення впливу екстракту Astragalus та екстракту Glycyrrhiza на продуктивність, швидкість метаболізму поживних речовин та якість м'яса бройлерів;

(2) вивчення впливу екстракту Astragalus та екстракту Glycyrrhiza на імунну функцію, антиоксидантну функцію та фактори запалення у бройлерів;

(3) дослідження механізму впливу екстракту Astragalus та екстракту Glycyrrhiza на кишковий бар'єр та бар'єр дихальних шляхів у бройлерів.

(4) вивчення впливу екстракту Astragalus та екстракту Glycyrrhiza при додаванні його до раціону курчат-бройлерів на якість м'яса, яка була обробленна за технологією високо-температурного варіння, а також на такі показники як колір, склад поживних речовин та вміст жирних кислот.

У другій частині (P64-74), наведена методика проведення експерименту відповідно до теми дослідження даної роботи. Для дослідження були використанні 720 добових курчат-бройлерів Arbor Acres, які були відібрані та випадковим чином розділені на 6 груп по 6 повторень, що складалися з 20 бройлерів у кожній групі. З усіма тваринами поводилися згідно з інструкціями щодо догляду та використанням експериментальних тварин, схваленими Комітетом з етики Хенанського сільськогосподарського університету (№ HNND2021031503). Контрольну групу (CON) годували основною дієтою без антибіотиків, а групу антибіотиків (ANT) годували основною дієтою, доповненою 500 мг/кг терраміцину кальцію, групу екстракту Astragalus (AE) годували основною дієтою, доповненою 300 мг/кг екстракту Astragalus, групу екстракту Glycyrrhiza (GE) годували основною дієтою, доповненою екстрактом Glycyrrhiza 150 мг/кг, екстракт Astragalus + екстракт Glycyrrhiza Ⅰ групу (AE+GE Ⅰ) годували базовою дієтою, доповненою екстрактом Astragalus 300 мг/кг і екстракт Glycyrrhiza 150 мг/кг, екстракт Astragalus+екстракт Glycyrrhiza II групи (AE+GE II) отримували базову дієту, доповнену екстрактом Astragalus 150 мг/кг і екстрактом Glycyrrhiza 75 мг/кг. Дослідні раціони годували 42 дні.

У третьому розділі (P76-117) були проаналізовані отримані виробничі показники, такі як: маса тіла, середньодобовий приріст, середньодобове споживання корму та коефіцієнт конверсії корму бройлерів, результати досліджень показали, що групи, які вживали рослинні екстракти, покращили середньодобовий приріст ваги та швидкість енергетичного метаболізму бройлерів,

а також зменшили співвідношення корму і ваги, яке нічим не відрізнялося від впливу додавання антибіотика на продуктивність бройлерів. За допомогою газової хроматографії визначали вміст жирних кислот у м'язах, а також вимірювали значення pH, силу зсуву та колір м'язів. Результати показали, що групи, яким додавали екстракти рослин, підвищили вміст поліненасичених жирних кислот у м'язах, і ефект був кращим, ніж у групах, де додавали антибіотики. Імуноферментний аналіз використовували для визначення імунних індексів, антиоксидантних індексів та факторів запалення у сироватці крові. Результати показали, що група AE+GE Ⅰ та група AE+GE Ⅱ підвищили індекс тимуса та вміст IgA у сироватці, група GE та група AE+GE підвищили вміст IgM у сироватці, група AE, група GE та група AE+GE Ⅰ підвищили вміст IgG у сироватці, AE+GE Ⅱ група підвищений індекс селезінки. Групи, з додаванням рослинних екстрактів, підвищували вміст SOD в трахеї. Крім того, група AE збільшила вміст T-AOC в сироватці, група GE збільшила вміст SOD в сироватці, група AE і група GE збільшили вміст T-AOC в трахеї, вміст MDA в сироватці знизився, а вміст GSH-Px в трахеї збільшувався у групі GE і групі AE+GE. Додавання у раціон екстракту Astragalusta екстракту Glycyrrhiza було більш ефективним, ніж окситетрациклін кальцію, для покращення імунної та антиоксидантної функції бройлерів. Рівні TNF-α та IL-6 у сироватці були знижені у кожній групі, доповненої рослинними екстрактами, рівні IL-1β у сироватці були знижені в групі AE, групі GE та групі AE+GE II, а також IL-1β та Вміст ІЛ-6 у легенях був знижений у групі GE та групі AE+GE II. Додавання рослинних

екстрактів було таким же ефективним для попередження запалення, як і додавання антибіотиків. Зрізи кишківника виготовляли шляхом фарбування гематоксилін-еозином, за морфологію слизової оболонки кишкового тракту спостерігали за допомогою електронного мікроскопа. Висота ворсинок і відношення висоти ворсинок до глибини крипти у групах з додавання рослинних екстрактів були вищими, ніж у ANT. Експресію білка щільних з'єднань і муцину в слизовій оболонці кишківника визначали мікроспектрофотометром, а вміст DAO в сироватці – імуноферментним аналізом. Експресія *мРНК NRF2* у дванадцятипалої кишки, порожній кишки, клубовій кишки та трахеї, експресія мРНК *SOD1, SOD2, GSH-Px* у дванадцятипалій кишці, експресія мРНК *SOD1,* GSH- *Px у порожній* кишці, експресія мРНК *SOD1, SOD2, GSH-Px* у клубовій кишці була підвищена у кожній групі з додаванням екстракту Astragalusta екстракту Glycyrrhiza, а вміст DAO був знижений. Результати показали, що додавання екстракту Astragalus та екстракту Glycyrrhiza було ефективнішим, ніж застосування антибіотику, для покращення антиоксидантної функції кишечника та проникності. Використовуючи високопропускну здатність секвенування для аналізу мікробної кількості вмісту сліпої кишки, результати показали, що додавання екстракту Astragalus та екстракту Glycyrrhiza збільшує різноманітність і насиченість кишкових мікроорганізмів, покращує структуру кишкової флори, збільшує кількість корисних бактерій та зменшує кількість шкідливих бактерій. Ефект був кращий, ніж від використання антибіотиків у поліпшенні кишкової флори. Кореляційний аналіз показав, що екстракт Astragalus та екстракт

Glycyrrhiza впливали на продуктивність, антиоксидантну функцію сироватки, імунну функцію, фактори запалення, морфологію слизової оболонки кишечника та проникність кишківника шляхом покращення структури кишкової флори. Показники якості м’яса бройлерів визначали методом високотемпературного проварювання. Результати показали, що технологія високотемпературного варіння може покращити показники якості м’яса бройлерів, яких годували екстрактом астрагалу та екстрактом солодки.

У четвертому та п'ятому розділах (P119-139) на підставі вищенаведених досліджень були зроблені такі висновки: додавання в раціон екстракту Astragalus та екстракту Glycyrrhiza покращує продуктивність, підвищує швидкість енергетичного обміну, поліпшує якість м’яса, антиоксидантну та імунну функцію у бройлерів, покращує роботу кишківника, морфологію слизової оболонки, бар'єрну функцію кишківника і дихання, пригнічує виникнення прозапальних факторів. Таким чином, технологія високотемпературного варіння може покращити показники якості м’яса бройлерів, яких годували екстрактом астрагалу та екстрактом солодки. Є припущення, що екстракт Astragalus та екстракт Glycyrrhiza можуть впливати на продуктивність, антиоксидантну дію сироватки, імунну функцію, фактори запалення, морфологію слизової оболонки кишківника та проникність кішківника шляхом покращення структури кишкової флори. Екстракт Astragalus та екстракт Glycyrrhiza можна використовувати у птахівництві як альтернативу антибіотикам. Серед них ефект годівлі від додавання 150 мг/кг екстракту Astragalus+ 75 мг/кг екстракту licorice був найкращим, а ефект

годівлі від додавання окремо екстракту Glycyrrhiza був кращим, ніж при додаванні окремо екстракту Astragalus. Крім того, додавання лише екстракту Glycyrrhiza має найкращі економічні переваги.

У роботі вперше системно досліджується вплив екстракту Astragalus, екстракту Glycyrrhiza та їх комбінованого використання на продуктивність птиці, якість м'яса, антиоксидантну функцію, імунну функцію та бар'єрну функцію кишечника бройлерів. Вперше шляхом аналізу мікробного різноманіття кишківника було досліджено можливий механізм впливу екстракту Astragalus та екстракту Glycyrrhiza на бройлерів шляхом регуляції кишкових мікробів. Вперше досліджено вплив технології високотемпературного варіння на показники якості м'яса бройлерів, яких годували екстрактом астрагалу та екстрактом солодки. Результати дослідження дають теоретичну основу для заміни антибіотиків екстрактом Astragalus та екстракту Glycyrrhiza та їхнього застосування у виробництві бройлерів, а також є ефективним способом виробництва безпечного корму для бройлерів.

У майбутньому ми будемо додатково вивчати механізм використання екстракту Astragalus та екстракту Glycyrrhiza та їхнього комбінованого застосування на респіраторну бар'єрну функцію бройлерів, а також механізм впливу екстракту Astragalus та екстракту Glycyrrhiza та їхнього комбінованого застосування на мікробно метаболічні показники кишківника бройлерів.

Ключові слова: *екстракт Астрагала, екстракт Гліцирізи, бройлер, інтенсивності росту, забій, метаболізм, якість м'яса, продуктивність, технологія, антиоксидант, птахівництво*

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CONTENT

ABBREVIATION LIST

English abbreviations	English full name
AE	Astragalus Extract
GE	Glycyrrhiza Extract
ANT	Antibiotic
ADG	Average Daily Ggain
ADFI	Average Daily Feed Intake
FCR	Feed Conversion Ratio
CP	Crude Protein
EE	Ether Extract
T-AOC	Total antioxidant capacity
SOD	Superoxide dismutase
GSH-Px	Glutathione peroxidase
MDA	Malondialdehyde
DAO	Diamine Oxidase
SOD1	Copper and zinc superoxide dismutase
Nrf2	Nuclear factor-erythroid 2-related factor-2
Ig	Immunoglobulin
TNF-α	Tumor necrosis factor
LPS	Lipopolysaccharide
IFN-γ	Interferon
IL	Interleukin

VH	Villus height
CD	Crypt depth
D-LA	D-lactate
ZO1	Zonula occludins 1
MUC	Mucin
OTU	Optical Transform Unit
KEGG	Kyoto Encyclopedia of Genes and Genomes
PCoA	Principal Coordinate Analysis
NMDS	Nonmetric multidimensional scale analysis
APS	Astragalus Polysaccharide
SFA	Saturated Fatty Acid
USFA	Unsaturated Fatty Acid
MUFA	Monounsaturated Fatty Acid
PUFA	Polyunsaturated Fatty Acid
EFA	Essential Fatty Acids

INTRODUCTION

Poultry products are an indispensable food source in people's daily life and ensuring their green, environmental protection and safety is an important part of food safety. However, in recent years, as the poultry breeding industry has become increasingly intensive, farmers use antibiotics extensively to improve economic benefits, which has brought great harm to human health and environmental safety [1]. In the context of comprehensive "anti-antibiotics", exploring new, efficient, safe and green antibiotic alternatives has gradually become a research hotspot in the feed industry. In recent years, many studies have found that plant extracts have strong immunomodulatory activity, can enhance the body’s immune function, have the advantages of being green, environmentally friendly, safe, and non-toxic, and can act on immune cells in the body, thereby regulating the body’s immune state, play an important role in resisting the infestation of various pathogenic bacteria. *Astragalus* is a plant with dried roots of the genus *Astragalus* in the leguminous family *Astragalus* membranaceus (Fisch.) Bge. var. Mongholicus (Bge.) and *Astragalus* membranaceus (Fisch.) Bge. It is one of the traditional Chinese medicines. *Astragalus* polysaccharide is an immunologically active polysaccharide isolated from the rhizome of *Astragalus* membranaceus. It has the functions of regulating animal immune function, anti-oxidation, anti-tumor, and anti-infection. As one of the traditional Chinese herbal medicines, *Glycyrrhiza* has anti-oxidation, anti-bacterial, anti-virus, anti-cancer, anti-inflammatory, and immune regulation, lowering blood sugar and other biological activities, and has attracted people's attention. In addition, *Glycyrrhiza* also has

antitussive, expectorant, anti-asthmatic, lung protection, and anti-pathogen effects on the respiratory tract.

At present, most research on *Astragalus* extract and *Glycyrrhiza* extract has focused on the effects on animal performance, and there are relatively few studies on intestinal health and respiratory health, as well as combined use. Therefore, it is of great significance to study the effects and mechanism of *Astragalus* extract and *Glycyrrhiza* extract according to their safety, and green and environmental characteristics. In the first part of this paper, physiological function of *Astragalus* and its application in poultry production, Physiological function of Licorice and its Application in Poultry Production, Intestinal barrier of poultry, respiratory barrier of Poultry and high temperature cooking technology were reviewed, with the purpose of providing theoretical basis for the development and utilization of *Astragalus* extract, licorice extract and high temperature cooking technology. The second part of the paper mainly introduces the experimental materials and methods. In the third part of the thesis, the results of the experiment are analyzed. The fourth part of the paper lists the previous research contents and discusses and analyzes the experimental results. The fifth part of the paper lists the conclusion, innovations, research Prospects.

SECTION 1 LITERATURE REVIEW

1.1 Physiological functions of *Astragalus* and its application in poultry production

Astragalus is one of the traditional Chinese medicines. *Astragalus* contains polysaccharides, proteins, alkaloids, amino acids, flavonoids, trace elements, and other

active substances. *Astragalus* polysaccharides (APS) are extracted from *Astragalus* and are the main bioactive component in *Astragalus*. Many studies have shown that APS can enhance animal immunity [2], and promote animal growth and other functions. Therefore, it has been widely used in poultry production.

1.1.1 Physiological functions of *Astragalus*

Anti-inflammatory function APS can inhibit the occurrence of inflammatory cytotoxicity through its structure-activity regulation [3]. Tissue damage caused by cytotoxic reactions such as inflammation and oxidative stress is the main source of pathogenic microorganism infection and toxicity. The molecular pattern of the marker pathogen of the Gram-negative bacteria is Lipopolysaccharide (LPS). The immune recognition receptor TLR4 can specifically recognize LPS. After recognition of LPS and TLR4, it can activate MAPK/NF-κB downstream of the TLR4 signaling pathway of immune-related cells such as lymphocytes and activates the expression of IL-1β and TNF-α, which in turn induces inflammation in the TLR4 pathway [4-5]. The core immune activation structures of APS and LPS are consistent and can be recognized by TLR4. APS can improve its structural effect and competitively inhibit the phosphorylation of ERK and JNK activated by LPS, thereby inhibiting NF-κB activation and IL-1β and TNF -α expression of pro-inflammatory cytokines, inhibiting the inflammatory response and inflammation-related tissue damage [6]. Abuelsaad, et al. [7] found that APS can significantly improve the phagocytic function of neutrophils in mice infected with Aeromonas, increase the number of CD4+ T cells in the intestine and thymus, reduce the number of inflammatory-related CD8+ T cells, and inhibit the

occurrence of inflammation. In addition, in a mouse colitis model induced by sodium dextran sulfate, APS can reduce the pro-inflammatory cytokines TNF-α, IL-1, IL-6, and IL-17 by inhibiting the NF-κB-related DNA phosphorylation process. The expression of cytokines inhibits the activation of intestinal inflammation [8]. Immune cell activation related to inflammatory response is a “dual signal” induced response. In addition to PAMP, cytokines act as costimulatory signals to jointly activate lymphocyte inflammatory response. APS can also antagonize the inflammatory response induced by cytokines. Kim, et al. [9] pointed out that APS can reduce the level of NF-κB by inhibiting the PI3K/Akt and JAK/STAT signaling pathways downstream of TLR4, but it can promote CD4+ Th0 cells to the differentiation of the Th2 and Th17 groups of T cells, thereby inhibiting the inflammatory response induced by IL-23 injection. APS can also suppress the colitis-inhibiting mice synthesize IL-1 and TNF-α cytokines, reduce the expression of T cell transcription factor NFATc4, and play a role in suppressing inflammation [10]. In short, APS can effectively inhibit the inflammatory response induced by pathogenic molecules such as LPS and pro-inflammatory cytokines, and inhibit the tissue damage caused by the cytotoxic response to the animal body.

Immunomodulatory function Animal immune organs consist of central and peripheral immune organs. Central immune organs include bone marrow, thymus, and avian bursa, which are responsible for the production, proliferation, differentiation, and maturation of immune active cells, and regulate the development of peripheral lymphoid organs and systemic immune function; peripheral immune organs include lymph nodes and spleen, etc., whose role It is a place for immune cell aggregation and immune

response. The development of immune organs will directly affect the level of immunity of the body. Many studies have shown that adding APS to poultry diets can effectively increase the quality of immune organs, improve the organ index, and at the same time promote the development of some organs [11-14]. In addition, Gao Xu, et al. [15] studied the effect of different concentrations of APS on the immune function of mice, and the results showed that: with the increase in APS concentration, the weight of mouse thymus and spleen increased significantly. Wang Junli, et al. [12] found that the effect of APS on organs was affected by both gender and growth stage.

Regulation effect of mucosal immune barrier The intestine is the most important digestion and absorption organ of animals, and the intestinal mucosa is the body’s largest "atypical" immune organ. It plays an important role in the regulation of animal immune function. In particular, the intestinal mucosal immune system plays an important role in the immune barrier and digestion and absorption of the intestinal canal. APS may be converted into oligosaccharides by acid hydrolysis, which cannot be directly absorbed by the body through the small intestinal mucosa. Its immune response occurs in the intestinal mucosal epithelium, which can activate the activity of the intestinal mucosal immune system and exert its immunoregulatory effect. A moderately activated intestinal mucosal immune system can promote the renewal of intestinal epithelial cells, thereby effectively improving mucosal integrity. In addition, APS can also inhibit the inflammatory response induced by LPS in the intestine and inhibit the corresponding intestinal injury. APS has a moderate immune activation and regulation effect, and intestinal mucosal epithelial renewal also needs to rely on immune activation.

For example, intestinal mucosal renewal needs to rely on the immune stimulation of intestinal commensal microorganisms. The immune activation effect produced by APS can also promote intestinal epithelium cell renewal [16]. The study of Wang, et al. [17] found that 1 mg/mL APS can effectively inhibit the high expression of Caco2 TNF-α, IL-1β, and IL-8 inflammatory cytokines induced by LPS and can increase the expression level of Caco2 cell tight junction protein and *Occludin*, thereby improving mucosal integrity. In addition, by inducing the activation of ornithine decarboxylase, APS can promote the proliferation, migration, and differentiation of small intestinal epithelial cells and ensure the mechanical barrier function of the intestinal mucosa [18]. The integrity of the animal's intestinal mucosa is an important prerequisite for its intestinal immune barrier function, thereby ensuring that the body can effectively resist the infection of intestinal pathogenic microorganisms [19]. In addition to promoting the renewal of the intestinal mucosa and improving the barrier function of the intestinal mucosa, APS can also improve the intestinal immune function by regulating the mucosal immune activity status. The study of Guo, et al. [20] found that APS can effectively improve the IgA, IgM, and IgG levels of Eimeria tenella infection broilers in the cecum. enhance mucosal immune function. Yin, et al. [21] found that APS can enhance the innate immunity of bladder epithelial mucosa by promoting the expression of TLR4 in bladder epithelial cells. In addition to direct regulation, APS can also optimize the structure of the intestinal flora by interacting with Lactobacillus and Bacillus to improve the stability of the intestinal microflora, thereby indirectly exerting the intestinal mucosal immune regulation effect of APS [22]. LPS challenge can significantly increase

the levels of serum pro-inflammatory cytokines IL-1β, IL-6, IL-8, and TNF-α, and cause an intestinal loss in mice. In short, APS can effectively improve the health and production performance of livestock and poultry. This effect is based on its effect on improving the function of the intestinal mucosal immune system and the morphological integrity of intestinal epithelial tissue.

Antioxidant function APS can exert biological effects such as anti-oxidation and anti-cancer through its immune regulation effect. Abuelsaad, et al. [7] found that APS can change the intestinal cellular immune status of mice caused by Aeromonas infection and inhibit the synthesis of reactive oxygen in the intestine, and is beneficial to protecting the tissue integrity of the intestinal mucosa. EA.hy926 cell-based model studies have found that APS can significantly reduce the expression of inflammation-related nuclear factor NF-κB and pro-inflammatory cytokine IL-8, thereby reducing the content of reactive oxygen ROS and malondialdehyde in cells, and significantly increasing cell superoxide dismutation enzyme activity [23]. Apoptosis of cardiac stem cells and progenitor cells can be induced by activating oxidative stress in streptozotocin-induced diabetic mouse models and affects myocardial viability. APS can alleviate the apoptosis of cardiac stem cells by increasing the level of SOD protein and enzyme activity and reducing ROS and malondialdehyde content [24]. In addition, in the absence of myocardium blood reperfusion injury and abnormal adrenaline, APS can inhibit ROS synthesis and down-regulate the synthesis of related signals PI3K/AKT, Bax/Bcl-2, and caspase 3 to prevent oxidative damage-induced apoptosis of cardiomyocytes [25-26]. Also, through its antioxidant regulation effect, APS can

prevent neuronal damage in Parkinson's disease mice and maintain neuronal mitochondrial stability [27]. The research results of Zhang, et al. [28] are consistent with this. Addition of APS to broiler diets can improve its production performance and antioxidant capacity. The smaller the particle size of APS crushing treatment (in the range of 37 to 300um), the stronger its activity [29]. In short, APS can affect the biological state of the body through its structure-activity regulation, increase the antioxidant enzyme activity of the body's circulatory system and liver tissue, reduce the level of reactive oxygen species, and enhance the body's antioxidant capacity [30].

Anti-cancer function The cancer suppression effect of APS stems from its immune activation regulation activity, and a moderately activated immune system can effectively improve its recognition and clearance of "different" components. The cell surface immune recognition molecules TLRs of macrophages, dendritic cells, etc. can recognize the surface-specific antigens of cancer cells, thereby activating the immune system's phagocytic clearance effect and inhibiting the occurrence of cancer [31-33]. In addition, APS can also exert an anti-cancer effect by inhibiting the expression of CD40 in cells [34]. The studies in gastric cancer [22], liver cancer [35], and lung cancer [36-37] have confirmed that APS does have a tumor suppressor effect, which is beneficial to slowing down the cancerous process of tissues and organs. Based on its biological regulation activity, APS can be used for the treatment of various diseases such as cardiac hypertrophy and diabetes [38]. APS can increase the expression level of nuclear factor IκB, reduce the activity of NF-κB, inhibit streptozotocin-activated diabetic conditions in rat models, and effectively alleviate diabetic complications such

as lethargy, polyuria, and weight loss [39]. In addition, APS can inhibit the high expression of protein tyrosine phosphatase, improve the insulin recognition of type II diabetic mice, and promote the blood glucose intake by activating AMPK expression to alleviate diabetes [40-41]. APS can also alleviate the metabolic abnormalities of APP swe/PS1dE9 mice by improving obesity, fatty liver disease, neuroinflammation, and cognitive impairment and is one of the possible clinical treatments for Alzheimer's disease [42]. APS can inhibit the activation of calcium-related phosphatase/NFATc3 and CaMKII pathways, and inhibit isoproterenol-induced cardiac hypertrophy [43]. In short, APS exerts anti-oxidation, anti-cancer, and other biological effects through its immune activation effect, and improves animal health.

1.1.2 Application of *Astragalus* in poultry production

Poultry is prone to stress during the breeding process, which increases the probability of disease. APS improves poultry growth performance, improves gut health, and boosts immunity. The addition of 800 mg/kg of AE to the diet can increase the body weight of broilers on 42 days and the weight gain of broilers from 15 to 42 days [44]. Adding 10 g/kg of *Astragalus* root powder can increase ADG and reduce FCR in broilers [45]. Wang Q, et al.[46] the study showed that adding *Astragalus* polysaccharide to the poultry diet can improve ADFI and reduce FCR, and *Astragalus* polysaccharide can also alleviate the growth performance of broiler chickens under immune stress with cyclophosphamide and lipopolysaccharide (LPS). The research of Wang Y, et al. [47] showed that adding 1.74% compound *Astragalus* granules to the broiler diet can significantly improve the meat quality. Studies have shown that

Astragalus polysaccharides can scavenge free radicals in time by activating a variety of enzyme activities in the body, reducing oxidative stress in animals, and enhancing animal immune responses [48]. Adding 0.5-1.0 g/kg of *Astragalus* polysaccharide to the diet can improve the growth performance and serum SOD, GSH-Px, IgG, IgM, and IgA levels of broilers, and reduce MDA levels [49]. Adding *Astragalus* root powder can improve growth performance, antioxidant status, and serum metabolites of broilers [50] and improve liver and kidney function by improving antioxidant status [51].

1.2 Physiological function of *Glycyrrhiza* and its application in poultry production

Glycyrrhiza is the dry roots and stems of leguminous *Glycyrrhiza* (*Glycyrrhiza* uralensis Fisch), *Glycyrrhiza* inflates Bat and *Glycyrrhiza* glabra L. It has excellent cold resistance, heat resistance, drought resistance, and salinity resistance. It is a perennial herb of the leguminous family. *Glycyrrhiza* has the functions of tonifying qi, relieving cough, clearing heat and detoxifying, and relieving pain. The main active components in *Glycyrrhiza* extract include saponins, flavonoids, glycyrrhizin, etc., which have various biological functions such as anti-virus, anti-inflammatory and bactericidal, antioxidant, and enhancing animal immunity [21].

1.2.1 Physiological function of *Glycyrrhiza*

Antivirus *Glycyrrhizin* has an obvious inhibitory effect on various viruses such as varicella, zoster virus, SARS, HIV, HBV, etc., because it can directly destroy viral cells in test tubes [52]. Studies have shown that *Glycyrrhiza* polysaccharides extracted from *Glycyrrhiza* have antiviral effects on 7 kinds of RNA and DNA viruses in vitro. The

results show that *Glycyrrhiza* polysaccharides can directly inactivate 4 kinds of viruses, and also have an effect on intracellular viruses, which can prevent viruses. Adsorption into cells [53]. The current treatment of hepatitis is mainly achieved by anti-virus, inducing interferon, regulating body immunity, and anti-inflammatory [54]. Ye et al., [55] found that glycyrrhizin and glycyrrhetinic acid can significantly reduce hepatocyte necrosis and ballooning in acute hepatitis, decrease aspartate aminotransferase and alanine aminotransferase, and also had good effects on hepatocytes of chronic hepatitis. Ding, et al. [56] studied the protective effect of liquidity on carbon tetrachloride liver virus and found that liquidity has a significant protective effect on liver toxicity.

Anti-inflammatory Inflammation is an immune response dominated by defense responses that occur when body tissues are damaged or infected by external stimuli. *Glycyrrhiza* glabra extract can achieve an anti-inflammatory effect by regulating the expression of immune-related factors. The study found that after the addition of *Glycyrrhiza* extract to the diet, the activity of alkaline phosphatase in the serum of weaned piglets was increased, the activity of aspartate aminotransferase was decreased, and the expression of related pro-inflammatory factors such as IL-6 and IL-8 was inhibited to varying degrees, thereby reducing the activity of aspartate aminotransferase. Improve the anti-inflammatory ability of weaned piglets [57]. *Glycyrrhiza* glabra extract mainly exerts anti-inflammatory and bactericidal effects through active components such as *Glycyrrhiza* acid and flavonoids. *Glycyrrhiza* glabra extract and flavonoids isolated from it can reduce the pro-inflammatory factors IL-1β, IL-6, and tumor necrosis factor-α (TNF-α) in RAW264.7 cells stimulated by lipopolysaccharide (LPS). Inhibition

induces the expression of induced nitric oxide synthase, (iNOS) and cyclooxygenase-2, (COX-2), which in turn interferes with nitric oxide, (NO) and prostaglandin E2 (PGE2), mediated inflammatory cascade response and exert an anti-inflammatory effect [58].

Antioxidant function Under normal circumstances, the production and scavenging of oxygen free radicals in the body are in a state of dynamic equilibrium. The antioxidant activity of *Glycyrrhiza* polysaccharide is mainly manifested in the following two aspects: on the one hand, it enhances the activity of antioxidant enzymes in the body, and on the other hand, it scavenges oxygen free radicals. Lian Yijun, et al.[59] and other experiments showed that *Glycyrrhiza* polysaccharide can scavenge DPPH free radicals and hydroxyl free radicals, and has strong antioxidant activity. Isoliquiritigenin and liquiritigenin have certain scavenging effects on hydroxyl radicals, superoxide anions, and DPPH. The study found that H2O2. ROS production was induced after stimulation of IPEC-J2 cells, whereas pretreatment of cells with *Glycyrrhiza* extract reduced ROS production [60]. Using doxorubicin alone to act on H9c2 cells, the level of ROS was significantly increased, inducing cardiotoxicity, while the level of ROS was reduced by three times after treatment with *Glycyrrhiza* glabra extract, indicating that *Glycyrrhiza* glabra extract can scavenge ROS and restore the cell's cytotoxicity. Antioxidative capacity [61]. In animal experiments, dietary supplementation of *Glycyrrhiza* extract increased total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD), and glutathione peroxidase (GSH- Px) activity, decreased malondialdehyde (MDA) content and enhanced antioxidant capacity in weaned piglets [57].

Immunomodulatory function *Glycyrrhizic* acid is an effective biological response modifier, and its immune function is manifested in many aspects such as immunocompetent cells, cytokines, complements, and so on. *Glycyrrhizic* acid can enhance the proliferation and activity of helper T lymphocytes, promote the production of IL-2, IFN-γ, IL-1, and other cytokines by lymphocytes, and inhibit the production of IL-4, IL-10, IL-8, etc. At the same time, it has complement activity and can selectively inhibit the activation pathway of the complement system [62]. *Glycyrrhiza* polysaccharides play an immunomodulatory effect mainly by activating the body's immune system [63]. Wang Lirong, et al. [64] test results show that *Glycyrrhiza* polysaccharide can significantly increase the weight of mice and increase the formation rate of E2 rosettes of lymphocytes, thereby improving cellular immune function. Hong, et al. [65] studied the effect of *Glycyrrhiza* polysaccharide on the proliferation of spleen lymphocytes and serum antibody levels in mice with a high-fat diet and found that *Glycyrrhiza* polysaccharide can promote the proliferation of mouse spleen lymphocytes and increase the levels of various antibodies in serum.

Improve gut health *Glycyrrhiza* also helps to regulate intestinal flora, which can inhibit the growth of harmful bacteria in the intestinal tract and promote the reproduction of beneficial bacteria. Jia Chunying, et al. [66] found that adding 0.1% *Glycyrrhiza* powder can reduce the incidence of E. coli in piglets, improve intestinal health, and improve the survival rate and disease resistance of piglets. Dong Yongjun, et al. [67] added *Glycyrrhiza* polysaccharide to the feed to study its regulating effect on the intestinal microflora of broilers. The results showed that the number of intestinal

Escherichia coli and Salmonella in the 1.0g/kg *Glycyrrhiza* polysaccharide group were lower than those in the control group ($P < 0.05$), the number of Lactobacillus and Bifidobacterium in the intestinal dominant flora were higher than those in the control group.

1.2.2 Application of *Glycyrrhiza* in poultry production

Adding 80 mg/kg of *Glycyrrhiza* extract to the diet of laying hens can improve the production performance and egg quality of laying hens in the late stage of laying, significantly reduce the feed-to-egg ratio, improve the color of egg yolk, average egg quality, and reduce the content of cholesterol in egg yolk [68]. Dietary supplementation of 500 mg/kg of *Glycyrrhiza* extract significantly increased the body weight gain of broilers throughout the growth stage under high-density feeding conditions [69]. Adding 3000mg/kg *Glycyrrhiza* extract to the diet of aflatoxin-challenged broilers can effectively alleviate the negative effects of aflatoxin on the growth performance, blood indexes, and immunity of broilers. As a bio converter, *Glycyrrhiza* extract can effectively combine Aflatoxin B1, thereby mitigating the negative effects of aflatoxin B1 on broiler growth [70]. Adding 1000mg/kg *Glycyrrhiza* extract to the diet of Ross chicks can increase body weight gain and feed conversion rate at 1-35 days of age, reduce mortality, and up-regulate *Occludin,* junctional adhesion molecule-2 (JAM-2), and Gene expression of glucagon-like peptide-2 (GLP-2); dietary supplementation of *Glycyrrhiza* extract upregulates intestinal mucin-2 (MUC-2) after challenge with Campylobacter jejuni in ROSS broilers at 35 days of age), down-regulated the gene expression of intestinal inflammatory factors TLR4 and IL-β, reduced the number of

Campylobacter jejuni, maintained intestinal barrier integrity, and reduced production loss [71]. It can be seen that adding an appropriate concentration of *Glycyrrhiza* extract to the diet can help improve the performance of poultry and reduce the impact of harmful bacterial infections and mycotoxins on poultry health.

1.3 Poultry's intestinal barrier

1.3.1 Overview of the intestinal barrier

As the largest digestive organ in the animal body, the intestine is essential to ensure the body absorbs and metabolizes nutrients. At the same time, the intestine is also the largest immune organ that can effectively resist the invasion of harmful substances such as bacteria, lipopolysaccharide (LPS), or toxins in the intestinal cavity. Therefore, it is particularly important to maintain the normal function of the intestine.

However, during the breeding process, broiler chickens are susceptible to pathogen stimulation, harsh environments, uneven diets, maternal and other factors, causing intestinal inflammation, thereby destroying intestinal homeostasis, impairing intestinal function, and leading to nutrient absorption obstructed, weakening immunity, decreasing growth performance, etc. At the same time, in modern broiler production, intensive cage breeding techniques are commonly used to improve the utilization of poultry houses. However, due to the intensive breeding environment, broilers with poorly developed intestinal functions are prone to intestinal inflammation and diarrhea. This will greatly reduce the growth performance of broilers. Under the action of various factors such as external stimulation and early immune system failure, the intestines of

broilers are extremely vulnerable to attack and infection, resulting in economic losses such as reduced production efficiency.

As the longest and most important section of the digestive tract, the intestine is an important digestive and immune organ in the body, so the maintenance of intestinal homeostasis plays an important role in the health of the body. The intestine is not only the main place for the digestion and absorption of nutrients but also the largest barrier to isolating the host's internal and external environments. The intestinal barrier shoulders two important but contradictory functions of "opening" and "closing". On the one hand, it must maintain a certain degree of permeability to maximize the entry of nutrients to ensure the growth and development needs of the host, and it also has good compactness and prevents the invasion of pathogenic bacteria and antigens. In a narrow sense, this barrier refers to a single layer of epithelial cells that separates the host from the external environment. In a broad sense, the intestinal barrier is a concept of a multi-layer barrier. Current research generally believes that the intestinal barrier is composed of a physical barrier, a chemical barrier, a microbial barrier, and an immune barrier (Figure 1-1). These four barriers are independent and mutually dependent on different biological functions, signal pathways, and regulatory mechanisms, from the local to the system jointly defending the health of the host's intestines and the body.

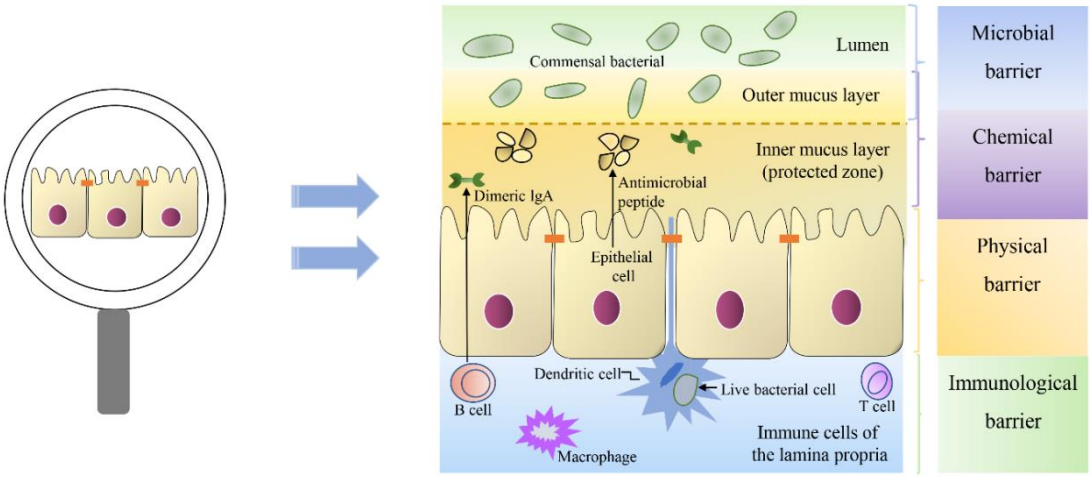

Fig. 1-1 Intestinal barrier

Source: prepared by the author based on Gao, et al. (2020) [80]

Intestinal physical barrier The physical barrier, also known as the intestinal epithelial barrier, is the main place for the intestine to absorb nutrients, and it is also the first barrier for the intestine to prevent bacteria, antigens, endotoxins, and other toxic and harmful substances from entering the intestinal submucosa and blood [72]. The intestinal epithelial barrier is mainly composed of intestinal epithelial cells and tight junctions [73].

The connection structure formed between the intestinal epithelial cells is called the intestinal tight junction structure (also known as the zonule atresia), which is the top structure between the cells and is an important part and structural basis for maintaining the physical barrier of the intestine. Tight junction (TJ) maintains cell polarity by restricting the movement of proteins in the plasma membrane and regulating paracellular solute and water flux. At the same time, TJ can also act as a signal hub [74-75]. The lack of TJ structure and function can lead to chronic inflammation and even intestinal diseases [76]. Studies have shown that TJ is mainly composed of three

transmembrane proteins (*occludin*, OCLN), *claudins* (CLDN), junctional adhesion molecule (JAM), and zonula *occludens* (ZO) [74]. *Claudin* family proteins are the most important members of tight junctions, helping to control paracellular movement by forming a barrier on the epithelial cell monolayer. The *Claudin* family has different members in different organizations, and they perform corresponding functions at the same time. Claudin1 is mainly expressed at the apex of epithelial cells in the colon. Claudin1 mainly acts as a seal and is essential for maintaining intestinal homeostasis [77]. *Occludin* is the first TJ membrane protein identified, and its C-terminus can bind to ZO1 to maintain tight junction integrity. The ZO family mainly plays a role as a scaffold, including ZO1, ZO2 and ZO3. The terminal of ZO1 can bind to actin and stress fibers, helping to regulate and maintain intestinal permeability [78]. TJ protein is involved in maintaining cell polarity and epithelial barrier function, preventing the invasion of macromolecules and microorganisms, and recruiting signal proteins to regulate cell proliferation, differentiation, migration, and other important functions [79].

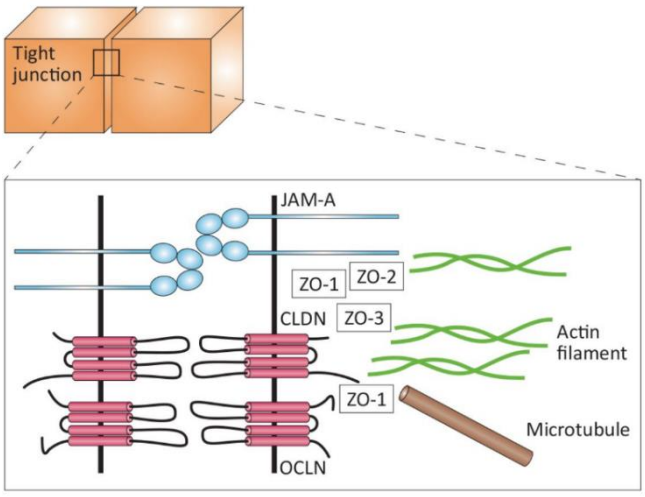

Fig. 1-2 Schematic representation of the major tight junction proteins.

Source: prepared by the author based on Guo, et al. 2014 [81]

Intestinal chemical barrier The intestinal chemical barrier is composed of a variety of chemical components, including gastric acid, bile, lysozyme, antimicrobial peptides, mucopolysaccharides, glycoproteins, and glycolipids [82]. Gastric acid can kill most of the bacteria that enter the digestive tract with food. Bile can reduce the damage of endotoxin to the body. For example, bile salts can combine with endotoxin to prevent it from being absorbed by the intestine. Bile salts and bile acid can decompose endotoxin into non-toxic subunits or form micro-polymers. Mucus is the most effective part of the chemical barrier [83]. The main component of intestinal mucus is the mucoprotein (MUC) formed by gel, mainly MUC2, which buffers the invasive injury of digestive enzymes and digestive juices. There is another type of mucin at the top of intestinal epithelial cells—transmembrane mucins, including MUC1, MUC3, MUC4, MUC14, etc., which have protective, sensory, and signal transduction functions. In addition, the mucin molecule contains specific protein binding domains, which can bind and stabilize the key factors of intestinal epithelial nutrition and repairment, contributing to the repair of intestinal epithelial cells [84].

Intestinal microbial barrier The intestinal microbial barrier is an important part of the intestinal barrier, which can affect the metabolism and proliferation of the intestinal epithelium. The intestinal microbial barrier is a micro-ecological system formed by the micro-space structure of intestinal symbiotic bacteria and the host. When the stability of this micro-ecosystem is destroyed, it will lead to the colonization and

invasion of potential pathogens (including conditional pathogens) in the intestine. The intestinal resident flora inhibits the colonization and growth of pathogenic bacteria by competing for adhesion on the intestinal mucosa, secreting antibacterial substances, and increasing mucus secretion [85]; it can also secrete lactic acid and short-chain fatty acids (such as Acetic acid, propionic acid, and butyric acid, etc.) to lower the intestinal pH and redox potential; among them, butyric acid, as the main energy substance of epithelial cells, affects the proliferation and differentiation of intestinal epithelial cells, and helps to strengthen the intestinal tight junction [86].

Intestinal immune barrier As the largest immune organ in the animal body, the intestine bears the dual tasks of tolerance to dietary antigens and immune defense. The immune defense system of the intestine is mainly composed of gut-associated lymphatic tissue (GALT), which is the body's largest lymphatic organ and important mucosal-associated lymphatic tissue. It is the first line of defense against pathogens invading the body during the feeding process. Chicken GALT is mainly composed of lymphocytes distributed in the lamina propria and submucosa of the intestinal mucosa [87]. The well-developed GALT of chickens makes up for the lack of lymph nodes. The main effector in the intestinal immune response system is the secretory immunoglobulin A (secretory IgA, sIgA), secreted by plasmablasts, which can bind strongly to antigens to prevent harmful antigens such as viruses and bacteria adhering to the intestinal epithelium and then promote the humoral and cellular immunity of the intestinal tract, and effectively reject or eliminate the harmful antigens ultimately.

1.3.2 Evaluation of poultry intestinal barrier function

Intestinal permeability As a normal function of the intestinal wall, intestinal permeability not only allows nutrients to pass through the intestinal wall but also acts as a barrier to prevent potentially harmful substances from entering the body through the intestinal wall. Abnormal intestinal permeability indicates that the intestinal mucosa may be damaged and destroyed to a certain extent. Therefore, intestinal permeability has been considered to be the most important indicator reflecting the intestinal barrier function [81]. And it is widely used in human and animal research. Diamine oxidase is a highly active intracellular enzyme found in the villi of the intestinal mucosa of all mammals. When the intestinal mucosa suffers damage, a large amount of diamine oxidase is released, which leads to increased diamine oxidase activity in the blood and intestinal lumen and decreased diamine oxidase activity in the intestinal mucosa. Therefore, detection of changes in blood and intestinal mucosal diamine oxidase (diamine oxidase) activity can reflect the intestinal barrier function.

Morphology of intestinal mucosa Intestinal mucosal histological observation is the most common and direct method to evaluate the intestinal barrier function using an optical microscope, scanning electron microscope, transmission electron microscope, or other tools. It can directly observe the intestinal epithelial cell morphology, villi structure, arrangement, epithelial cell connection, and complex physical conditions. The changes in these intestinal mucosal indicators can reflect the damage of the intestinal mechanical barrier to a certain extent and are suitable for experimental research in various animals.

1.4. Poultry's respiratory barrier

The respiratory tract of livestock and poultry is connected to the environment in the livestock and poultry house. It is constantly stimulated by the temperature, humidity, bacteria, virus, dust particles, and a variety of harmful gases in the house. Harmful substances enter the animal body through damage to the respiratory mucosa or through the trachea epithelial cell gap to cause respiratory diseases. With the rapid development of intensive farming, the respiratory diseases of livestock and poultry have gradually increased, leading to a decline in animal production performance and a decrease in immune function [88-89]. The large-scale outbreaks of respiratory syndromes, infectious bronchitis, influenza, and other respiratory infectious diseases have attracted people's attention. The prevention and treatment of respiratory diseases in livestock and poultry have become particularly important. As an important part of the mucosal barrier, the respiratory mucosal barrier is directly connected to the external environment and isolates various harmful substances in the external environment from the body environment. It protects the health of livestock and poultry, especially the respiratory tract of livestock and poultry from pathogenic microorganisms, dust, etc. The mucosal barrier of the respiratory tract can remove pathogenic bacteria, dust, and other foreign matter in the trachea, resist pathogenic bacteria infection and maintain the health of the respiratory tract. Therefore, an in-depth understanding of the structure and function of the respiratory mucosal barrier and the pathogenesis of respiratory diseases is of great significance to the treatment of respiratory diseases in livestock and poultry, the development of new drugs, and the development of healthy breeding. At present, most reports on the mucosal barrier focus on the intestinal mucosa, while there

are few reports on the respiratory mucosa. The respiratory mucosa is an important barrier for the respiratory system to resist external harmful substances and an important line of defense to protect the health of the body.

1.4.1. Overview of the respiratory tract mucosal barrier

The animal body mucosal system mainly includes the respiratory tract and gastrointestinal mucosa, and the respiratory tract mucosa, as the body's second-largest mucosal system, is an effective barrier to maintaining the health of the respiratory tract. The respiratory mucosal barrier is composed of a mechanical barrier, a chemical barrier, a microbial barrier, and an immune barrier, each of which combines through different molecular regulation mechanisms, signal pathways, and biological functions to protect the respiratory barrier of the body and resist the attack of pathogenic bacteria and other harmful substances [90]. When various external pathogenic factors interact, the integrity of the mucosal barrier of the respiratory tract is destroyed, leading to trachea oxidative stress, inflammation, and infection, causing respiratory diseases, reducing immune function, and even causing lung damage in livestock and poultry, which seriously endangers the health of animals [91-92].

1.4.2 The structure and function of the mucosal barrier of the respiratory tract

Mechanical barrier Respiratory tract mucosal epithelial cells and tight junctions (TJ) between cells constitute a mechanical barrier to the respiratory tract mucosa [93-94]. The various parts of the mechanical barrier interact to prevent pathogenic microorganisms from penetrating the mucosa of the respiratory tract into the deep

tissues of the body, which is the structural basis of the mucosal barrier of the respiratory tract. Respiratory mucosal epithelial cells (Figure 1-3) mainly include ciliated cells, goblet cells, basal cells, and submucosal secretory glands [95-96]. Ciliated cells are the main cells of the mucosal epithelium of the respiratory tract. Ciliated cells are attached to a certain number of cilia and oscillate at a certain frequency. Through the oscillating action of the cilia, the bacteria and dust entering the nasal cavity and trachea can be removed in time [90]. When cilia cells are stressed by external harmful factors such as bacteria, viruses, dust, etc., the motor function of cilia decreases, resulting in a decrease in the removal of harmful substances in the respiratory tract, and the residence time of pathogenic bacteria and dust in the respiratory tract is prolonged, which in turn leads to oxidative stress in the respiratory tract inflammation and infection [94]. Ciliated cells are also plastic and can be transformed into goblet cells [97]. Goblet cells and Clara cells between ciliated cells can continue to differentiate into ciliated cells under certain conditions. Basal cells are fixed on the basement membrane with the help of adhesion molecules, which can proliferate and differentiate to form ciliated cells [98]. Goblet cells and submucosal glands are the main secretory cells, which protect the respiratory mucosa by secreting chemicals such as mucin.

Fig.1-3 Components of airway tract epithelium

Source: prepared by the author based on Ganesan, et al. 2013 [96]

Respiratory tract mucosal cells maintain the normal structure and function of epithelial cells through various connexins and signaling molecules to form tight junctions, adhesion junctions, and desmosome connections. Cells interact with each other through a variety of connexins to maintain the integrity and regulation of epithelial cells and the effective transport of various substances [99]. The connections between epithelial cells mainly include tight junctions, adhesion junctions, and gap junctions (Figure 1-4). Tight junctions are the most important connection method [92]. Adhesion connections play a role in cell-to-cell communication and adhesion and intracellular signal transmission [100]. Gap junctions are channels for the exchange of materials and information between cells, which are essential for cell proliferation, differentiation, and growth and development of the body. A tight junction is a composite structure formed by the interaction of a variety of proteins. It is mainly located at the top of the connection between adjacent cells. It is the most basic and common form of tissue structure between cells. By sealing the gaps between adjacent cells, it prevents the respiratory tract from foreign harmful substances such as pathogenic bacteria penetrate the body tissues

through the intercellular space to ensure the relative stability of the animal's internal environment [101]. Tight junction proteins mainly include transmembrane proteins and cytoplasmic proteins. Among them, transmembrane proteins include Occludin, Claudins, and junction adhesion molecules (JAM) [81], Cytoplasmic proteins are zonula occludens (ZO), including ZO-1, ZO-2, and ZO-3. These transmembrane proteins are connected to the actin cytoskeleton through cytoplasmic proteins to form tight junctions complex and form a complete epithelial structure with epithelial cells and regulate the cell's barrier function [102,104]. Tight junction proteins can regulate the permeability and permeability of a variety of macromolecular substances between respiratory epithelial cells, prevent pathogens, dust particles, and other harmful foreign substances from entering the body, and participate in the body's signal transduction and innate immunity [103]. The stability of tight junctions between cells is related to the complex interactions between claudins and between claudins and other tight junction proteins. Claudins are the main skeleton proteins that constitute tight junctions. Occludin can seal the gaps between epithelial cells, maintain the body's permeability barrier and the polarity of epithelial cells, regulate cell adhesion, and accept and transmit cell signals and other biological functions [105]. ZO has the functions of connecting transmembrane proteins and cytoskeleton, transmitting signal molecules, regulating and transporting intracellular substances, and maintaining the polarity of epithelial cells. It is the basis of a tight junction support structure [106].

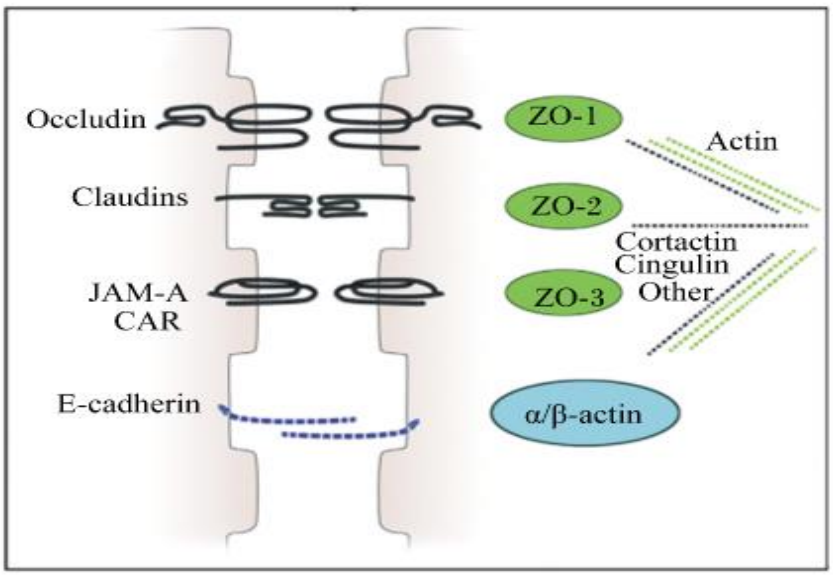

Fig.1-4 Cartoon diagram of airway epithelial cells indicating junction structures. ZO-1 and ZO-2 make claudins and actin tightly bound together to form a stableconnection system. Source: prepared by the author based on Georassn, et al. 2014 [107]

Chemical barrier The respiratory tract mucosal chemical barrier is mainly composed of a certain elastic and viscous surface liquid covering the entire respiratory tract surface, mainly including a mucus layer and a serous layer, which can keep the trachea moist and prevent the invasion of a variety of harmful factors. The mucous layer is mainly composed of submucosal glands, epithelial cell secretions, and tissue exudates, such as defensins, lysozyme, antimicrobial peptides, and other chemical components, which can destroy the cell walls of various bacteria and eliminate invading pathogens [108]. The main component of mucus is water, accounting for 84% to 94%, in addition to protein, carbohydrates, lipids, etc., among which mucin is the most important protein component in mucus [109]. Mucins can be divided into membrane-bound mucins and

secretory mucins. Membrane-bound mucins exist on the surface of epithelial cells and have the functions of protecting cells, adhering to bacteria, and promoting the proliferation of epithelial cells. Secreted mucins can lubricate and protect the mucosal barrier [110]. Goblet cells and submucosal gland cells are respiratory tract mucus [111]. The main source of protein, MUC5AC is mainly produced by goblet cell secretion, and MUC5B is mainly produced by the serous cells of submucosal glands. Under normal circumstances, there is a small amount of mucus in the respiratory tract, which protects and lubricates the respiratory mucosa, and maintains the normal function of the respiratory tract. However, under the stress of external harmful substances, goblet cells are hypertrophy and proliferation, and the expression of respiratory mucin MUC5AC is up-regulated, causing trachea mucus secretion to increase and leading to trachea obstruction [112]. The serous layer, as a chemical barrier of the respiratory tract, can provide a good environment for the growth of cilia, which is of great significance for maintaining the movement of cilia.

Microbial barrier A certain number and types of microorganisms reside on the mucosal surface of the respiratory tract at all times, and the interaction between these microorganisms and between the microorganisms and the host forms a stable micro ecological environment of the animal body. Generally, the micro-ecological environment is in a state of dynamic equilibrium, forming a microbial barrier against pathogen infection. The respiratory tract is colonized by many bacteria to form the normal flora of the upper respiratory tract. This flora helps the host's food metabolism and nutrient absorption, and also participates in the maintenance of mucosal immune

homeostasis and the resistance and elimination of foreign pathogens by competing with the body in adhesion on the mucous membrane of the respiratory tract, secretion of antibacterial substances, and increased mucus secretion to inhibit the colonization and growth of pathogenic bacteria. It plays an important role in exerting local immune function and resisting the invasion of foreign bacteria. As the age of broilers increases, the Shannon index and richness of the larynx and tracheal flora gradually increase. At the microbial level, the phylum of Firmicutes, Actinomycetes, and Bacteroides are the main phyla [113-114]. Pan K, et al. [115] reported that the microorganisms in the respiratory tract of broilers are mainly Enterobacteriaceae, followed by non-cultivable microorganisms. Luan S, et al. [116] used 16S high-throughput sequencing method to determine the respiratory tract microbial community in broilers. The results showed that at 21 days of broilers, the respiratory tract microorganisms were mainly Firmicutes. At 42 days of broilers, the respiratory tract microbial flora mainly includes Firmicutes and Proteobacteria. The main dominant flora at the level of family in respiratory microbiology is Lactobacillus, Bacillus, Enterococcus, Staphylococcus, Streptococcus, and Enterobacteriaceae. The special structure of the respiratory tract determines that it is easily affected by various factors in the environment. Once the respiratory tract flora is imbalanced, it will induce the body to produce a variety of respiratory diseases.

Immune barrier The respiratory tract mucosal immune system mainly includes mucosa-associated lymphoid tissue and diffuse lymphoid tissue. Mucosal-associated lymphoid tissue is the afferent lymphatic area of the immune response. Foreign antigens enter the respiratory tract mucosa from the mucosal-associated lymphoid tissue and are

extracted by respiratory epithelial cells and delivered through antigens presenting cells to T cells and B cells to trigger an immune response [117]. Diffuse lymphoid tissue is the efferent lymphatic area of the immune response. Through the homing mechanism, plasma cells and sensitized lymphocytes migrate to diffuse Lymphatic tissue and perform biological functions [118]. The mucosa-associated lymphoid tissue of the respiratory system is mainly composed of bronchial-associated lymphoid tissue and nasal-associated lymphoid tissue, Yang S, et al. [119] reported that the respiratory mucosa-associated lymphoid tissues of broilers appeared at 4 and 7 days of age, forming the basis of the tissue structure of the respiratory tract immunity. With the increase of age, the antigen-presenting cells of the respiratory tract-associated lymphoid tissues and T, B lymphocytes, etc. continue to develop. Before 35 days of age, it is mainly to exert cellular immune function, and after 35 days of age, it is mainly to exert humoral immunity. Yan M, et al. [120] reported that there are abundant nasal-associated lymphoid tissues in the nasal cavity of chickens. Feng X, et al. [121] reported that chicken nasal glands were formed at the age of 18 embryos, and the nasal mucosa changed significantly after husks. At 21 days of age, chicken nose-related lymphoid tissues were fully developed and mature. Diffuse lymphoid tissue is the effective site of an immune response, mainly in the lamina propria of the mucosa, including intraepithelial lymphocytes and lamina propria lymphocytes [122]. Intraepithelial lymphocytes participate in cell-mediated mucosal immunity and maintain the integrity of epithelial cells. They can express CD and B integrins, and secrete interferon (IFN), interleukin (lL)-2, lL-5, and other cytokines, and with antigen-specific auxiliary

functions and natural killer cell (NK cell) activity [123]. There are a variety of immune-related cells in the lamina propria lymphocytes, which are mainly located in the lamina propria of the mucosal epithelium, such as B cells, T cells, NK cells, and macrophages. When stimulated by external antigens, B cells secrete a large amount of secreted immunoglobulin A (sIgA), which plays a role through the mediation of secretion tablets. T cells secrete transforming growth factor (TGF), IL-4, IL-5, IL-6, IL-10, etc. through CD4 and CD8 cells to exert immune function [28]. SIgA plays an important role in the defense of respiratory mucosa [124]. Li P, et al. [125] reported that the respiratory tract of pigs contains more antibody-secreting cells, and immunoglobulin A (IgA) secreting cells are most distributed in the trachea, followed by pharyngeal tonsils, soft palate tonsils, and the least in the lungs. SIgA is produced by plasma cells and consists of one J chain and one secretory sheet and two IgA monomers. It can prevent the adhesion of pathogenic microorganisms, combine with secretory sheets secreted by mucosal epithelial cells, neutralize antigenic substances, and dissolve bacteria [28].

1.5. High temperature boiled technology

Most of the meat needs to be processed before eating, so as to obtain the expected taste and flavor, remove the peculiar smell and bloody smell of the meat itself, which is more conducive to human digestion and absorption of more nutrients. Common traditional processing methods include salting, steaming, boiling, frying, frying, roasting, drying, etc., but the quality of meat will change after processing, and different processing methods have different effects on the quality of meat. Research shows that

the meat cooked in high temperature water can not only kill harmful microorganisms in the meat, improve the taste, but also retain the nutrition of meat more comprehensively.

High-temperature water boiled technology is a processing method using water as the heat transfer medium. The raw materials and ingredients are put into a large amount of boiling water together. First, the food is cooked with high fire, and then the food is cooked with mild fire. After heating for a certain time, it can be eaten. After cooking, the food tastes light and delicious, and the nutrients are well preserved. The control of cooking time and temperature is the key to the cooking process, and the time and temperature of different raw materials should be controlled reasonably to avoid the food being too soft and rotten due to too long processing time and too high processing temperature, and the taste of the food becoming bad, and the loss of nutrients is too much, which is not conducive to the absorption of nutrients by the human body. However, if the processing time is too short, the temperature is too low, the food processing is insufficient, and the microorganisms and bacteria are difficult to inactivate, there is potential harm to human body.

1.5.1Effect of High Temperature boiled technology on Nutrient Content in Chicken

The nutritional quality of meat is one of the four major qualities of meat, which has an important impact on the nutritional balance and health of human body. After processing by different processing methods, the nutritional quality changes differently. In order to find suitable processing conditions and obtain high-quality food, many studies have been done by predecessors. Wang Ruihua et al. [126] compared and

analyzed the effect of boiled processing methods on the nutritional quality of pork, and found that boiled meat can improve the nutritional value of meat. Gao Tianli et al. [127] studied the changes of fatty acids in mutton using cooking, microwave and ultrasonic as processing methods and mutton as raw materials. The results showed that the nutritional value of fatty acids in mutton after cooking was significantly improved. After analyzing relevant literature reports, it was found that there were many studies on the nutritional quality of livestock and poultry meat by water cooking technology, but there was no report on the effect of the comprehensive cooking technology of adding *Astragalus* extract and *Glycyrrhiza* extract to the diet of broilers during the feeding process on meat processing. Therefore, this experiment mainly studied the effects of adding Astragalus extract and *Glycyrrhiza* extract to the diet on the performance, meat quality, serum index, intestinal barrier of broilers, and the effects of high temperature boiling treatment on meat quality traits.

1.6 The purpose, significance and content of this test

The purpose of this experiment is to study the feeding effect of AE and GE on broiler chickens and the impact on intestinal health and respiratory health and to explore their potential mechanism of action and To explore the effect of high temperature water boiled technology on meat quality traits of broilers fed with AE and GE. So that to provide a theoretical basis for AE and GE to be used as new antibiotic substitutes in broiler feed to produce pollution-free animal products.

Research content:

(1) Effects of AE and GE on broiler performance, nutrient metabolic rate and meat quality

(2) Effects of AE and GE on the immune function, antioxidant function and inflammatory factors of broilers

(3) Study on the potential mechanism of AE and GE on the intestinal barrier and respiratory barrier of broilers

(4) Effects of adding AE and GE to broiler diets on meat quality traits such as meat color, shear strength, nutrient composition and fatty acid content of chicken after high temperature boiled

SECTION 2 MATERIALS AND METHODS

2.1 Preparation of *Astragalus* extract (AE) and *Glycyrrhiza* extract (GE)

AE and GE are provided by Inner Mongolia Hengguang Pharmaceutical Co., Ltd.（Inner Mongolia, China） The extraction process is water extraction at 90℃, double-effect at 70℃ concentrated, and dried under vacuum at 70℃. AE contains 70.23% of *Astragalus* Polysaccharides and GE contains 61.36% of *Glycyrrhiza* Polysaccharides.

2.2 Experimental animals and experimental design

In the experiment, 720 1-day-old healthy Arbor Acres broilers (purchased from Henan Yue Poultry Agriculture and Animal Husbandry Co., Ltd.) were selected and randomly divided into 6 treatments, with 6 replicates in each treatment, and 20 chickens in each replicate polyculture of male and female. The control group (CON) was fed a basal diet, the antibiotic group (ANT) was supplemented with Terramycin calcium 500 mg/kg (based on 50 mg/kg Terramycin calcium active ingredient) based on the basal diet, and the *Astragalus* extract group (AE) was supplemented with *Astragalus* extract 300 mg/kg based on basal diet, and the *Glycyrrhiza* extract group (GE) was added 150 mg/kg *Glycyrrhiza* extract to the basal diet, *Astragalus* extract + *Glycyrrhiza* extract Ⅰ group (AE+GE Ⅰ) was supplemented with 300 mg/kg of *Astragalus* extract and 150 mg/kg of *Glycyrrhiza* extract based on the basal diet. *Astragalus* extract + *Glycyrrhiza* extract group Ⅱ (AE+GE Ⅱ) was added to the basal diet *Astragalus* extract 150 mg/kg and *Glycyrrhiza* extracts 75 mg/kg based on the basal diet. The test period was 42 days. The composition and nutritional level of the basal diet are shown in Table 2-1.

Table 2-1 Composition and nutrient levels of basal diets (air-dry basis) %

Items	1 to 21 days of age	22 to 42 days of age
Ingredients		
Corn	54.50	55.42
Soybean meal	29.70	25.30
Corn gluten meal	8.00	8.00
$CaHPO_4$	1.30	1.20
Limestone	1.40	1.40
NaCl	0.30	0.30
Soybean oil	2.80	6.50
Soda	0.15	0.15
L-Lys•HCl	0.87	0.80
DL-Met	0.25	0.21
Threonine	0.13	0.12
Premix[1)]	0.60	0.60
Total	100.00	100.00
Nutrient levels[2）]		
ME/（MJ/kg）	12.61	13.59
CP	23.39	21.19
Ca	0.77	0.72
TP	0.56	0.54
Lys	1.50	1.34
Met	0.62	0.55
Thr	0.97	0.88

1) Premix is provided per kilogram of feed：1-21d：VA，12000 IU；VD_3，4500 IU；VE，30 IU； VK_3， 4.5 mg；VB_1，2.8 mg；VB_2，9.6 mg；VB_6，3.75 mg；VB_{12}，30μg；Niacin，49.5 mg；Calcium pantothenate，20 mg；Folic acid，1.5 mg；Biotin，0.18 mg；

Choline，500 mg；Zn，100 mg；Fe，110 mg；Cu，20 mg；；Mn，120 mg；I，0.7mg；Se，0.3 mg。22-42d：VA，10000 IU；VD_3，3750 IU；VE，25 IU； VK_3， 3.75 mg；VB_1，2.3 mg；VB_2，8 mg；VB_6，3.1 mg；VB_{12}，25 μg；Niacin，41.2 mg；Calcium pantothenate，20 mg；Folic acid，1.25 mg；Biotin，0.12 mg；Choline，400 mg；Zn，100 mg；Fe，110 mg；Cu，20 mg；；Mn，120 mg；I，0.7mg；Se，0.3 mg。

2）ME was a calculated value, while the others were measured

2.3 Test time and place

The feeding experiment is from October to November 2020, and the feeding location is the Xinxiang Broiler Breeding Experimental Base of Henan Agricultural University. Laboratory determination and analysis are completed in the Animal Nutrition Laboratory of the College of Animal Science and Technology of Henan Agricultural University.

2.4 Feeding management

Before the start of the experiment, clean the chicken house and all feeding utensils, fumigate and disinfect the chicken house with trichloroisocyanuric acid powder, seal the chicken house for 24 hours, ventilate for 12 hours and then perform spray disinfection (bromogeramine water 1:15), airtight for 12 hours and natural ventilation for 48 hours. The broiler chickens were raised in a three-layer vertical cage with free intake and drinking. The brooding temperature of the chicken house was maintained at 35～38℃ for the first week and then dropped by 1℃ every two days until it was maintained at 25～28℃. Natural light with the artificial light supplement, relative humidity 50-60%, natural ventilation combined with longitudinal negative pressure ventilation.

Immunization program: 7 d Newcastle disease-infectious bronchitis combined live vaccine nasal drops and eye drops; 14 d drinking water for immunization against bursal disease live vaccine; 21 d drinking water Newcastle disease-infectious bronchitis combined live disease; 28 d drinking water Immunization with live bursal disease vaccine; other disinfection management measures follow the normal procedures of the chicken farm. Observe and record the food intake, drinking water, and growth and health status of the test chickens every day, and record the death and feed consumption of the test chickens in time.

Table 2-2 The main test instrument

Instrument name	Model	Origin or manufacturer
Electronic balance	HY769	Shanghai Ding Leng Industrial Development Co., Ltd.
Analytical balance	Ser14228147	South China National Metrology and Testing Center
Colorimeter	CR-400	Japan
Digital Meat Tenderness	C-LM3B	China
pH meter	Testo 205	Germany
FOSS Automatic Kjeldahl nitrogen analyzer	Kjeltec 8400	Denmark
Soxhlet extraction system	Soxtec2055	Denmark
Muffle furnace	SR-5000	Australia
High performance liquid chromatography	TRACE1310	United States
Gas chromatograph	Waters 2695	United States
UV-Visible Spectrophotometer	UV-2102	Yunnan Sainz Experimental Equipment Co., Ltd.
Electric heating blast drying oven	101-2A	Tianjin Test Instrument Co., Ltd.
Centrifugal sedimentation machine	80-2	Jiangyan Xinkang Medical Instruments Co., Ltd.

2.5 Measurement indicators and methods

2.5.1 Growth performance

Fasting at 20:00 in the evening on the 21st and 42nd days and drinking water freely. The broilers were weighed in repeat units at 8:00 in the morning on the 22nd and 43rd days, and the weight and the feed consumption of the broilers were accurately recorded.

Actual feed consumption = total consumption-total consumption × dead chicken weight / (live chicken weight + dead chicken weight)

Average daily gain (ADG) = (average weight of each chicken in each repetition at the end of the experiment-average weight of each chicken in each repetition at the beginning of the experiment) / number of days of the experiment

Average daily feed intake (ADFI) = actual feed consumption / (number of days in the test × number of chickens on hand at the end of the test)

Feed conversion rate (FCR) = average daily feed intake/average daily gain

2.5.2 Determination of meat quality indicators

After slaughter, samples of the pectoral muscles on the same side were taken, and the following meat quality indicators were determined.

Muscle pH: Testo 205 pH meter (Testo AG, Lenzkirch, Germany) was directly inserted into the pectoralis muscle at 45 min and 24 h after slaughter, and the pH meter was calibrated with standard buffers of pH 4.01 and pH 6.86 before use.

Meat color: 45 minutes after slaughter, cut 3 pieces of breast muscle samples (5cm×5cm×0.5cm) vertically, and measure the L*， a*, and b* values repeatedly 3

times use a colorimeter (CR-400, Konica Minolta Holdings, Inc., Japan), and the average value was taken as the final color value.

Muscle drip loss rate: After slaughter, take about 2 g of pectoral muscles for each chicken to weigh (W1), place it in a sealed plastic bag, inflate the plastic bag to prevent the muscle mass from sticking to the wall, hang it in the refrigerator at 4 ℃ for 24 hours, dry the surface water of the muscle with filter paper and weigh it (W2) to calculate the drip loss: drip loss=(W1-W2)/W1 ×100%

Muscle shear force: The pectoral muscle samples after slaughter are packaged in plastic bags and placed in a constant temperature water bath at 80 ℃ for heating. When the core temperature of the meat reaches 70 ℃, it is taken out and cooled to room temperature. Then trim the length, width, and height of 3 cm, 1 cm, and 1 cm strips along the direction of the muscle fibers, and cut them perpendicular to the direction of the muscle fibers with a digital meat tenderness meter (model C-LM3B, Northeast Agricultural University), and measure the shear force.

Fatty acid content: with gas chromatography method (GB/5009.168-2016): After the sample is hydrolyzed, the fat is extracted from the diethyl ether + petroleum ether (1:1) solution, saponified and methyl esterified under alkaline conditions to generate fatty acid methyl. The esters were analyzed by capillary column gas chromatography and the content of fatty acids was quantitatively determined by the external standard method.

2.5.3 Determination of nutrient metabolic rate

After the end of the feeding experiment, 2 broilers with good body condition and close to average weight were randomly selected from each repetition, and raised in a single cage, and the whole manure metabolism test of broilers was carried out for 5 days (see Table 2-3). On the 43rd day at 15:00, fasting was started to eliminate the influence of intestinal chyme on the metabolism test. During the fasting period, free drinking water (glucose and vitamin C were added to the water to relieve stress), and the rest of the feeding conditions remained unchanged. After 17 hours of fasting, start feeding and perform 55 hours of manure collection. After 55 hours, start to cut off feed, record feed intake, continue to collect manure for 17 hours (during this period, chickens stop feeding and keep watering), and collect and weigh excrement in time (pay attention to removing feathers, dander, and other debris), add 10% hydrochloric acid (100 g of fresh manure use 10 mL) to the collected excrement to fix nitrogen and prevent it. Then put the fresh manure in a 65 ℃ blast drying oven to dry to constant weight, place it at room temperature for 24 hours after regaining moisture, and then weigh it. After crushing, it is passed through a 40-mesh sieve to determine the content of crude protein, crude fat, calcium, and total phosphorus in the feces. The preparation method of the air-dried sample of the test diet is the same as the preparation method of the fecal sample. After the relevant nutrient content in the diet and feces is determined, the apparent nutrient metabolic rate is calculated according to the relevant formula.

Crude Protein (CP); The detection method is based on: GB/T 6432-1994 Determination of crude protein in feed

Crude fat (Ether Extrac, EE); the detection method is based on: GB/T 6433-2006 Determination of crude fat in feed

Calcium (Calcium, Ca); the detection method is based on: GB/T 6436-2002 Determination of calcium in feed

Total Phosphorus (TP); the detection method is based on: GB/T 6437-2002 Determination of total phosphorus in feed by spectrophotometer method

Apparent nutrient metabolic rate (%) = (diet intake × diet nutrient content-excrement amount × excrement nutrient content)/(diet intake × diet nutrient content) ×100

Table 2-3 Metabolic test time

Date	Time	Task
The first day	15：00	Pick chicken and stop feeding
The second day	8：00	Start feeding and collecting manure
The fourth day	15：00	Stop feeding, count the amount of feed, continue to collect excrement
The fifth day	8：00	Manure collection stopped and the test ended

2.5.4 Determination method of high temperature boiled technology

After the chicken is completely thawed, remove the epidermis, fascia and connective tissue, take 200g of meat sample, wash and dry it, boil it in 800ml of water for 60min, cool it to room temperature, and use it for standby. The determination method of meat color, nutritional composition and fatty acid content is the same as 2.5.2 and 2.5.3.

2.5.5 Serum indicators and intestinal permeability

After 42 days of feeding, one broiler was randomly selected for each repetition. The blood was collected from wing veins with a heparin sodium anticoagulation tube after 12 hours of fasting. The blood was centrifuged at 3000r/min for 15min, serum was separated at 4℃, and stored at -20℃. T-AOC, SOD, GSH-Px, MDA, IgA, IgM, IgG, TNF-α, IFN-γ, IL-1β, IL-6, DAO, and D-LA were detected using reagents Box of (China) Nanjing Jiancheng Institute of Bioengineering.

2.5.6 Observation of Intestinal Tissue Morphology

After the broilers were euthanized, intestinal samples of about 1 cm from the duodenum, jejunum, and ileum were taken and stored in a 4% formaldehyde solution for tissue section preparation. The contents of the duodenum, jejunum, ileum mucosa, and cecum were collected with centrifuge tubes. The samples were quickly immersed in liquid nitrogen for quick freezing and then stored at -80 ℃ for subsequent testing. The duodenum, jejunum, and ileum tissues fixed in 4% formaldehyde solution are washed, dehydrated, waxed, embedded, sectioned, patched, etc., and then stained with hematoxylin and eosin. Observe the morphology of the intestinal tissue under an optical microscope, measure the intestinal villi height (VH) and crypt depth (CD), and calculate the villus height and crypt depth ratio (VH/CD).

2.5.7 qRT-PCR detection of intestinal tight junction protein gene

The total RNA in the sample was extracted according to the instructions of the Trizol kit. Use a microspectrophotometer to determine the concentration of total RNA (OD260/280 value between 1.8-2.0). The extracted RNA was reverse transcribed into cDNA using Takara Reverse Transcription Kit. Use Takara fluorescent quantitative kit

for fluorescent quantitative PCR detection. The reaction system is 20 μL The reaction system and procedure of fluorescent quantitative PCR are shown in Tables 2-4 and 2-5. The primers are all synthesized by Shanghai Shenggong Biological Co., Ltd. (Shanghai, China), and the specific information of the primers is shown in Table 2-6. The gene detected in this experiment uses the GAPDH gene as an internal reference, and the $2^{-\triangle\triangle CT}$ method is used to calculate the relative expression of the target gene.

Table 2-4 Reaction system

Reaction system	Volume
TB Green Premix Ex Taq II	10 μL
PCR forward primer (10 Mm)	0.8 μL
PCR reverse primer (10 Mm)	0.8 μL
ROX Reference Dye	0.4 μL
Template[c]	2 μL
RNase Free Water	20μL

Table 2-5 Reaction procedures

Program	Cycle	Temperature	Time
predegeneration	1	95℃	30 s
PCR reaction	40	95℃	5 s
		60℃	34 s

Table 2-6 Primer sequences used for the quantitative real-time PCR

Gene	Sequence	Product size (bp)
Occludin	F: GATGGACAGCATCAACGACC R: CTTGCTTTGGTAGTCTGGGC	142
Claudin1	F: ACACCCGTTAACACCAGATTT R: GCATTTTTGGGGTAGCCTCG	152
ZO-1	F：TC GTCGCATTGTTGAGTCTGA	129

	R：TATAGCGTGTCCACAACCCG	
	R: GGGGCCTTGTGAGGAATGTT	
MUC2	F: ATTGAAGCCAGCAATGGTGT R: TGACATCAGGGCACACAGAT	214
SOD1	F: AAATGGGTGTACCAGCGCA R: CTTTGCAGTCACATTGCCGA	109
SOD2	F: GAGGGGAGCCTAAAGGAGAAT R: TCTTGATTTGCACAGGCTGC	175
GSH-PX1	F: TCACCATGTTCGAGAAGTGC R: ATGTACTGCGGGTTGGTCAT	124
	F: CTTCAGGGGTAGCAAGGTATGA	
Nrf2	R：TTCCCAGTTCGGTGCAGAAG	169
	R: AGTCCACAACACGGTTGCTGTAT	

2.5.8 Analysis of microbial abundance of cecal contents

Four treatments were selected: CON group, ANT group, AE group, and GE group. For each treatment, 4 duplicate samples of cecal contents were selected. The high-throughput sequencing method was used to analyze the microbial abundance of the cecal contents, and the sequencing was completed by Parsonox Biotechnology Co., Ltd. (Shanghai, China). Extract DNA from the cecum content, use the Illumina platform to sequence paired-end DNA fragments in the V3~V4 region, and use the DADA2 method (Callahan et al., 2016) to perform depriming, quality filtering, and denoising (denoise), splicing and de-chimerism. Analysis software: QIIME2 (2019.4). Analysis steps: first call the qiime cutadapt trim-paired excision sequence primer fragment, discard the unmatched primer sequence, then use qiime dada2 denoise-paired to call DADA2 for quality control, denoising, splicing, and dechimerism. Primer information: F: ACTCCTACGGGAGGCAGCA R: CGGACTACHVGGGT.

2.6 Data Statistics and Analysis

Test data use SPSS 26.0 software for one-way analysis of variance (one-way ANOVA), using Duncan method to perform multiple comparisons, and the results were expressed as mean and standard error, and P <0.05 was used as the criterion for judging the significance of the difference. The bar graph was drawn with GraphPad 8.0.

SECTION 3 RESULTS AND ANALYSIS

3.1 The effects of *Astragalus* extract and *Glycyrrhiza* extract on broiler performance

The effects of AE and GE on the performance of broilers in the early growth period (1-21d) are shown in Table 2-7. Compared with the CON group, the average weight and ADG of the GE group and the AE+GE II group at 21d were significantly higher ($P<0.05$), and the difference between the AE group and the AE+GE I group was not significant ($P>0.05$). Compared with the ANT group, the difference between the groups added with plant extracts was not significant ($P>0.05$). Compared to the groups added with plant extracts, the 21d average weight and ADG of the AE+GE II group were significantly higher than that of the AE group ($P<0.05$). The difference between the other groups was not significant ($P>0.05$). There was no significant difference between ADFI and FCR in each group ($P>0.05$). The effect of AE and GE on the production performance of broilers in the late growth period (22-42d) showed that compared with the CON group, the body weight on 42 days of the AE group, the GE group, the AE+GE I group, and the AE+GE II group increased significantly ($P<0.05$), the ADG of the AE group and the AE+GE II group increased significantly ($P<0.05$), The difference between GE group and AE+GE I group was not significant ($P>0.05$). Compared with the ANT group, the difference between the groups added with plant extracts was not significant ($P>0.05$). In the comparison between the groups added with plant extracts, the 42d average weight and ADG of the AE+GE II group were significantly higher than those of the GE group ($P<0.05$), and the difference between the other groups was not

significant (P>0.05). There was no significant difference between ADFI and FCR in each group (P>0.05). The effect of AE and GE on the production performance of broilers during the whole period (1-42d) showed that compared with the CON group, 42d ADG of AE group, GE group, AE+GE I group, AE+GE II group significantly increased(P<0.05), FCR significantly decreased (P<0.05). Compared with the ANT group, there was no significant difference between the groups added with plant extracts (P>0.05). The ADG of the AE+GE II group was significantly higher than that of the GE group (P<0.05), and the difference between the other groups added with plant extracts was not significant (P>0.05).

Table 2-7 Effects of AE and GE on Performance of Broilers

Items	CON	ANT	AE	GE	AE+GE I	AE+GE II	SEM	*P*-value
Day1-21								
Initial weight(g)	46.18	46.27	46.63	46.07	46.20	45.97	0.18	0.79
21day weight(g)	841.67^{c}	866.27^{abc}	853.42^{bc}	874.12^{ab}	870.14^{abc}	885.78^{a}	5.23	0.04*
ADFI (g/d)	48.10	50.19	47.17	47.29	46.56	47.24	0.75	0.47
ADG (g/d)	37.88^{c}	39.05^{abc}	38.42^{bc}	39.43^{ab}	39.23^{abc}	39.99^{a}	0.28	0.03*
FCR (g ∶g)	1.27	1.29	1.23	1.20	1.19	1.18	0.02	0.35
Day22-42								
42day weight(g)	2488.77^{c}	2578.15^{ab}	2580.35^{ab}	2567.63^{b}	2585.83^{ab}	2657.41^{a}	13.44	＜0.01*
ADFI (g/d)	142.08	138.68	141.80	139.14	142.55	148.05	1.51	0.27
ADG (g/d)	78.43^{c}	81.52^{abc}	82.23^{ab}	80.64^{bc}	81.70^{abc}	84.36^{a}	0.56	0.03*
FCR (g ∶g)	1.81	1.70	1.72	1.73	1.75	1.76	0.02	0.34
Day1-42								
ADFI (g/d)	95.09	94.43	94.48	93.22	94.55	97.64	0.71	0.30
ADG (g/d)	58.16^{c}	60.28^{ab}	60.33^{ab}	60.04^{b}	60.47^{ab}	62.18^{a}	0.33	＜0.01*
FCR (g ∶g)	1.64^{a}	1.57^{b}	1.57^{b}	1.55^{b}	1.57^{b}	1.57^{b}	0.01	0.047

Note: The data in the same line marked with different lowercase letters indicate significant differences, $P<0.05$; the marked with the same lowercase letters indicates that the differences are not significant, $P>0.05$, the same in the table below.

3.2 The effect of *Astragalus* extract and *Glycyrrhiza* extract on the apparent metabolic rate of nutrients in broilers

The effects of AE and GE on the apparent metabolic rate of nutrients in broilers are shown in Table 2-8. Compared with the CON group, the apparent metabolic rate of energy in the AE group, the GE group, and the AE+GE II group were significantly increased ($P<0.05$), and the difference in the AE+GE I group was not significant ($P>0.05$). The CP apparent metabolic rate in the GE group was significantly increased ($P<0.05$). Compared with the ANT group, the difference between the groups added with plant extracts was not significant ($P>0.05$). There was no significant difference between the groups added with plant extracts ($P>0.05$). The apparent metabolic rates of EE, Ca and P in each group were not significantly different ($P>0.05$).

Table 2-8 Effects of AE and GE on Apparent Metabolism Rate of Nutrients in Broilers （%）

Items	CON	ANT	AE	GE	AE+GE I	AE+GE II	SEM	*P*-value
Energy	64.15^{b}	74.05^{a}	71.07^{a}	74.81^{a}	69.02^{ab}	71.18^{a}	0.64	0.03*
CP	46.20^{b}	49.98^{ab}	50.63^{ab}	54.25^{a}	50.33^{ab}	52.36^{ab}	0.61	0.01*
EE	70.95	75.97	76.63	75.72	72.18	78.29	0.46	0.22
Ca	40.20	47.03	47.61	46.27	43.32	48.49	0.56	0.13
P	42.74	45.31	42.88	46.30	45.05	50.64	0.59	0.20

3.3 The effects of *Astragalus* extract and *Glycyrrhiza* extract on the meat quality of broilers

The effects of AE and GE on the meat quality of broilers are shown in Table 2-9. Compared with the CON group, the pH of the AE group and the GE group increased significantly ($P<0.05$), and the difference between the AE+GE I group and AE+GE II group was not significant ($P>0.05$), the shear force and drip loss of the AE+GE II group was significantly reduced ($P<0.05$). Compared with the ANT group, the pH of the AE group and the GE group was significantly increased at 45 min ($P<0.05$), and the difference between the AE+GE I group and AE+GE II group was not significant ($P>0.05$). The pH45min of the AE group and the GE group was significantly higher than that of the AE+GE I group and the AE+GE II group ($P<0.05$). The difference between the AE group and the GE group was not significant ($P>0.05$). There was no significant difference between AE+GE I group and AE+GE II group ($P>0.05$). The pH24h, L, a*, and b* values between each group had no significant difference ($P>0.05$).

Table 2-9 The effect of AE and GE on the quality of broiler chicken

Items	CON	ANT	AE	GE	AE+GE I	AE+GE II	SEM	*P*-value
pH_{45min}	6.22^{b}	6.22^{b}	6.88^{a}	6.89^{a}	6.49^{b}	6.23^{b}	0.05	＜0.01*
pH_{24h}	5.81	5.90	5.82	5.92	5.74	5.82	0.02	0.10
L	51.81	52.60	49.91	47.77	46.34	49.50	0.63	0.22
a*	6.81	6.30	4.90	4.76	5.68	6.92	0.24	0.18
b*	8.53	6.34	6.71	6.30	4.33	8.64	0.53	0.36
Shear force	27.13^{a}	26.64^{ab}	24.77^{ab}	26.19^{ab}	26.38^{ab}	24.33^{b}	0.37	0.04*
Drip loss	1.61^{a}	1.07^{ab}	1.36^{ab}	1.44^{ab}	1.38^{ab}	0.95^{b}	0.07	0.04*

3.4 Effects of *Astragalus* Extract and *Glycyrrhiza* Extract on Fatty Acid Content in Broiler Muscles

The effects of AE and GE on the fatty acid content in breast muscles of broilers are shown in Table 2-10. Compared with the CON group, the SFA of the GE group was significantly reduced ($P<0.05$), and the USFA content of the AE group, GE group, AE+GE I group, AE+GE II group was significantly increased ($P<0.05$), The MUFA of AE+GE II group increased significantly ($P<0.05$), the PUFA of AE group, GE group, and AE+GE group II increased significantly ($P<0.05$), and the EFA of group AE and GE increased significantly ($P<0.05$). Compared with the ANT group, USFA and MUFA in the AE+GE II group were significantly increased ($P<0.05$), but the difference in SFA, PUFA, and EFA was not significant ($P>0.05$). The SFA of the AE+GE I group and the AE+GE II group was significantly higher than that of the AE group and the GE group, and the MUFA of the AE+GE II group was significantly higher than that of the AE group and the GE group ($P< 0.05$), the PUFA and EFA of the GE group were significantly higher than those of the AE+GE I group, and the EFA and USFA between each group were not significantly different ($P>0.05$).

Table 2-10 Effects of AE and GE on Fatty Acid Content in Breast Muscles of Broilers

Items	CON	ANT	AE	GE	AE+GE I	AE+GE II	SEM	*P*-value
C16:0	18.92^{ab}	19.08^{ab}	19.44^{a}	18.23^{b}	19.77^{a}	20.01^{a}	0.23	0.02*
C16:1	1.28^{b}	1.43^{ab}	1.56^{a}	1.43^{ab}	1.41^{ab}	1.60^{a}	0.02	0.04*
C18:0	9.53^{abc}	8.82^{abc}	8.30^{bc}	8.04^{c}	10.11^{a}	9.59^{ab}	0.15	0.04*
C18:1n9c	22.25^{b}	23.51^{a}	23.69^{a}	24.13^{a}	23.00^{ab}	24.07^{a}	0.18	0.03*
C18:2n6	33.52^{c}	35.74^{abc}	36.51^{ab}	38.02^{a}	34.77^{bc}	35.63^{abc}	0.28	0.02*
C18:3n3	2.09^{c}	2.50^{abc}	2.78^{a}	2.68^{ab}	2.27^{bc}	2.28^{bc}	0.07	0.03*
C20:2	0.86^{bc}	0.73^{c}	0.88^{bc}	0.78^{c}	1.12^{ab}	1.20^{a}	0.05	0.02*
C22:0	0.64^{ab}	0.55^{bc}	0.46^{bc}	0.45^{c}	0.58^{bc}	0.75^{a}	0.03	0.01*
C20:3n6	034	0.31	0.38	0.30	0.30	0.40	0.01	0.16
C22:1n9	5.86	5.08	4.21	4.11	6.18	6.49	0.24	0.15
C24:1	1.30	1.19	1.12	1.08	1.56	1.68	0.05	0.09
C22:6n3	0.77^{ab}	0.63^{ab}	0.51^{b}	0.47^{b}	1.05^{a}	1.0^{a}	0.06	0.03*
SFA	29.09^{ab}	28.45^{abc}	28.20^{bc}	26.73^{c}	30.45^{a}	30.34^{ab}	0.32	0.01*
USFA	68.24^{c}	71.12^{b}	71.62^{ab}	72.98^{ab}	71.64^{ab}	74.33^{a}	0.33	＜0.01*
MUFA	30.68^{b}	31.21^{b}	30.58^{b}	30.74^{b}	32.14^{ab}	33.83^{a}	0.36	0.02*
PUFA	37.57^{c}	39.91^{abc}	41.05^{ab}	42.24^{a}	39.50^{bc}	40.50^{ab}	0.24	0.02*
EFA	35.61^{c}	38.24^{abc}	39.28^{ab}	40.69^{a}	37.04^{bc}	37.91^{abc}	0.34	0.01*

3.5 Effects of *Astragalus* Extract and *Glycyrrhiza* Extract on Immune Organ Indexes of Broilers

The effects of AE and GE on the immune organ indexes of broilers are shown in Table 2-11. Compared with the CON group, the thymus index of the AE+GE Ⅰ group and the AE+GE Ⅱ group was significantly increased ($P<0.05$), and the spleen index of the AE+GE Ⅱ group was significantly increased ($P<0.05$). Compared with the ANT group, the thymus index of the AE+GE Ⅰ group was significantly increased ($P<0.05$). The thymus index of the AE+GE Ⅰ group was significantly higher than that of the AE group ($P<0.05$), and the spleen index of the AE+GE Ⅱ group was significantly higher than that of the AE group ($P<0.05$). The bursal index of each group was not significantly different ($P>0.05$).

Table 2-11 Effects of AE and GE on Immune Organ Indexes of Broilers

Items	CON	ANT	AE	GE	AE+GE I	AE+GE II	SEM	*P*-value
Thymus Index	0.16^{c}	0.16^{bc}	0.17^{bc}	0.18^{abc}	0.20^{a}	0.19^{ab}	0.02	0.04*
Spleen index	0.13^{c}	0.18^{ab}	0.15^{bc}	0.16^{abc}	0.15^{bc}	0.20^{a}	0.01	0.02*
Bursal Index	0.06	0.06	0.06	0.06	0.05	0.05	0.02	0.66

3.6 Effects of *Astragalus* Extract and *Glycyrrhiza* Extract on Broiler's Tracheal Cilia and Lung Structure

The effects of *Astragalus* extract and *Glycyrrhiza* extract on the status of broiler tracheal cilia are shown in Figure 2-1. The cilia in the CON group are sparsely arranged, and the ANT group, AE group, GE group, AE+GE I group and AE+GE II group are

arranged evenly and densely. As shown in Figure2- 2, the effects of *Astragalus* extract and *Glycyrrhiza* extract on the lung structure of broilers. The alveoli in the CON group were relatively intact, with a small amount of inflammatory exudation in the alveoli, and the capillaries around the alveoli were congested. The alveoli in the ANT group, AE group, GE group, AE+GE I group and AE+GE II group were intact, and there were no alveoli inflammatory exudation, no congestion of the capillaries around the alveoli.

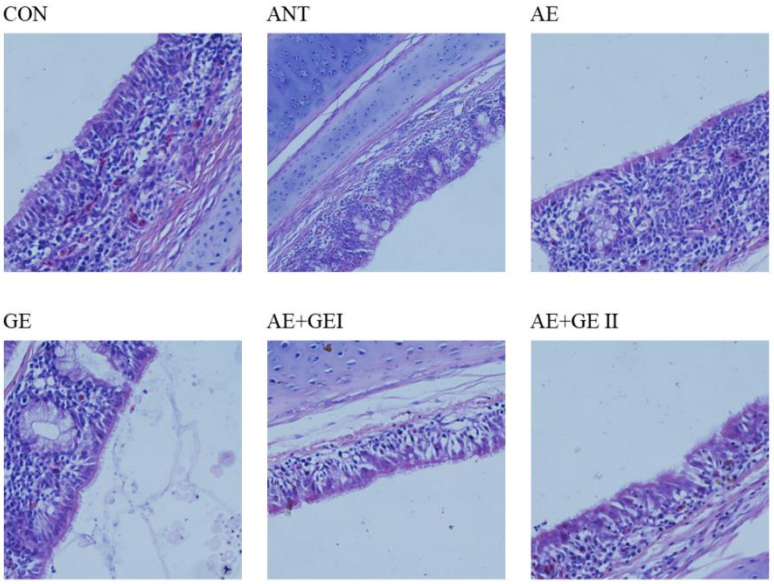

Fig.2-1 Effects of AE and GE on tracheal cilia state of broilers

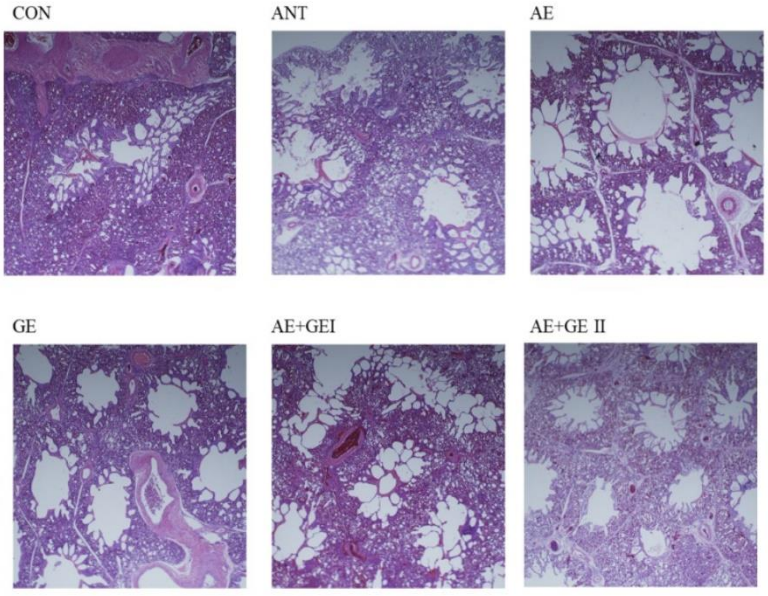

Fig. 2-2 Effects of AE and GE on lung structure of broilers

3.7 Effects of *Astragalus* Extract and *Glycyrrhiza* Extract on Serum Immune Indexes of Broilers

The effects of *Astragalus* extract and *Glycyrrhiza* extract on serum immune indexes of broilers are shown in Figure 2-3. Compared with CON group, serum IgA of AE+GE I group and AE+GE II group significantly increased ($P<0.05$), serum IgM of GE group and AE+GE II group significantly increased ($P<0.05$), Serum IgG of GE group and AE+GE I group was significantly increased ($P<0.05$). Compared with the ANT group, the serum IgM of the AE+GE I group was significantly reduced ($P<0.05$), the serum IgG of the AE group was significantly increased ($P<0.05$). The serum IgG of the AE group was significantly higher than that of the GE group, AE+GE I group and AE+GE II group ($P<0.05$).

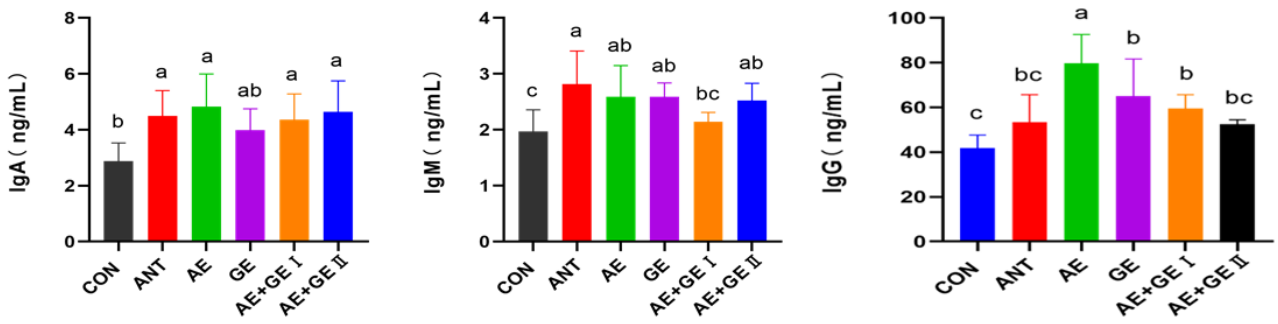

Fig. 2-3 Effects of Astragalus Extract and Glycyrrhiza Extract on Serum Immune Indexes of Broilers

3.8 Effects of *Astragalus* Extract and *Glycyrrhiza* Extract on Antioxidant Function of Broiler's Serum and Trachea

The effects of AE and GE on the antioxidant properties of broiler serum and trachea are shown in Figure 2-4 (A). Compared with the CON group, T-AOC content in the AE group, SOD content in GE group, GSH-Px content in AE+GE I group, and AE+GE II group were significantly increased ($P<0.05$), the MDA content of AE group and GE group was significantly reduced ($P<0.05$). Compared with the ANT group, the GSH-Px content of the AE group, the GE group, the AE+GE I group, and the AE+GE II group was significantly increased ($P<0.05$). In addition, the T-AOC content of the AE group and the SOD content of the GE group increased significantly ($P<0.05$). The MDA content of the AE group and GE group was significantly reduced ($P<0.05$). The SOD content of the GE group was significantly higher than that of the AE+GE I group ($P<0.05$), and the MDA content of the AE group and the GE group was significantly lower than that of the AE+GE I group and AE+GE II Group ($P<0.05$). As shown in Figure 3-4 (B) for trachea, compared with the CON group, the T-AOC content of the AE group and the GE group, the SOD content of the AE group, the GE group, the AE+GE I group, and the AE+GE II group, the GSH-Px content of the GE group and the AE+GE II group were significantly increased ($P<0.05$), and the MDA content of the AE+GE II group was significantly decreased ($P<0.05$). Compared with the ANT group, the GSH-Px content of the AE+GE II group was significantly increased ($P<0.05$). The T-AOC content of the AE group and the GE group was significantly higher than that of the AE+GE I group ($P<0.05$), and the GSH-Px content of the AE+GE II group was significantly higher than that of the AE group, GE group and AE+GE I group ($P<0.05$),

and the MDA content of AE+GE II group was significantly lower than that of AE group and AE+GE I group ($P<0.05$).

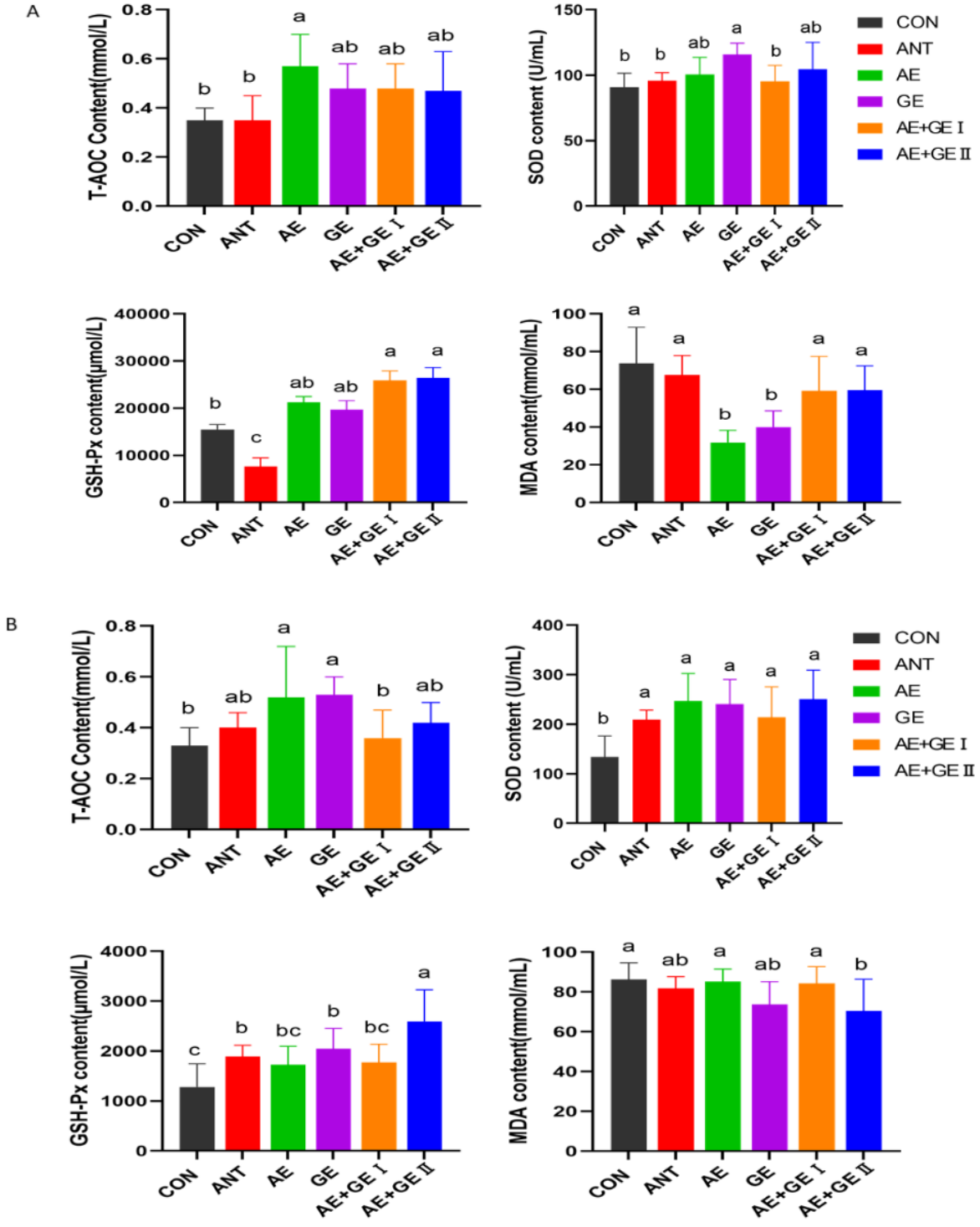

Fig. 2-4（A-B） Effects of AE and GE on Antioxidant Function of Broiler's Serum and Trachea

3.9 Effects of *Astragalus* Extract and *Glycyrrhiza* Extract on Serum and Lung Inflammatory Factors of Broiler's

The effects of AE and GE on serum inflammatory factors in broilers are shown in Figure 2-5(A). Compared with the CON group, AE group, GE group, AE+GE I group, and AE+GE II group serum TNF-α and IL-6 were significantly reduced ($P<0.05$). in addition, the AE group, GE group, and AE+GE II group serum IL-1β significantly decreased ($P<0.05$). Compared with the ANT group, the serum IL-1β of the AE+GE I group was significantly increased ($P<0.05$). The serum IL-1β of the AE+GE I group was significantly higher than that of the other groups ($P<0.05$), and the serum IFN-γ of each group was not significantly different ($P>0.05$). As shown in Figure 2-5(B), compared with the CON group, the levels of IL-1β in the lungs of the GE group and the AE+GE II group, and the content of IL-6 in the lungs of the GE group and the AE+GE II group were significantly reduced ($P<0.05$). There was no significant difference between the ANT group and the groups added with plant extracts ($P>0.05$). There was no significant difference between the groups added with plant extracts ($P>0.05$). In addition, the difference between TNF-α and IFN-γ in the lungs of each group was not significant ($P>0.05$).

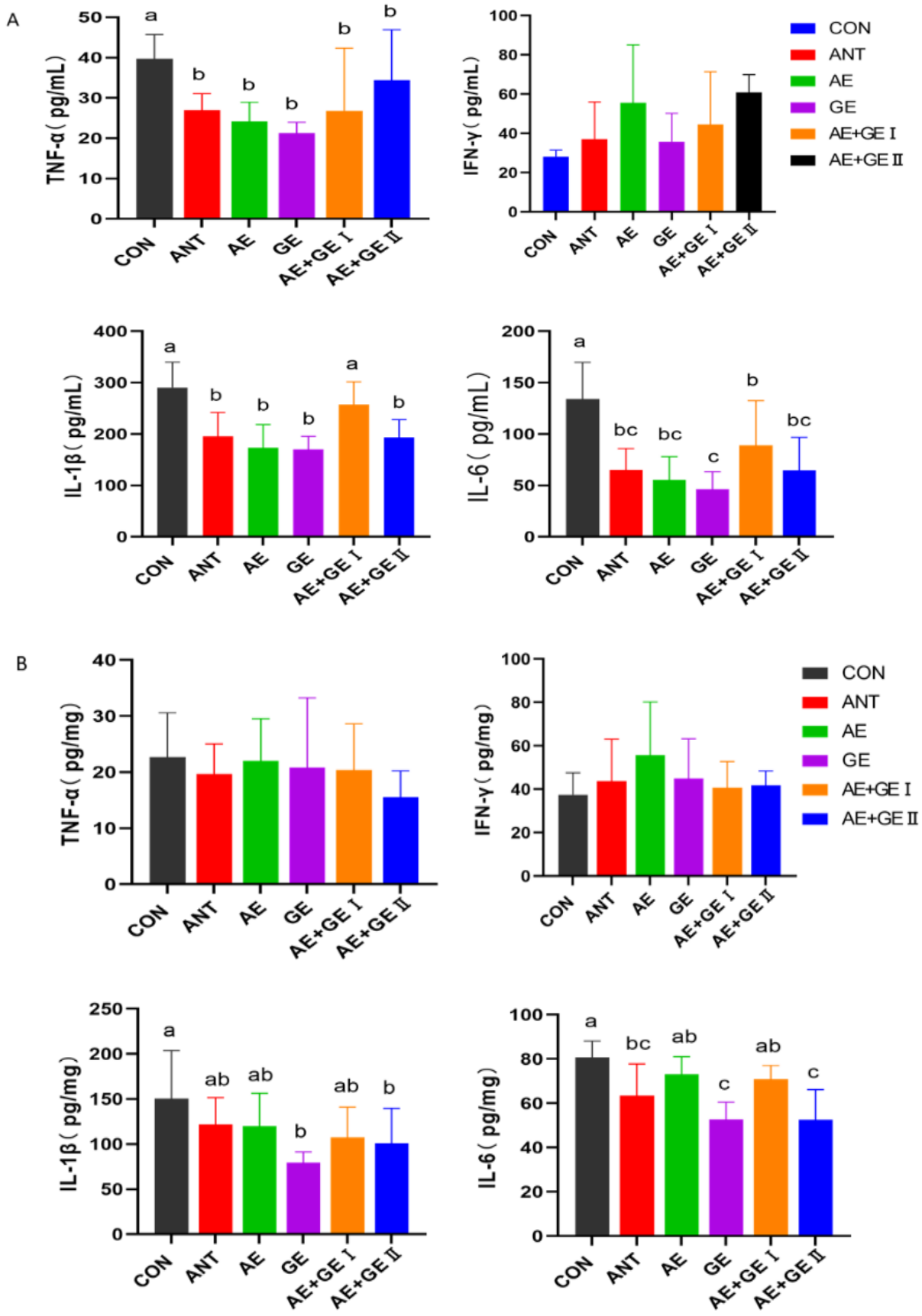

Fig.2-5（A-B） Effects of AE and GE on Serum and Lung Inflammatory Factors

in Broilers

3.10 Effects of *Astragalus* Extract and *Glycyrrhiza* Extract on Intestinal Mucosal Structure of Broilers

The effects of AE and GE on the structure of intestinal villi of broilers are shown in Table 2-11 and Figure 2-6. Compared with the CON group, the VH and VH/CD of the duodenum in the AE group, GE group, AE+GE I group, and AE+GE II group were significantly increased ($P<0.05$) . Compared with the ANT group, the VH and VH/CD of the duodenum in the AE+GE II group were significantly increased ($P<0.05$). The VH of the duodenum in the AE+GE II group was significantly higher than that of the other groups ($P<0.05$), and the difference in the CD of the duodenum in each group was not significant ($P>0.05$). Compared with the CON group, the VH/CD of the jejunum of the GE group, AE+GE I group, and AE+GE II group were significantly higher ($P<0.05$). Compared with the ANT group, the VH/CD of the jejunum of the GE group was significantly higher ($P<0.05$). The CD of the jejunum in each group was not significantly different ($P>0.05$). Compared with the CON group, AE group, GE group, AE+GE I group, and AE+GE II group ileum VH was significantly increased ($P<0.05$). There was no significant difference between the CD and VH/CD of the ileum in each group ($P>0.05$).

Table 2-11 Effects of AE and GE on the structure of intestinal villi in broilers

Items	CON	ANT	AE	GE	AE+GE I	AE+GE II	SEM	*P*-value
Duodenum								
VH	1378.24^{c}	1957.11^{b}	2112.92^{b}	2011.39^{b}	2052.53^{b}	2472.51^{a}	14.56	＜0.01
CD	412.94	399.56	396.67	331.72	307.93	307.82	3.55	0.06
VH/CD	3.40^{c}	5.09^{bc}	5.62^{ab}	6.30^{ab}	6.89^{ab}	8.50^{a}	0.05	＜0.01
Jejunum								
VH	1165.01^{b}	1265.67^{ab}	1268.98^{ab}	1435.09^{a}	1347.80^{a}	1323.20^{ab}	18.64	0.03*
CD	300.92	282.78	237.90	235.15	212.04	229.02	6.56	0.08
VH/CD	3.95^{c}	4.54^{bc}	5.53^{abc}	6.87^{a}	6.36^{ab}	6.39^{ab}	0.12	0.04*
Ileum								
VH	842.29^{b}	1128.26^{a}	1053.82^{a}	1019.04^{a}	998.42^{a}	993.91^{a}	16.74	0.01*
CD	295.45	283.26	222.67	215.83	233.06	198.77	4.11	0.08
VH/CD	2.92	4.08	5.19	5.14	4.82	5.45	0.13	0.11

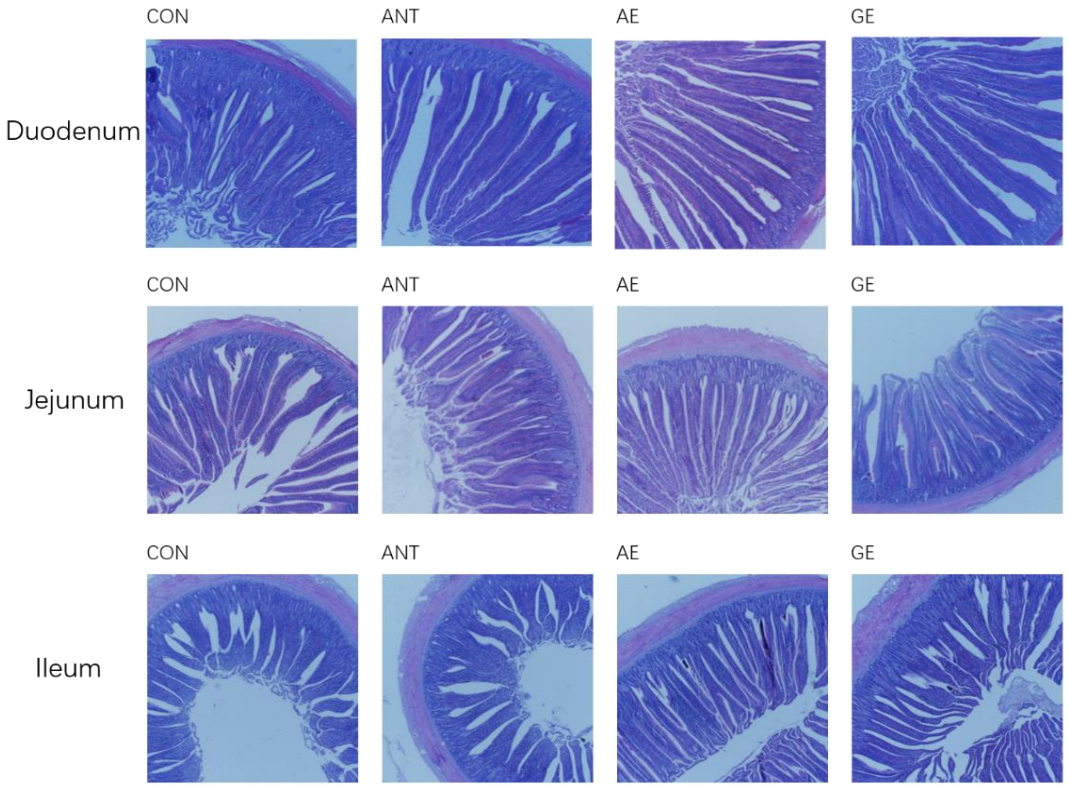

Fig. 2-6 Intestinal villi sections of each group

3.11 Effects of *Astragalus* Extract and *Glycyrrhiza* Extract on Serum DAO and D-Lactate in Broilers

The effects of AE and GE on serum DAO and D-LA in broilers are shown in Figure 2-7 (A-B). Compared with the CON group, the serum DAO of each group added with plant extracts was significantly reduced, and the serum D-LA of the AE+GE I group and AE+GE II group was significantly reduced ($P<0.05$). Compared with the ANT group, the serum DAO and D-LA of the AE+GE I group and the AE+GE II group were significantly reduced ($P<0.05$). The serum DAO and D-LA of the AE+GE I and AE+GE II groups were significantly lower than those of the AE and GE groups

(P＜0.05). There was no difference between the AE and GE groups (P>0.05), AE+GE I and AE+GE II group (P>0.05).

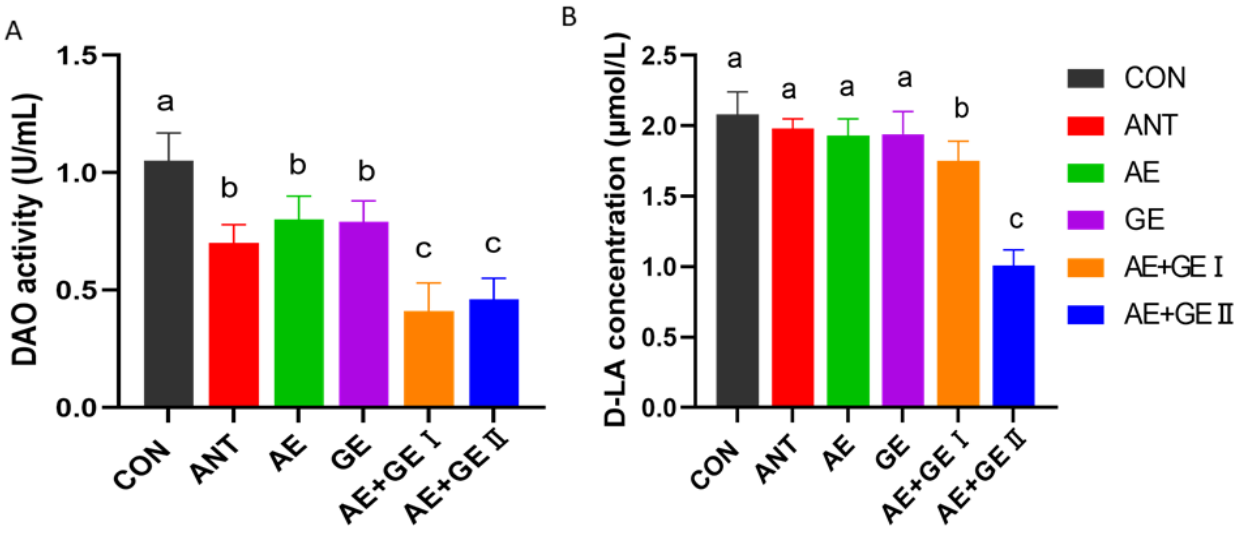

Fig. 2-7（A-B） Effects of AE and GE on Serum DAO and D-LA in Broilers

3.12 Effects of *Astragalus* Extract and *Glycyrrhiza* Extract on the Expression of Tight Junction Protein Related Genes in the Intestine and Respiratory Tract of Broilers

The effects of AE and GE on the expression of tight junction protein-related genes in broilers are shown in Figure 2-8. Compared with the CON group, the expression of *Occludin*, *Claudin1*, *ZO-1* mRNA of the duodenum in each group was significantly up-regulated (P<0.05). Compared with ANT group, *Claudin1* mRNA expression of the duodenum in GE group and AE+GE II group was significantly up-regulated (P<0.05), and *Occludin* mRNA expression in AE group was significantly down-regulated (P<0.05). The expression of *Occludin* mRNA of the duodenum in the GE group, the AE+GE I group, and the AE+GE II group was significantly higher than that in the AE

group ($P<0.05$), and the Claudin1 and *ZO-1* mRNA expression of the duodenum between each group were not significant ($P>0.05$). Compared with the CON group, the expression of *Occludin* mRNA of the jejunum in the GE group, the AE+GE I group and the AE+GE II group was significantly up-regulated ($P<0.05$), and the expression of *Claudin1* mRNA of the duodenum in the AE group, the GE group, the AE+GE I group and AE+ GE II group was significantly up-regulated ($P<0.05$), and the expression of *ZO-1* mRNA in the AE+GE I group and AE+GE II group was significantly up-regulated ($P<0.05$). There was no significant difference between the ANT group and the groups added with plant extracts ($P>0.05$). The expression of *Occludin* and *ZO-1* mRNA in the AE+GE II group and the expression of *Occludin* in the AE+GE I group were significantly higher than those in the AE group ($P<0.05$). Compared with the CON group, the expression of *Occludin* and *Claudin1* mRNA of the ileum in the groups added with plant extracts was significantly up-regulated ($P<0.05$). in addition, the expression of *ZO-1* mRNA in the GE group and the AE+GE II group was significant up-regulated ($P<0.05$). The *ZO-1* mRNA expression in the ANT group was significantly higher than that in the AE group ($P<0.05$), and there was no difference between plant extract groups ($P>0.05$). The expression of Claudin1 and *ZO-1* mRNA in the GE group and the AE+GE II group was significantly higher than that in the AE group ($P<0.05$). Compared with the CON group, the expression of *Occludin*, *Claudin1* and *ZO-1* mRNA of the trachea in each group added with plant extracts was significantly up-regulated ($P<0.05$). Compared with the ANT group, the expression of *ZO-1* mRNA of the trachea in each group with plant extracts, and the expression of *Occludin* mRNA in the GE group and

AE+GE II group were significantly up-regulated (P<0.05). The expression of *Claudin1* mRNA of the trachea in the GE group was significantly higher than that in the AE group and AE+GE I group (P<0.05), while the other groups were not significantly different (P>0.05).

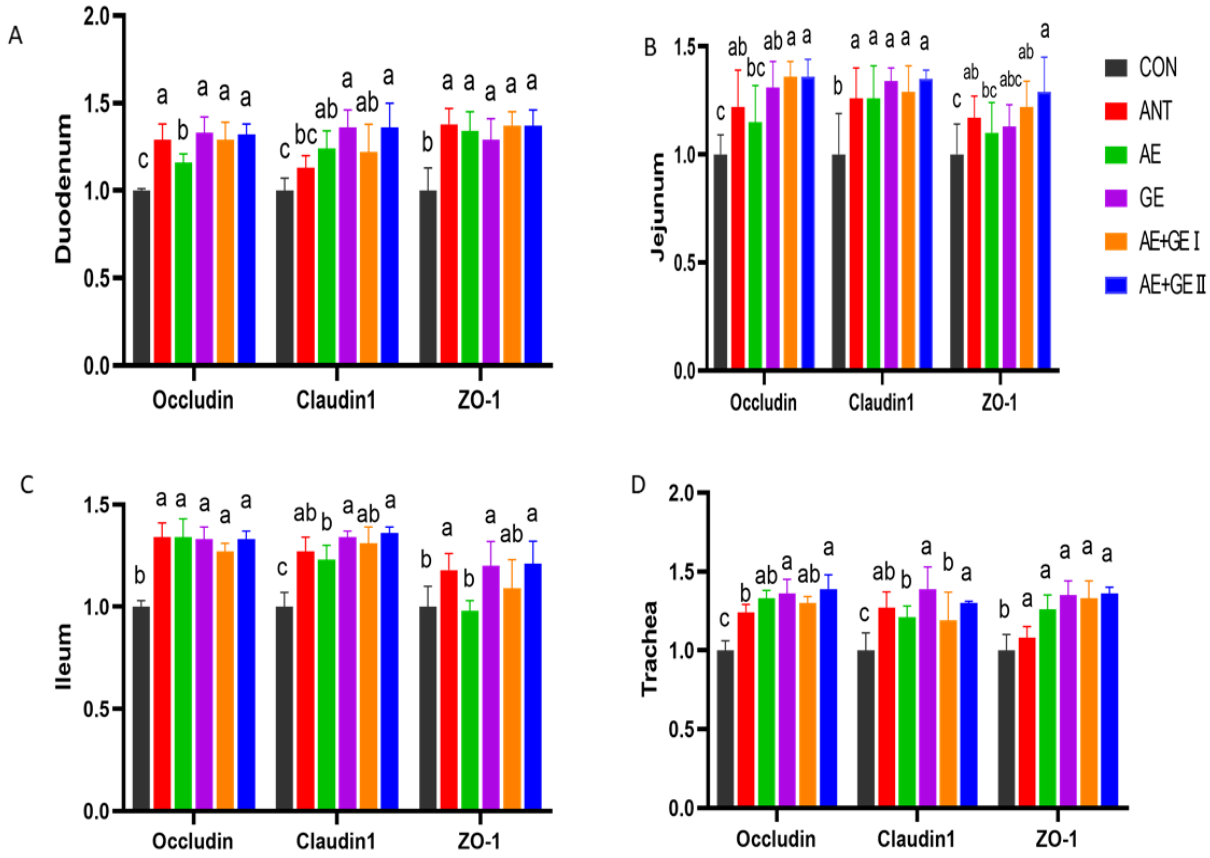

Fig. 2-8 Effects of AE and GE on the expression of tight junction protein-related genes in broilers

3.13 Effect of *Astragalus* Extract and *Glycyrrhiza* Extract on the Expression of Antioxidant Enzyme Related mRNA in Broilers

The effects of AE and GE on the expression of antioxidant enzyme-related mRNA in broilers are shown in Figure 2-9 (A-D). The results of comparative analysis of the

duodenum in each group see Figure 3-9 (A). Compared with the CON group, the expression of *SOD1, SOD2* and *GSH-Px* mRNA in each group with plant extracts was significantly up-regulated ($P<0.05$). Compared with the ANT group, the expression of *SOD2* and *GSH-Px* mRNA in each group added with plant extracts was significantly up-regulated ($P<0.05$). In addition, the expression of *SOD1* mRNA in the GE group and AE+GE II group was significantly up-regulated ($P<0.05$). *SOD1* mRNA expression in GE group and AE+GE II group was significantly higher than AE group and AE+GE I group ($P<0.05$), GE group, the expression of *SOD2* mRNA in AE+GE I group and AE+GE II group was significantly higher than that of the AE group ($P<0.05$). The results of comparative analysis of the jejunum in each group see Figure 3-9 (B). Compared with the CON group and the ANT group, the expression of *SOD1* and *GSH-Px* mRNA in each group added with plant extracts was significantly up-regulated ($P<0.05$), and the expression of *SOD2* mRNA in each group was not significant ($P>0.05$). The results of comparative analysis of the ileum in each group see Figure 3-9 (C). Compared with the CON group and the ANT group, the expression of *SOD1, SOD2* and *GSH-Px* mRNA in each group added with plant extracts was significantly up-regulated ($P<0.05$). There was no significant difference between the groups added with plant extracts ($P>0.05$). The results of comparative analysis of the trachea in each group see Figure 3-9 (D). Compared with the CON group and the ANT group, the expression of *SOD1, SOD2* and *GSH-Px* mRNA in each group added with plant extracts was significantly up-regulated ($P<0.05$). *SOD1* mRNA expression in GE group and AE+GE II group was significantly higher than that in AE group and AE+GE I group

($P<0.05$), There were no significant difference between each group for *SOD2* and *GSH-Px* mRNA expression ($P>0.05$).

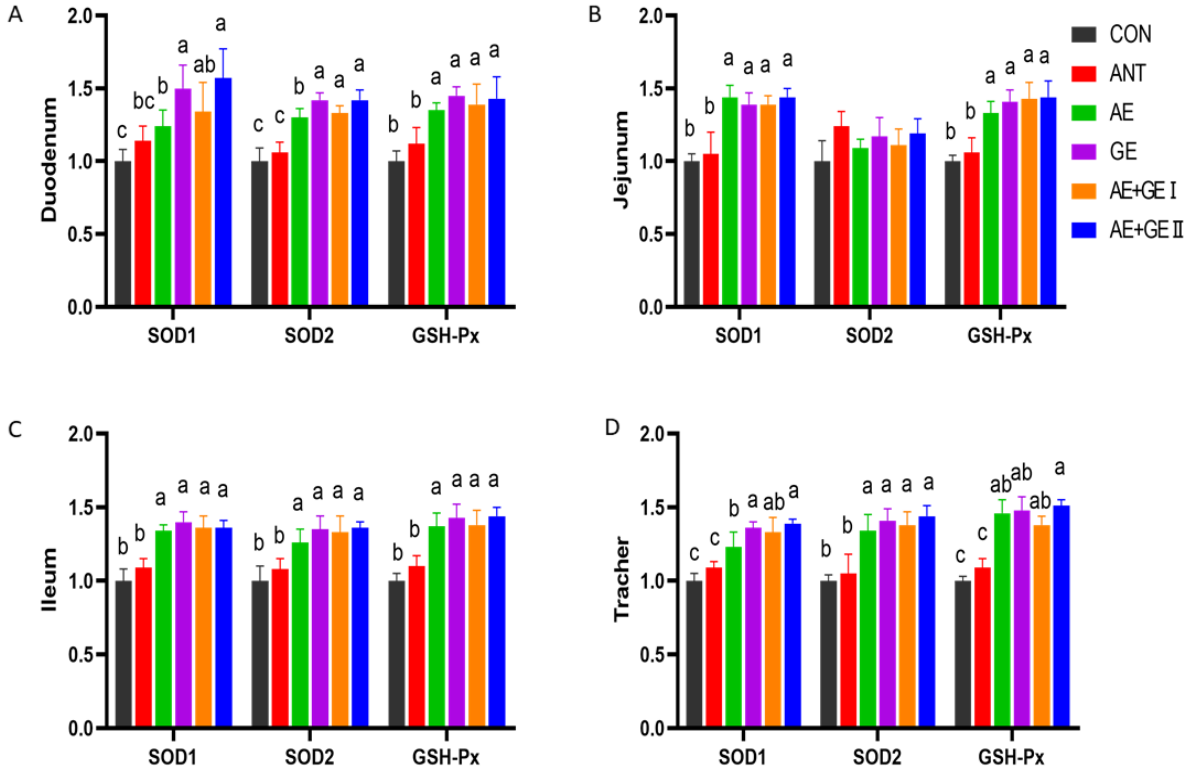

Fig. 2-9 （A-D）Effect of AE and GE on the Expression of Antioxidant Enzyme Related mRNA in Broiler Intestine

3.14 Effects of *Astragalus* Extract and *Glycyrrhiza* Extract on *MUC2* mRNA Expression in Intestine and Respiratory Tract of Broilers

The effects of AE and GE on the expression of *MUC2* mRNA in the intestine and respiratory tract of broilers are shown in Figure 2-10. Compared with the CON group, the expression of *MUC2* mRNA in the duodenum, jejunum and ileum of each group added with plant extracts was significantly up-regulated ($P<0.05$). Compared with ANT group, *MUC2* mRNA expression in duodenum and ileum of AE group was significantly

down-regulated (P<0.05). The expression of *MUC2* mRNA in the duodenum of the GE group, the AE+GE I group and the AE+GE II group was significantly higher than that of the AE group (P＜0.05), and the *MUC2* mRNA in the ileum of the AE+GE II group was significantly higher than that in the AE group (P＜0.05). Compared with the CON group, the expression of *MUC2* mRNA of trachea in each group added with plant extracts was significantly up-regulated (P<0.05).

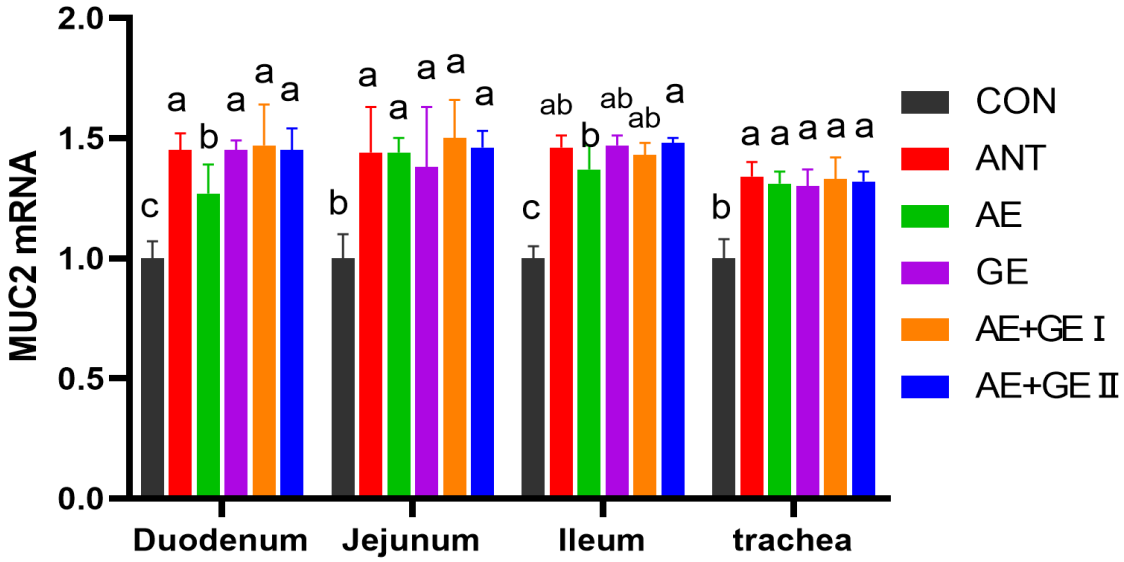

Fig. 2-10 Effects of AE and GE on MUC2 mRNA Expression in Intestines and Respiratory Tracts of Broilers

3.15 Effects of *Astragalus* Extract and *Glycyrrhiza* Extract on the Expression of *NRF2* mRNA in the Intestine and Respiratory Tract of Broilers

The effects of AE and GE on the expression of *NRF2* mRNA in the intestine and respiratory tract are shown in Figure 2-11. Compared with the CON group, the expression of *NRF2* mRNA in the duodenum, jejunum, ileum and trachea of each group

added with plant extracts was significantly up-regulated (P<0.05). Compared with the ANT group, the expression of *NRF2* mRNA in the trachea of the AE+GE II group was significantly up-regulated (P＜0.05). There was no significant difference between the groups added with plant extracts (P>0.05).

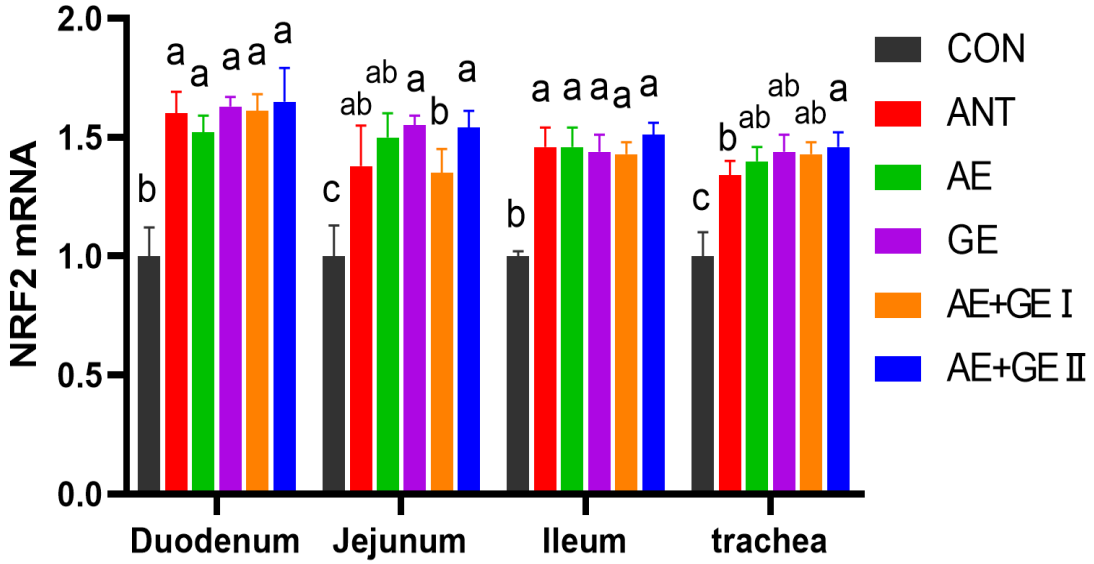

Fig. 2-11 Effects of AE and GE on the expression of NRF2 mRNA in the intestine and respiratory tract

3.16 Effects of *Astragalus* extract and *Glycyrrhiza* extract on the intestinal microbial diversity of broilers

The Illumina Miseq high-throughput sequencing platform separately sequenced the 16S rRNA gene V3-V4 regions of 16 cecal contents samples (4 samples for each of the CON, ANT, AE, and GE groups). After removing the incorrect chimeric sequences, a total of 668,375 high-quality reads were generated. On average, 41,773 sequences were obtained for each sample, with an average length of 416 bp. At the species level, a

total of 13,963 OTUs were identified using the 99% sequence similarity criterion. According to the Greengenes classification, they all belong to the bacterial domain. As shown in Figure 2-12, each group has 754 OUTs. The OTU of each group is ordered as GE group (4258)>AE group (3435)>ANT group (2768)>CON group (2748).

Fig. 2-12 Venn diagram analysis of cecum microbial species in broilers

3.16.1 Effects of *Astragalus* Extract and *Glycyrrhiza* Extract on Alpha Diversity of Cecal Microbes in Broilers

Alpha diversity is also called within-habitat diversity, which refers to the diversity within a specific area or ecosystem, including Chao1 index, Shannon index, Simpson index, Observed species index. Chao index and Observed species are used to evaluate the abundance of the flora. The larger the Chao or Observed species index, the higher the abundance of the community. Shannon and Simpson are used to evaluate the diversity of

the flora. The larger the Shannon value, the higher the community diversity. The larger the Simpson index value, the lower the community diversity. As shown in Table2-12, compared with the CON group, the Chao1 index, Shannon index and Observed species of the GE group were significantly increased (P<0.05), and the Shannon index of the AE group was significantly increased (P<0.05). Compared with the ANT group, the Chao1 index and Observed species of the GE group were significantly increased (P<0.05). The difference between the AE group and the GE group was not significant (P>0.05).

Table 2-12 Effects of AE and GE on Alpha Diversity of Cecal Microbes in Broilers

Items	CON	ANT	AE	GE	SEM	*P*-value
Chao1	1758.65^{b}	1838.56^{b}	1898.76^{ab}	2099.48^{a}	46.55	0.04
Shannon	7.88^{b}	8.1^{a}	8.21^{a}	8.09^{a}	0.03	0.02
Simpson	0.99	0.98	0.98	0.98	0.004	0.48
Observed - species	1613.98^{b}	1597.35^{b}	1775.28^{ab}	1926.23^{a}	47.78	0.02

As shown in Fig. 2-13 (A), the Shannon sparse curve reflects the index of microbial diversity in the sample. The microbial diversity index of each sample's sequencing volume at different sequencing depths is used to construct the curve to reflect the different levels of each sample. When the curve tends to be flat, it indicates that the amount of sequencing data is large enough to reflect most of the microbial species information in the sample. In this study, the cecum content curve tends to be flat,

indicating that the amount of sequencing data is large enough and the sequencing results are reasonable. And in the Shannon curve, the Shannon index of the AE group is the highest, indicating that the AE group has the highest microbial diversity. As shown in Fig.3-13(B), the OTU abundance grade curve is used to explain two aspects of sample diversity at the same time, that is, the abundance and uniformity of the species contained in the sample. In the OTU abundance level graph, the abscissa indicates the OTU number in descending order of the sequence number, and the ordinate indicates the relative abundance of the (OTU) species. The longer the span of the curve on the horizontal axis, the higher the abundance of the sample species, and the smoothness of the curve on the vertical axis represents the uniformity of the communities in the sample. As shown in Figure 3-13 (B), the GE group curve has the longest span on the horizontal axis, indicating that the GE group flora has

the highest abundance of species.

3.16.2 Effects of *Astragalus* Extract and *Glycyrrhiza* Extract on the Beta Diversity of Cecal Microbes in Broilers

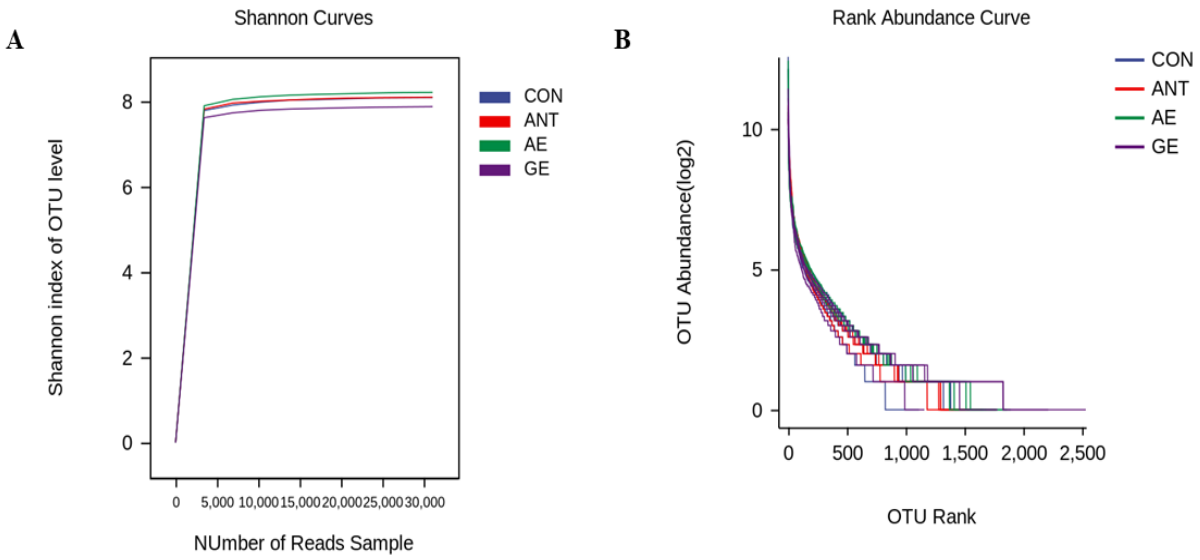

Fig.2-13（A-B）Dilution curves and Rank-Abundance curves

Beta diversity is used to analyze the similarity of the cecum flora among different groups. Among them, principal coordinate analysis (PCoA) Fig.2-14 (A) and non-metric multidimensional scaling analysis (NMDS) Fig.3-14 (B) based on Weighted UniFrac distance perform a comprehensive analysis of differences or similarities. PCoA is based on the selected distance matrix for mapping, sorts all the eigenvalues and eigenvectors, selects the most important eigenvalues in the top, and displays them in the coordinate system, comprehensively considering the effect of bacterial species on the overall flora Influence. NMDS represents a graph based on the value of evolution or quantitative distance matrix in a two-dimensional table. Each point in the figure represents a sample. The same color points represent the same group. The closer the distance between the two points, the smaller the difference in community composition between the two. The three groups of ANT group, AE group and GE group were completely separated from the CON group, indicating that the ANT group, AE group and GE group changed the flora structure. The ANT group, the AE group and the GE group have crossovers, indicating that the structure of the cecal microbial flora is similar among the three groups.

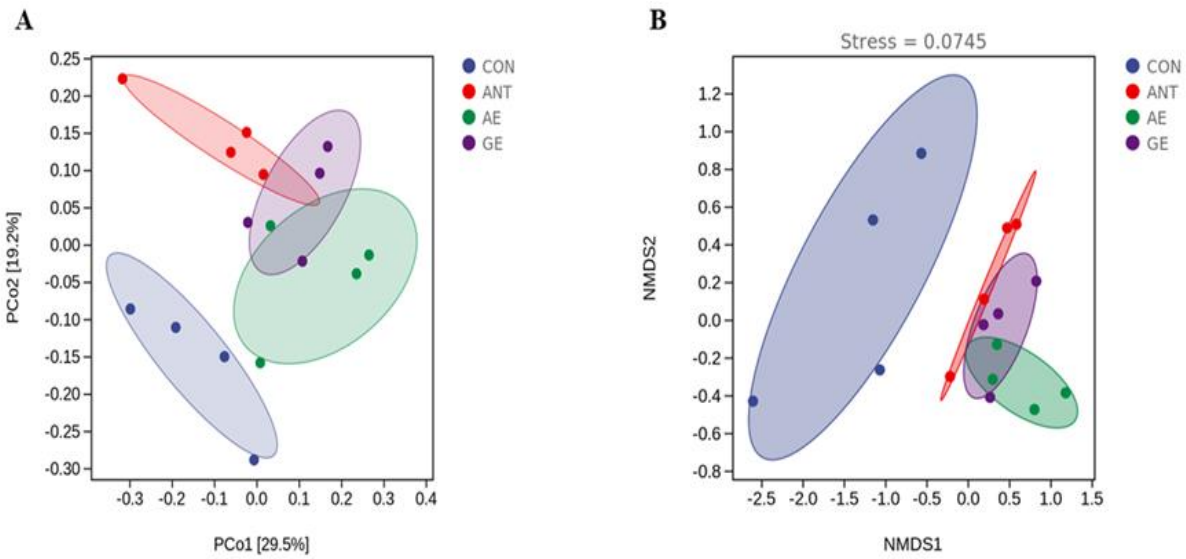

Fig.2-14 （A-B） Principal coordinate analysis and non-metric multidimensional scale analysis.

3.16.3 Effects of *Astragalus* Extract and *Glycyrrhiza* Extract on the Structure of the Cecal Microflora of Broilers

As shown in Figure 2-15 (A-E), the dominant phyla of the four groups are *Bacteroidetes, Firmicutes, Synergistetes, Proteobacteria* and *Fusobacteria* in order. The relative abundance of *Bacteroidetes* in each group accounted for the CON group (48.05%), the ANT group (53.18%), the AE group (37.07%), and the GE group (37.81%). The relative abundance of *Firmicutes* in each group accounted for the CON group (37.67%), the ANT group (32.42%), the AE group (51.75%), and the GE group (44.53%). Further statistical analysis of the dominant bacteria at the phylum level showed that the relative abundance of *Bacteroidetes* in the AE group and the GE group was significantly reduced compared with the CON group and the ANT group ($P<0.05$). The difference between the AE group and the GE group was not significant ($P>0.05$). Compared with the CON group, the relative abundance of *Firmicutes* in the AE group was significantly increased ($P<0.05$). Compared with the ANT group, the relative abundance of *Firmicutes* in the AE group and the GE group was significantly increased ($P<0.05$). The AE group was not significant compared with the GE group ($P>0.05$). Compared with the CON group and the ANT group, the F/B of the AE group and the GE group were significantly higher ($P<0.05$). Compared with the CON group, the relative abundance of *Synergistetes* in the GE group was significantly increased ($P<0.05$). Compared with the ANT group, the relative abundance of *Synergistetes* in the AE group

was significantly lower (P<0.05). The relative abundances of *Proteobacteria* and *Fusobacteria* in each group were not significantly different (P>0.05).

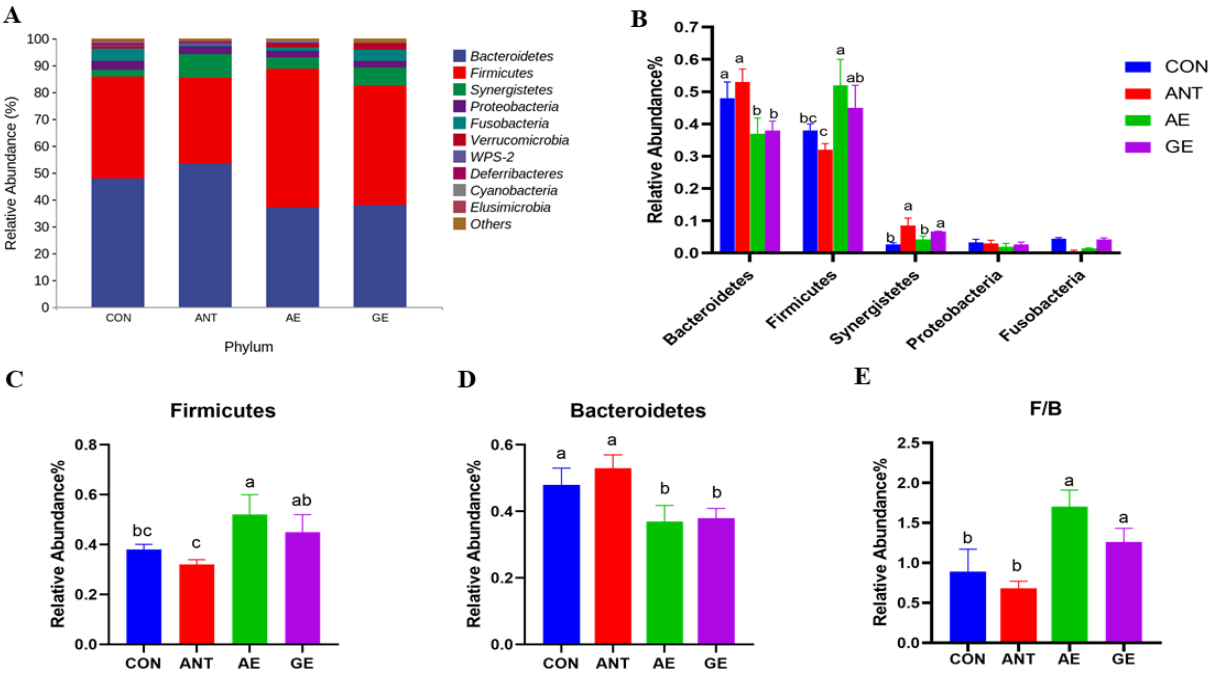

Fig. 2-15（A-E）Effects of AE and GE on phylum level in cecum of broilers

(A) Phylum level relative abundance statistics; (B) Differential comparison of microbiota; (C-E) Comparison of Firmicutes and Bacteroidetes.

As shown in Figure 2-16 (A), the dominant bacteria in the four groups are *Bacteroides, Oscillospira, Phascolarctobacterium,* and *Faecalibacterium.* Figure 3-16 (B) shows the difference analysis of the top 15 dominant bacteria genera. There are 9 different strains, namely *Bacteroides, Phascolarctobacterium, Faecalibacterium, Prevotella, Desulfovibrio, Parabacteroides, Ruminococcus, Alistipes, Butyricicoccus.*

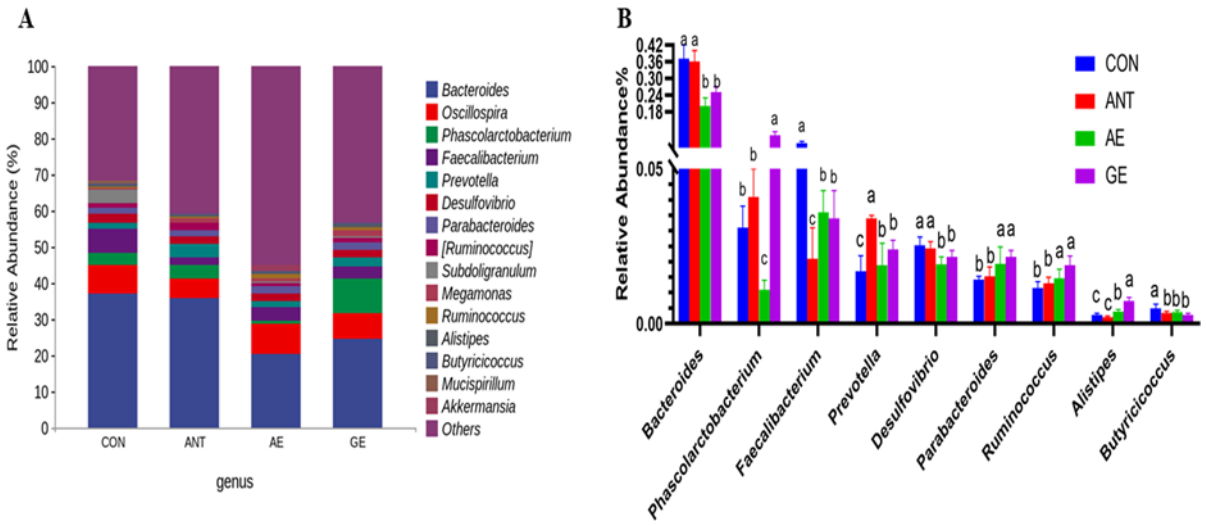

Fig. 2-16（A-B）Effects of *Astragalus* Extract and *Glycyrrhiza* Extract on the Abundance of Caecal Microorganism Genus in Broilers (A) Statistics of relative abundance of genus level; (B) Comparison of abundance of various species

3.16.4 LEfSe Differential Species Discriminant Analysis of Cecal Microorganisms

The analysis result of LEfSe includes two parts, respectively, a histogram of the LDA value distribution of significantly different species as shown in Figure 3-17 (A), which is used to show the significantly enriched species in each group and their degree of importance; species taxonomy branch map (Cladogram) is shown in Figure 3-17(B) to show the taxonomic hierarchical distribution of the marker species in each group of samples. In Figure 2-17 (A), the CON group was enriched with 11 differential markers, among which *Ruminococcaceae* was enriched in a higher abundance. The ANT group was enriched with 2 differential markers, among which *Veillonellaceac* was enriched with a higher abundance, and the AE group was enriched with 2 differential markers, among which *Sphingomonadaceae* was enriched with a higher abundance. The GE group was enriched with 7 differential markers, among which the abundance of

Prevotellaceae was higher. It showed that compared with the CON group, the intestinal flora of broilers in the ANT group, AE group and GE group changed significantly.

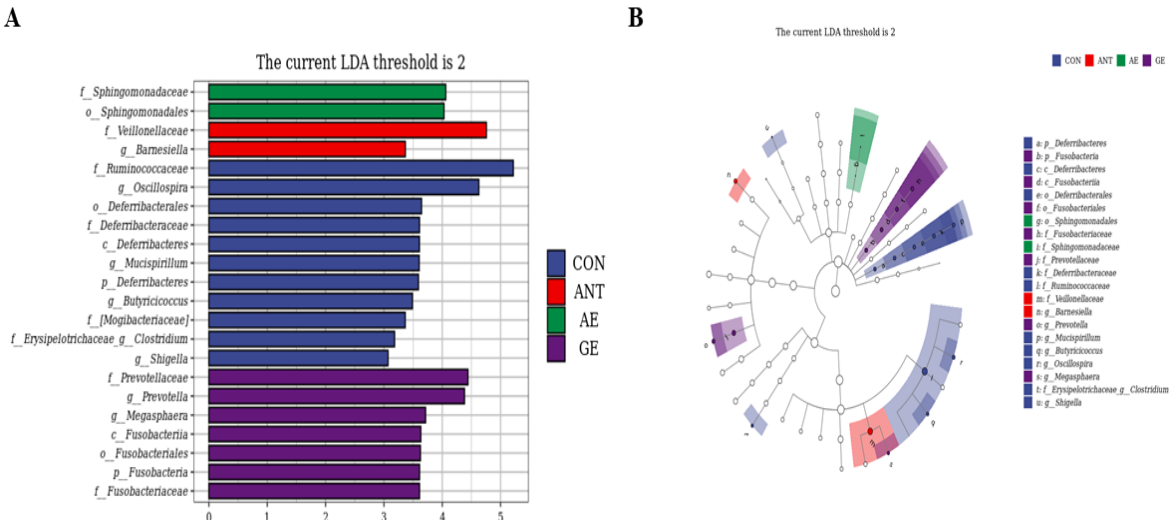

Fig.2-17（A-B）LEfSe Differential Species Discriminant Analysis (LDA> 2) (A) Histogram of LDA value distribution of significantly different species (B) Cladogram of species taxonomy

3.16.5 Predictive Analysis of Cecal Microbial Function

This experiment uses the KEGG database to predict the function of intestinal microbes. There are six major functions in pathway level 1. As shown in Table 2-13, in order of relative abundance, they are Metabolism, Genetic Information Processing, Cellular Processes, Environmental Information Processing, Organic Systems, and Human Diseases. In this experiment, AE and GE treatment only had a significant effect on Cellular Processes. According to the KEGG database Cellular Processes contains 5 pathways at the level 2 level, and 4 pathways at the level 2 level are involved in this

experiment, namely Transport and catabolism, Cell growth and death, Cellular community-prokaryotes and Cell motility, among which Cell Motility contains 6 pathways at level 3, as shown in Figure 2-18. Statistical analysis of the relative abundance of these 6 pathways found that only Bacterial chemotaxis and Flagellar assembly have significant differences, so we will focus on the study of these two pathways in subsequent experiments.

Table2-13 Pathway Level 1 relative abundance statistics

Items	CON	ANT	AE	GE	SEM	*P*-value
Metabolism	31392.72	31716	30139.64	30425.27	11.46	0.28
Cellular Processes	1265.11^{b}	1329.31^{b}	1609.58^{a}	1349.62^{b}	5.78	0.04
Environmental Information Processing	723.57	687.04	733.55	765.20	3.54	0.31
Genetic Information Processing	5348.97	5391.30	5441.08	5376.82	8.67	0.47
Human Diseases	61.36	63.82	67.59	63.06	3.56	0.12
Organismal Systems	149.04	142.52	145.20	138.62	5.43	0.84

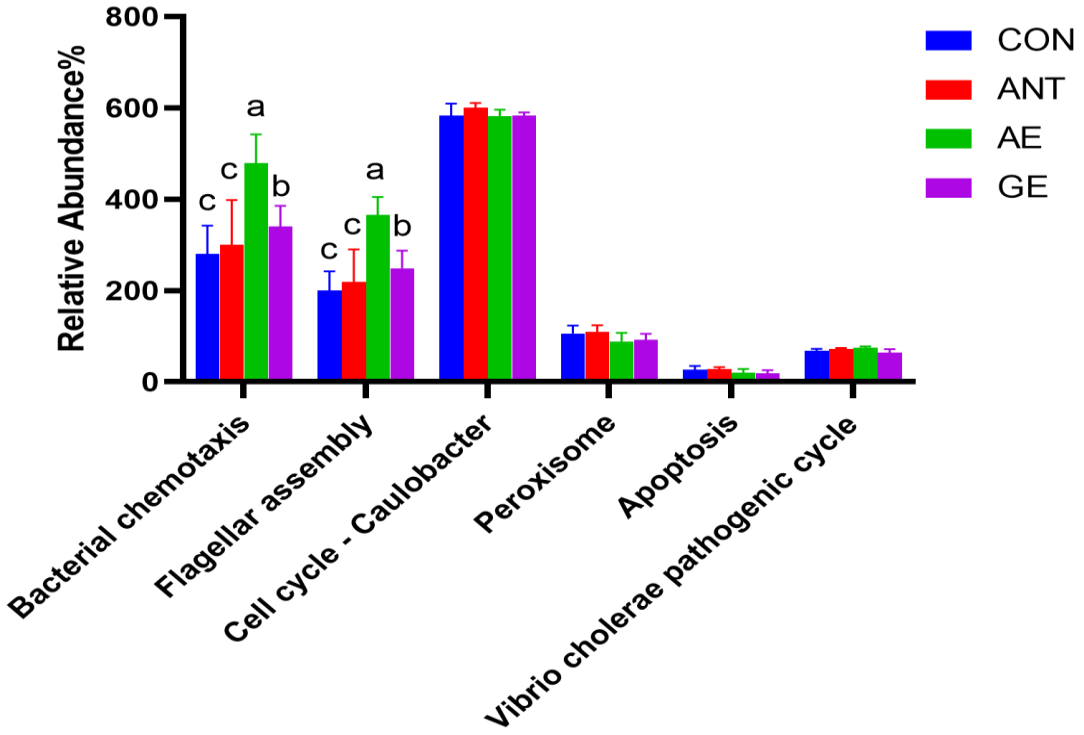

Fig. 2-18 KEGG function predicts the differential pathway in level 3

3.16.6 Correlation Analysis Between Cecal Microorganisms and Environmental Factors

The correlation analysis between cecal microbes and environmental factors in the AE group are shown in Figure 2-19 (A). The microbial flora at the genus level was selected for correlation analysis with 13 environmental factors with significant differences. *Bacteroides* was significantly positively correlated with FCR, TNF-α, IL-1β, and IL-6, and was significantly negatively correlated with ADG, T-AOC, GSH, IgM, and jejunum VH (P<0.05). *Faecalibacterium* was significantly positively correlated with FCR, and was significantly negatively correlated with ADG and jejunum VH (P<0.05). *Desulfovibrio* was significantly positively correlated with MDA and TNF-α, and was significantly negatively correlated with ADG and jejunum VH (P<0.05).

Butyricicoccus was significantly positively correlated with MDA, TNF-α, jejunum VH, and DAO, and was significantly negatively correlated with IgA (P<0.05). *Phascolarctobacterium* was significantly positively correlated with ADG, T-AOC, GSH, IgA, IgM, and jejunum VH, and significantly negatively correlated with MDA and TNF-α (P<0.05). *Prevotella* was significantly positively correlated with ADG, T-AOC, and IgM, and significantly negatively correlated with FCR, IL-1β, IL-6, and DAO (P<0.05). *Parabacteroides* was significantly positively correlated with ADG, T-AOC, IgA, IgM, and jejunum VH, and significantly negatively correlated with FCR, MDA, IL-1β, and DAO (P<0.05). *Ruminococcus* was significantly positively correlated with ADG, T-AOC, IgA, IgM, and was significantly negatively correlated with FCR, MDA, and DAO (P<0.05). *Alistipes* was significantly positively correlated with ADG and GSH, and significantly negatively correlated with TNF-α, IL-1β, and IL-6 (P<0.05).

The correlation analysis between cecal microbes and environmental factors in the GE group are shown in Figure 3-19 (B). The microbial flora at the genus level was selected for correlation analysis with 15 environmental factors with significant differences. *Bacteroides* was significantly positively correlated with MDA, TNF-α, IFN-γ, and IL-1β, and significantly negatively correlated with ADG, SOD, and IgM (P<0.05). *Faecalibacterium* was significantly positively correlated with MDA, and significantly negatively correlated with GSH and jejunum VH (P<0.05). *Desulfovibrio* was significantly positively correlated with MDA, TNF-α, IFN-γ, IL-1β, and IL-6, and was significantly negatively correlated with T-AOC, GSH, SOD, IgA, IgM, and IgG (P<0.05). *Butyricicoccus* was significantly positively correlated with TNF-α, IFN-γ,

IL-1β, and IL-6, and was significantly negatively correlated with T-AOC and jejunum VH (P<0.05). *Phascolarctobacterium* was significantly positively correlated with ADG, GSH, SOD, IgM, and jejunum VH, and significantly negatively correlated with MDA (P<0.05). *Prevotella* was significantly positively correlated with T-AOC, IgA, and jejunum VH, and significantly negatively correlated with TNF-α, IFN-γ, IL-1β, and IL-6 (P<0.05). *Parabacteroides* was significantly positively correlated with ADG, T-AOC, IgA, IgG, and jejunum VH, and significantly negatively correlated with IFN-γ, IL-1β, IL-6, and DAO (P<0.05). *Ruminococcus* was significantly positively correlated with ADG, T-AOC, and IgA, and significantly negatively correlated with FCR, TNF-α, IFN-γ, IL-1β, and IL-6 (P<0.05). *Alistipes* was significantly positively correlated with ADG, T-AOC, IgA, IgG, and jejunum VH, and significantly negatively correlated with FCR, IL-6, and DAO (P<0.05).

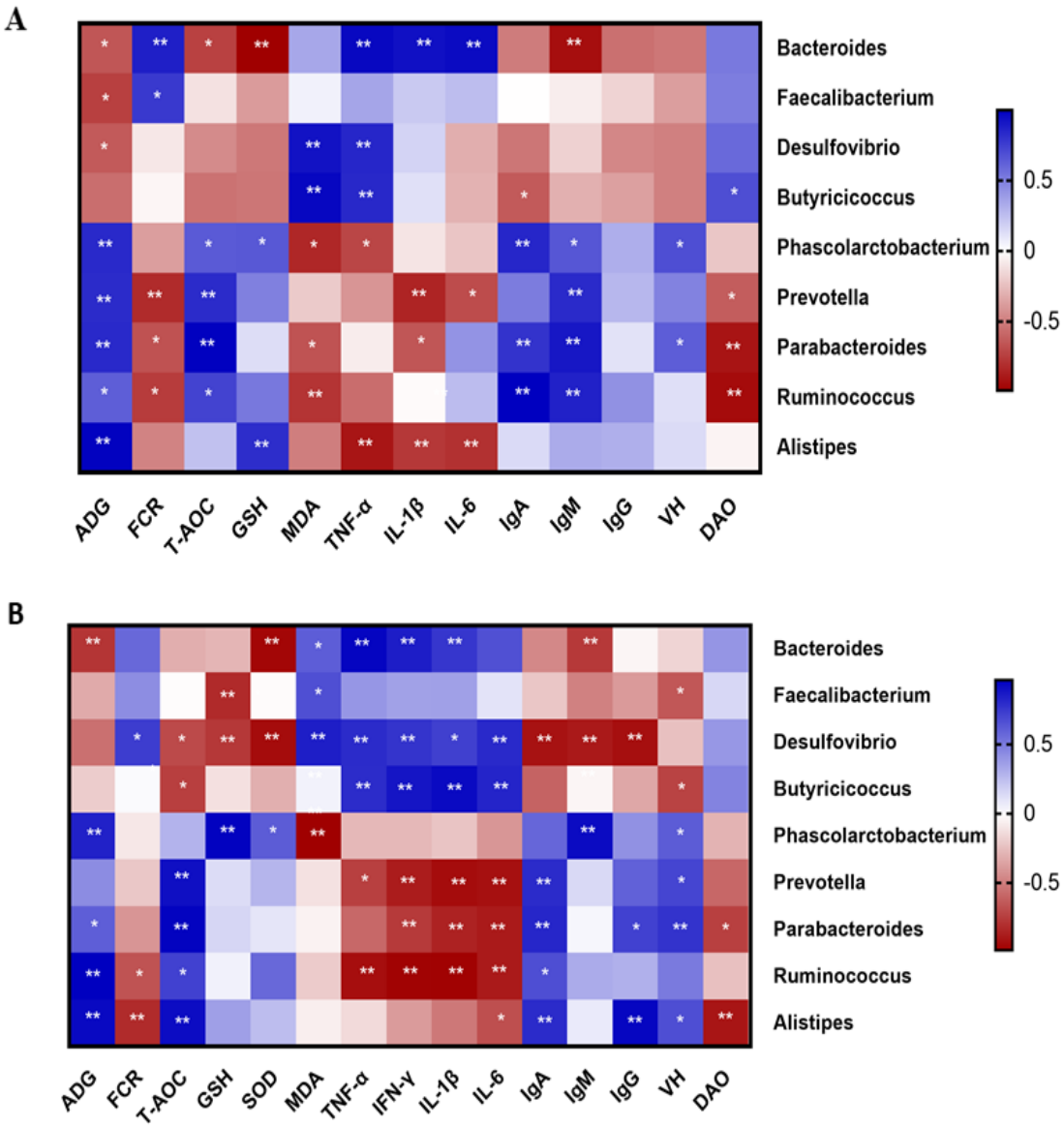

Fig.2-19 (A) Heat map of correlation analysis between different species of *Astragalus* extract genus level and different environmental factors (B) The heat map of the correlation analysis between *Glycyrrhiza* extract genus level difference species and difference environmental factors

3.17.1 Effect of high temperature boiled technology on meat color and shearing force

As shown in Table2-14, the effect of high temperature boiled technology on meat color and shear strength. Compared with CON group, the a * of ANT group, AE group,

GE group, AE+GE I group and AE+GE II group increased significantly, while the b * and shear force decreased significantly (P<0.05). Compared with ANT group, the a * of AE group, GE group, AE+GE I group and AE+GE II group were significantly higher (P<0.05). There was no significant difference in L value between groups (P>0.05).

Table 2-14 Effect of high temperature boiled technology on meat color and shearing force

Items	CON	ANT	AE	GE	AE+GE I	AE+GE II	SEM	*P*-value
L	55.57	53.60	52.91	56.77	56.34	54.50	0.63	0.22
a*	1.78^{c}	2.30^{b}	2.92^{a}	2.75^{a}	2.69^{a}	2.92^{a}	0.14	0.04*
b*	14.62^{a}	12.34^{b}	11.71^{b}	11.35^{b}	10.43^{b}	9.87^{b}	0.23	0.03*
Shear force	27.06^{a}	23.64^{b}	21.77^{b}	23.19^{b}	22.38^{b}	22.33^{b}	0.37	0.02*

3.17.2 Effect of High Temperature boiled technology on Nutrient Content in Chicken

As shown in Table 2-15, the effect of high temperature boiled technology on the nutrient content of chicken. Compared with CON group and ANT group, CP of AE group, GE group, AE+GE I group and AE+GE II group were significantly higher (P<0.05). There was no significant difference in EE, Ash, Ca and P in each group (P>0.05).

Table 2- 15 Effect of high temperature boiled technology on the content of nutrients in chicken

Items	CON	ANT	AE	GE	AE+GE I	AE+GE II	SEM	*P*-value
CP	24.15[b]	24.98[b]	26.78[a]	26.84[a]	27.87[a]	27.03[a]	0.67	0.03*
EE	1.54	1.51	1.58	1.51	1.56	1.56	0.09	0.13
Ash	2.23	2.13	2.46	2.25	2.19	2.32	0.21	0.12
Ca	0.78	0.82	0.79	0.84	0.81	0.78	0.03	0.09
P	3.44	3.54	3.48	3.51	3.49	3.58	0.14	0.15

3.17.3Effect of High Temperature boiled technology on the Content of Fatty Acids in Chicken

As shown in Table 2-16, the effect of high temperature boiled technology on the fatty acid content of chicken. Compared with CON group, the PUFA content of AE group, GE group, AE+GE I group and AE+GE II group and the MUFA content of AE+GE I group and AE+GE II group were significantly higher (P<0.05). Compared with ANT group, MUFA in AE+GE Ⅰ group and AE+GE Ⅱ group was significantly higher (P<0.05). There was no significant difference in SFA content in each group.

Table 2-16Effect of High Temperature boiled technology on the Content of Fatty Acids in Chicken

Items	CON	ANT	AE	GE	AE+GE I	AE+GE II	SEM	*P*-value
C16:0	20.13	20.14	20.64	20.89	20.87	21.32	0.21	0.07*
C16:1	2.26^{b}	2.64^{ab}	2.89^{a}	2.78^{a}	2.96^{a}	2.85^{a}	0.02	0.04*
C18:0	10.43	10.84	10.45	10.64	10.11	10.87	0.15	0.06*
C18:1n9c	24.26	24.57	25.01	24.98	24.09	24.98	0.19	0.08*
C18:2n6	35.43^{c}	37.88^{abc}	38.68^{ab}	40.14^{a}	36.89^{bc}	37.79^{abc}	0.29	0.02*
C18:3n3	2.10^{c}	2.54^{abc}	2.85^{a}	2.79^{ab}	2.38^{bc}	2.36^{bc}	0.07	0.03*
C20:2	0.86	0.89	0.82	0.83	0.87	0.84	0.05	0.12*
C22:0	0.74	0.78	0.75	0.81	0.79	0.78	0.03	0.14*
C20:3n6	0.36	0.38	0.38	0.37	0.37	0.40	0.01	0.16
C22:1n9	5.67	5.12	4.66	4.45	6.34	6.54	0.26	0.17
C24:1	1.38	1.45	1.43	1.42	1.39	1.44	0.05	0.09
C22:6n3	0.47^{b}	0.64^{ab}	0.54^{b}	0.78 ^{ab}	1.05^{a}	1.12^{a}	0.06	0.03*
SFA	31.3	31.76	31.84	32.34	31.77	33.03	0.32	0.07*
MUFA	33.57^{b}	33.78^{b}	33.99^{b}	33.63^{b}	34.78^{a}	35.81^{a}	0.26	0.02*
PUFA	39.22^{b}	42.33^{a}	43.27^{a}	44.91^{a}	41.56^{a}	42.51^{a}	0.24	0.02*

3.18 Economic benefit analysis of different groups

The economic benefit analysis was carried out with each chicken as a unit (as shown in Table 2-14). The price of each chick was 0.428$, the price of *Astragalus* extract was 8 $/kg, and the price of *Glycyrrhiza* extract was 10 $/kg. The gross income per chicken of CON group, ANT group, AE group, GE group, AE+GE I group, AE+GE II group was 0.561 $, 0.701 $, 0.639 $, 0.681 $, 0.605 $, and 0.674 $, respectively. Compared with the CON group, the gross income per chicken of ANT Group, AE group, GE group, AE+GE I group, and AE+GE II group increased by 0.140 $, 0.078 $, 0.120 $, 0.004 $, and 0.113 $, respectively. Compared with the CON group, the GE group had the best economic benefit, followed by the AE+GE II group, but neither achieved the effect of the ANT group. The results of the economic benefit analysis showed that adding *Astragalus* extract or *Glycyrrhiza* extract or a combination of both in broiler chickens' diets could improve the economic benefits of broiler chickens. Among them, adding *Glycyrrhiza* extract alone was the best but did not achieve the effect of adding Terramycin calcium economic benefits.

Table 2-17Economic benefit analysis of broilers in different groups (per chicken)

Item	CON	ANT	AE	GE	AE+GE Ⅰ	AE+GE Ⅱ
Chick price ($)	0.428	0.428	0.428	0.428	0.428	0.428
Feed Intake (kg)	3.993	3.966	3.968	3.915	3.971	4.100
Feed price($/kg)	0.643	0.644	0.660	0.654	0.670	0.657
Feed cost ($)	2.567	2.554	2.619	2.560	2.661	2.694
Body weight/(kg)	2.489	2.578	2.580	2.568	2.586	2.657
Chicken income ($)	3.556	3.683	3.686	3.669	3.694	3.796
Gross income ($)	0.561	0.701	0.639	0.681	0.605	0.674
Increase ($)		0.140	0.078	0.120	0.044	0.113

Note: Price of *Astragalus* polysaccharides: 8$/kg; Price of *Glycyrrhiza* polysaccharides:10$/kg; Price of broiler: 1.43$/kg; Feed cost= Feed intake* Feed price ; Chicken income = Body weight* Price of broiler ; Gross income = Chicken income-Feed cost -Chick price.

SECTION 4 DISCUSSION

4.1 The Effect of *Astragalus* Extract and *Glycyrrhiza* Extract on Broiler Performance

In the context of comprehensive "anti-antibiotics", exploring "new, efficient, safe, and green" antibiotic alternatives has gradually become a research focus in the feed industry. A number of studies have shown that adding plant extracts to poultry diets can improve the immunity of poultry, prevent the occurrence of diseases, and promote poultry growth [128-129]. Adding 800 mg/kg of AE to the diet can increase the body weight of broilers at 42 days and the weight gain of broilers at 15-42 days [130]. Adding 10g/kg of *Astragalus* root powder can increase the ADG of broilers and reduce FCR [45]. Wang Q, et al. [131] The study showed that adding *Astragalus* polysaccharides to poultry diets can increase ADFI and reduce FCR, and *Astragalus* polysaccharides can also alleviate the growth performance of broiler chickens under immune stress caused by cyclophosphamide and lipopolysaccharide (LPS) [132-134]. The study by Wu H, et al. [36] showed that adding 3% *Glycyrrhiza* residue in broiler diets can increase the ADG of broilers and reduce FCR. GE can reduce the negative impact of aflatoxin B1 on broiler performance and immunity [135]. Drinking water containing GE can improve the average body weight, ADFI, ADG, and FCR of broilers under heat stress [136]. Adding 500 mg/kg of GE to the diet of high-density broiler chickens can effectively improve body weight gain during the late and full growth period [137]. A study by Ocampo, et al. [138] found that adding *Glycyrrhizic* acid to drinking water can improve the growth performance of broilers and reduce mortality

by enhancing the immune system. Salary, et al. [139] study showed that adding 2% GE to drinking water at the early stage of feeding can increase the feed intake of broilers by increasing the palatability of feed and stimulating appetite. The results of this experiment show that the addition of AE and GE alone or in combination can increase the 42day weight and ADG of broilers, reduce FCR, thereby improve the growth performance of broilers, and achieve the effect of adding antibiotics on the growth performance of broilers. Among them, the combination of AE and GE in low doses has the best effect.

4.2 The Effect of *Astragalus* Extract and *Glycyrrhiza* Extract on the Apparent Metabolic Rate of Nutrients in Broilers

Nutrient metabolism rate is an important indicator to measure the digestion and absorption of nutrients by animals. Its level directly affects the growth performance of animals and also reflects the nutritional value of diets. Some studies have shown that adding *Astragalus* polysaccharides to diets can improve CP metabolism and energy utilization [133]. The study by Wu H, et al. [36] showed that adding 3% glycerin residue in broiler diets can increase the apparent metabolic rate of CP and reduce the apparent metabolic rate of EE. This experimental study showed that the addition of AE and GE increased the apparent metabolic rate of energy in the diet. In addition, the addition of GE also increased the apparent metabolic rate of CP, and the effect of addition was better than antibiotics. It is speculated that the increase in the apparent metabolic rate of nutrients may be related to the increase in production performance, which further

indicates that the increase in the production performance of broilers by AE and GE may be related to the increase in the apparent utilization of diets.

4.3 The Effect of *Astragalus* Extract and *Glycyrrhiza* Extract on the Meat Quality of Broilers

Muscle pH, shear force, and drip loss are indicators to evaluate the physical and chemical properties of meat quality. The content of fatty acids in muscle is an index to evaluate the nutritional value of meat quality. The acceptability of meat to consumers depends on the physical and chemical properties of the meat, and the chemical composition of the meat is closely related to its nutritional value. The decrease in pH value after animal slaughter is related to the fermentation of muscle glycogen. Stress accelerates *glycogenolysis* in the body and rapidly lowers muscle pH [140]. This study found that the addition of AE and GE to the diet significantly increased the pH of chicken at 45 min ($P < 0.05$), indicating that the addition of AE and GE can effectively alleviate *glycogenolysis* caused by stress in broilers after slaughter.

The color of meat is the most intuitive external performance that consumers evaluate the freshness of meat. It is usually expressed by L, a*, and b* values. L value represents the brightness of the meat. The normal range is 46-53. The larger the value inside this range, the better the gloss. The a* value represents the redness of the sample, the higher the value, the better and the fresher the meat. The b* value represents the yellowness of the meat, the higher the value is, the less fresh the meat is [141]. Zhang X, et al. [142] studies have shown that drinking fermented *Astragalus-Glycyrrhiza* water extract for broilers can significantly increase the a* value of the flesh color, and

significantly reduce the b* value and drip loss (P <0.05). Adding 3000 mg/kg ~ 4000 mg/kg *Glycyrrhiza* extract to the sheep diet can improve the physical quality of fresh meat [143]. This experimental study shows that the L* value is within the normal range, and there is no significant difference between the L value, a* value, and b* value, indicating that the addition of AE and GE under this test condition does not affect the freshness of the broilers.

Fatty acid is an important chemical substance that constitutes fat, and it is also an important factor that affects the flavor of meat. Among them, the content of unsaturated fatty acid (USFA) plays a key role in the formation of flavor substances. The higher the proportion of unsaturated fatty acids in the whole fat structure, the larger the proportion of soft fat in meat, and the more flavor substances produced during the cooking process, the better the palatability. USFA can be divided into monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Among them, PUFA is an important precursor of meat flavor and an indispensable nutrient for the human body. Wang Y, et al. [144] research shows that adding 1.74% compound *Astragalus* granules to broiler diets can significantly improve meat quality. Adding 900 mg/kg of *Glycyrrhiza* extract to the diet of finishing pigs can increase the content of unsaturated fatty acids in the muscles, thereby improving pork quality [145]. The results of this experiment are consistent with the results of the above studies, indicating that adding AE and GE to broiler diets can increase the content of USFA in muscle, thereby improving the flavor of broilers.

4.4 The Effect of *Astragalus* Extract and *Glycyrrhiza* Extract on the Antioxidant Function of Broilers

GSH-Px and SOD are important antioxidant enzymes in animals. GSH-Px can reduce the production of lipid peroxides and protect the structure and function of cell membranes. SOD can scavenge superoxide anion free radicals to protect cells from damage. Its activity indirectly reflects the body's ability to scavenge free radicals [146]. MDA is one of the products of lipid peroxidation, which has strong toxicity to cells and indirectly reflects the damage degree of cells attacked by free radicals [147]. T-AOC is a comprehensive index used to measure the body's antioxidant capacity [148]. Studies have shown that *Astragalus* polysaccharides can scavenge free radicals in time by activating a variety of enzyme activities in the body, reducing oxidative stress in animals, and enhancing animal immune responses [149]. Adding 0.5~1.0 g/kg of *Astragalus* polysaccharides to the diet can improve the growth performance of broilers and the levels of SOD, GSH-Px, IgG, IgM, and IgA in the serum, and reduce the level of MDA [49]. Adding Astragalus root powder can improve the growth performance, antioxidant status, and serum metabolites of broilers [50], and improve liver and kidney function by improving the antioxidant status [51]. Adding GE to chicken patties can reduce the production of MDA in the patties, and increase the pH and a* values of the patties, thereby improving the oxidation stability, quality attributes, and shelf life of the chicken patties [150]. This experimental study showed that the addition of AE and GE increased the levels of T-AOC, SOD, and GSH-Px in the broiler's serum and trachea, lowered the level of MDA in the serum and increased *SOD1*, *SOD2*, and *GSH-Px* mRNA in the intestine and respiratory tract. The expression level can activate the activities of various enzymes in the body, eliminate free radicals in time, and reduce the oxidative stress of

animals. It shows that adding AE or GE to broiler diets can improve the body's antioxidant function, and the antioxidant effect is better than that add antibiotics.

Some studies have shown that the release of antioxidant enzymes was regulated by the Nrf2 pathway, which is essential for regulating the oxidation/reduction state of cells. Nrf2 is an important nuclear transcription factor in cells and plays a key role in regulating oxidative stress in the small intestine and stomach [146]. Inhibition or deficiency of Nrf2 will cause the expression of downstream antioxidant enzymes to decrease, which will not be able to resist the toxicity caused by oxidative stress, leading to cell dysfunction, apoptosis, or necrosis [151]. Under normal physiological conditions, Nrf2 activity is inhibited in the cytoplasm by binding to Keap1. This combination maintains the basic levels of protective enzymes and antioxidants in the cell and keeps the cell in a stable state [152]. When free radicals continue to increase, Keap1 ubiquitination and/or Nrf2 phosphorylation are reduced, causing Nrf2 to separate from Keap1 and translocate to the nucleus, causing the release of intracellular protective genes and antioxidant enzymes [153]. In this study, the addition of AE and GE increased the expression of Nrf2 mRNA. This indicates that AE and GE may activate the Keap1-Nrf2 signaling pathway in the broiler's intestines, promote the release of antioxidant enzymes, improve the antioxidant capacity of the broiler's intestines, and effectively alleviate the intestinal barrier damage caused by stress.

4.5 The Effect of *Astragalus* Extract and *Glycyrrhiza* Extract on the Immune Function of Broilers

The thymus, spleen, and bursa of Fabricius are the main immune organs of birds. The relative weight of the thymus, spleen, and bursa of Fabricius can reflect the overall immune function of the body to some extent. It is generally believed that a large immune organ index indicates that the immune organs are well developed, and the body's immunity is high. A small immune organ index indicates that the immune organs are not well developed. The body's immunity is low. Studies have shown that adding 100, 200, and 300 mg/kg of *Astragalus* extract to the diet can increase the weight of the immune organs of broilers [154]. Zhang X, et al. [142] Studies have shown that broiler chickens drink fermented *Astragalus-Glycyrrhiza* water extract, the spleen index is significantly increased by 50.82%, and the bursa index is increased by 38.13% ($P < 0.05$). This experimental study showed that the addition of AE and GE increased the thymus index and spleen index of broilers, and the low-dose combined addition had a better effect. Serum immunoglobulins IgA, IgG, and IgM are the main immune molecules in the humoral immune response, which can improve the resistance of animals [155]. Kuang Z, et al. [156] research shows that *Astragalus* polysaccharides can improve immune organ development, improve immune organ index, promote immune cell proliferation, enhance immune cell function, and induce cytokine synthesis and secretion. The innate immunity and adaptive immunity of chickens. Poultry drinking *Astragalus* polysaccharide can be used as an immune adjuvant to increase the antibody level of serum immunoglobulins [157] and can increase serum IgG and IgM concentrations caused reduction by cyclophosphamide immunosuppression [132]. GE can reduce the negative effects of aflatoxin B1 on

broiler performance, blood indicators, and immunity [70]. This experimental study shows that the addition of AE and GE can increase the content of immunoglobulins IgA, IgM, and IgG in the serum of broilers, thereby improving the immune function of broilers, and the addition of AE can improve the immune function of broilers better than antibiotics.

4.6 The Effect of *Astragalus* Extract and *Glycyrrhiza* Extract on Inflammatory Factors in Broilers

Cytokine is a kind of regulatory factor with a variety of biological activities in the animal body. It is the bridge between the natural immune system and the specific immune system. It can activate and regulate the immune active cells and play an important role in the body's immune response, inflammatory response, etc. [158-159]. Cytokines such as IL-1β, IL-6, TNF-α, and IFN-γ are pro-inflammatory cytokines, which can activate the innate immunity and acquired immune system of the animal body, and mediate the generation and aggravation of inflammatory response [160]. Studies have shown that *Astragalus* Polysaccharides can reverse the negative effects of LPS on chickens by reducing pro-inflammatory cytokines (IL-6 and IL-1β) [133]. GE can reduce the increase of Toll-like receptors 4 (TLR4) and IL-1β after Clostridium jejuni infection in broilers [161]. *Glycyrrhiza* polysaccharides can also alleviate the increase of IL-1 β and IFN-γ in the liver of broilers induced by LPS, and reduce the stress response [162]. This experimental study shows that the addition of AE and GE can reduce the serum levels of TNF-α, IL-1β, and IL-6 in broilers, and the levels of

IL-1β and IL-6 in the lungs, inhibit inflammation and is beneficial to the health of broilers. The effect is the same as adding antibiotics to inhibit inflammation.

4.7 The Effect of *Astragalus* Extract and *Glycyrrhiza* Extract on Intestinal Mucosal Morphology of Broilers

As the body's largest immune organ, the intestine is not only responsible for the digestion and absorption of nutrients but also acts as a barrier to the immune system [163]. The normal intestinal mucosal structure is necessary to ensure the digestion and absorption of nutrients and the growth of the animal body [164]. The morphological indicators of the small intestine mucosa mainly include VH, CD, and VH/CD ratio. VH and VH/CD are common standards for estimating the absorption capacity of the small intestine. A higher amount of VH and VH/CD means that the absorption capacity of the small intestine is enhanced [165-166]. Studies have shown that the addition of 10 g/kg *Astragalus* polysaccharide in the sire line diet can promote the growth and histological changes of the offspring's jejunum, *Astragalus* polysaccharide can significantly reduce the duodenal CD of broilers, increase the VH and VH/CD of the jejunum [38], and increase the VH and VH/CD of the broiler jejunum under the immunosuppression state induced by cyclophosphamide [167]. The addition of 4 mg/kg of GE to the tilapia diet can increase the VH and CD of heavy metal pollution intestines [168]. This experimental study showed that the addition of AE and GE increased the VH and VH/CD of the duodenum and ileum, and the addition of GE also increased the VH of the jejunum. It further shows that AE and GE can improve the morphology of the

intestinal mucosa, promote the absorption of nutrients, and have the same effect as adding antibiotics to the morphology of the intestinal mucosa.

4.8 The Effect of *Astragalus* Extract and *Glycyrrhiza* Extract on Intestinal Permeability of Broilers

DAO is an intracellular enzyme mainly secreted by intestinal epithelial cells [169]. D-LA is a metabolite of bacteria and mainly exists in the intestinal mucosa [170]. A complete intestinal mucosal barrier can prevent DAO and D-LA from entering the blood circulation [171]. When the intestinal mucosal epithelial cells are damaged and shed, DAO and D-LA will enter the intestinal intercellular space, lymphatic vessels and blood, which will increase the DAO activity and D-LA concentration in the blood [172-173]. Therefore, the intestinal barrier function can be assessed indirectly by assessing the blood DAO activity and D-LA concentration [47]. Studies have shown that adding *Astragalus* polysaccharides can reduce the D-LA content in broiler serum [131]. The results of this experiment showed that the addition of AE and GE reduced the activity of DAO and the concentration of D-LA in the serum of broiler chickens, thereby reducing the permeability of the intestinal tract, and improving the integrity of the intestinal tract of broiler chickens.

4.9 The effect of *Astragalus* extract and *Glycyrrhiza* extract on the physical and chemical barriers of the intestines and respiratory tracts of broilers

Intestinal tight junction protein acts as a physical barrier between the intestine and the respiratory tract and plays a role in maintaining the homeostasis of the intestine. Tight junction proteins are mainly composed of *Claudins*, *Occludin*, and ZO-1 [174].

The expression of tight junction proteins is very important for maintaining the integrity of the intestinal epithelium and maintaining intestinal permeability [175]. As a chemical barrier for the intestine and respiratory tract, MUC2 is the main component of mucus on the surface of the intestinal mucosa and can repair intestinal mucosal damage caused by harmful factors [176]. The high expression of MUC2 in intestinal goblet cells is an important part of barrier integrity [177-178]. Studies have found that adding γ-irradiated *Astragalus* (IAPS) to the diet can significantly up-regulate the mRNA expression of *Occludin* and ZO-1 in the jejunum [46]. Adding 1 g/kg of GE to the diet can improve the growth performance of broilers, reduce the shedding of *Clostridium jejuni*, increase the gene expression of tight junction protein *Occludin*, and junctional adhesion molecules (JAM), and promote the MUC2 gene in the state of LPS immunosuppression, the expression of inflammatory markers such as TLR-4 and IL-1β are down-regulated to maintain the integrity of the intestine [179]. The results of this experiment showed that the addition of AE and GE up-regulated the mRNA expression of *Claudins*, *Occludin*, ZO-1, and MUC2 in the intestine and respiratory tract, maintained the integrity of the intestinal barrier and respiratory barrier of broilers, improved the digestive and respiratory tracts, and improved the body's defense against diseases. It is speculated that the mechanism of AE and GE on the growth performance of broilers may be through improving the expression of tight junction proteins, enhancing the integrity of the intestinal and respiratory immune systems and barriers, and improving the absorption of nutrients.

4.10 The Effect of *Astragalus* Extract and *Glycyrrhiza* Extract on the Intestinal Microbes of Broilers

When broilers suffer from environmental stress or viral infection and are in an immunosuppressive state, the microbial structure of the gut will change and eventually destroy its function of the gut [180]. Studies have shown that some plant polysaccharides that reach the distal end of the gastrointestinal tract can be fermented by the intestinal microbiota and further regulate the intestinal microenvironment [181]. Compared with the control group and the cyclophosphamide-treated broiler chickens fed γ-irradiated *Astragalus*, the number of intestinal microflora OUT increased [182]. This experimental study showed that the addition of AE and GE increased the number of intestinal flora OUT compared with the control group and the antibiotic group. Tea tree pectin *heteropolysaccharides* can increase the Chao and Shannon indexes of the microbial flora in the feces of mice treated with cyclophosphamide, indicating that plant polysaccharides can increase the abundance and uniformity of intestinal microbes under immunosuppressive conditions [183]. The addition of γ-irradiated *Astragalus* significantly increased the Shannon index of the intestinal microflora of broilers and decreased the Simpson index [182]. Communities with high species diversity and richness can often fill the niche by mobilizing species with similar functions when they are disturbed by the environment. This experiment shows from the Alpha diversity that the addition of AE increases the diversity of intestinal microbes, and the addition of GE increases the diversity and abundance of microorganisms in the tract. The results indicate that the addition of AE and GE has changed the structure of the intestinal flora,

and further show that the diversity and abundance of the intestinal flora of broilers with AE and GE are higher and better to maintain the ecological stability of the body.

At the Phylum level, the dominant phyla in this experiment are *Bacteroidetes*, *Firmicutes, Synergistetes, Proteobacteria,* and *Fusobacteria*. *Bacteroidetes* is one of the most important symbiotic bacteria in the animal gastrointestinal flora. It can inhibit the colonization of host intestinal pathogens. Many bacteria belonging to *Firmicutes* have been shown to participate in energy metabolism and maintain intestinal health [184]. The increase in the ratio of *Firmicutes* to *Bacteroidetes* is beneficial to the absorption of nutrients [185] and is closely related to the composition of the intestinal microflora and the ability of the host to obtain energy [186]. This experimental study shows that the addition of AE and GE increases the ratio of *Firmicutes* to *Bacteroidetes*, which is conducive to the absorption of nutrients by the intestinal microbes of broilers. Yin, et al. [187] study showed that the addition of lysine to the diet of piglets increased the abundance of *Synerggistetes* and promoted feeds. This is consistent with the result that the addition of GE in this experiment increased the abundance of *Synerggistetes*.

At the genus level, the dominant bacteria in this experiment were *Bacteroides, Phascolarctobacterium, Faecalibacterium,* and *Prevotella*. *Bacteroides* are one of the dominant anaerobic bacteria in the cecum of chickens [188]. Studies have shown that certain *Bacteroides* have special pathogenic potential, by producing enterotoxin on the side of intestinal cells, promoting the penetration of intestinal bacterial epithelial cells, and inducing diarrhea [189]. The study by Liu, Y, et al. [182] showed that the abundance of *Bacteroides* in broilers fed with γ-irradiated *Astragalus* was lower than

that in the cyclophosphamide-treated group, which was comparable to the results of adding AE and GE to reduce the abundance of *Bacteroides* in this experiment. Maier, et al. [190] reported that the activity of *Faecalibacterium* does not enhance the integrity of the epithelial barrier in an anaerobic co-culture model at the top of the large intestine. Plantain is a medicine widely used to treat constipation, and ulcerative colitis is an inflammatory disease that affects the colon and rectum. The study by Hou D, et al. [191] found that compared with healthy people, the levels of *Phascolarctobacterium* and *Prevotella* in gut microbes in patients with ulcerative colitis decreased significantly. Jalanka, et al. [192] found that plantain increased the abundance of *Phascolarctobacterium* in the fecal microorganisms of patients with constipation. This indicates that the increased abundance of *Phascolarctobacterium* and *Prevotella* has a protective effect on the body's health. In this experiment, the addition of GE increased the relative abundance of *Phascolarctobacterium*, and the addition of AE and GE increased the relative abundance of *Prevotella* and decreased the relative abundance of *Faecalibacterium*, indicating that AE and GE have a protective effect on the intestinal health of broilers and make the most of the beneficial bacteria increase and the harmful bacteria decrease so that the intestinal microbes develop in a favorable direction.

In the LEfSe differential species discriminant analysis, the addition of antibiotics, AE and GE caused significant changes in the differences in the intestinal flora of broilers, and it was found that *Veillonellaceae* was more enriched in the antibiotic group, *Sphingomonadaceae* was more enriched in the AE group, and *Prevotellaceae* was more

enriched in the GE group. Studies have shown that an increase in the proportion of *Veillonellaceae* can cause irritable bowel syndrome (IBS) [193]. Feeding succinic acid (SA) can increase the enrichment of *Sphingomonadaceae* in shrimp gut microbes, and *Sphingomonadaceae* may participate in nutrient metabolism and degradation of toxic compounds and pollutants [194]. The increased abundance of *Prevotellaceae* plays an important role in the prevention and treatment of Staphylococcus aureus infections by mixed lactic acid bacteria (LAB) [195].

Combining species abundance analysis and LEfSe difference discriminant analysis, it is concluded that the addition of AE and GE changes the dominant flora of intestinal microbes and significantly reduces pathogenic bacteria. And AE and GE are superior to antibiotics in improving intestinal flora.

Through the prediction of the cecal microbial function, it is found that AE and GE lead to changes in metabolic pathways. For example, the changes in the Cellular Processes pathway are predicted in this experiment, and the changes in the Bacterial chemotaxis and Flagellar assembly pathways are obtained in the database. In later trials, we will be able to focus on Bacterial chemotaxis and Research on the Flagellar assembly pathway.

This experiment uses bacteria with significant differences in the genus level of intestinal microbes and analyzes the correlation with significant differences in environmental factors. It is found that the addition of AE and GE is significantly positively correlated with ADG, most serum antioxidant indicators, immune indicators, and jejunum VH; Significantly negatively correlated with FCR, most serum

inflammatory factors, and serum DAO. It is speculated that AE and GE can affect production performance, serum antioxidant performance, immune function, inflammatory factors, intestinal mucosal morphology, and intestinal permeability by improving the structure of intestinal flora.

4.10 Effect of high temperature boiled technology on meat quality

When selecting meat products, most consumers mainly look at the color. Under the condition of no contact, the color of meat may be the only standard to judge the quality, which plays a more important role compared with other factors. Generally, it is considered that the color of fresh or unsanitary meat is darker [196]. In this study, there was no significant change in the L value of meat color in each group after boiling, but the a * value of meat color in broilers treated with astragalus and licorice significantly increased, and the b * value significantly decreased, indicating that eating AE and GE can improve the meat color after boiling at high temperature. This is consistent with the results of studying the color change of mutton under high temperature processing [196], and Jiang Xiuli et al. [197] have the same results when studying the effect of different drying time on the color of preserved meat. The reason for this result may be that during the processing, the meat sample is heated and oxidized in full contact with oxygen, which promotes the production of ferrimyoglobin and gradually darkens the color of the meat [198].

Protein is one of the nutrients that the human body cannot lack. Meat is an important source of protein, but the protein in meat is affected by various factors. This study found that compared with the control group, the crude protein content in the

meat of the treatment group added with AE and GE increased significantly after high temperature boiling, indicating that the *Astragalus* extract and *Glycyrrhiza* extract can improve the nutritional value of the meat after high temperature boiling, and the effect is better than that of antibiotics. The tenderness reflects the texture of meat. The structure and state of myofibril are the key factors that lead to the difference in meat tenderness. Calpain and connective tissue also have an impact on it [199]. This study found that the tenderness of chicken is greatly affected by the high temperature boiling treatment, which is consistent with the previous report that the high temperature boiling treatment has a significant impact on the shear strength of poultry meat [200-201]; After high temperature processing, the shear force increases gradually with the increase of processing time and processing temperature. During high temperature processing, part of the protein in the meat will undergo denaturation due to heat, resulting in changes in protein structure and meat tenderness [201]. In this study, the shearing force value of chicken fed with AE and GE decreased after boiling and heating treatment, indicating that eating AE and GE would affect the process of dissolving meat collagen into gel, improve the tenderness, and thus improve the palatability of meat.

4.11Effect of high temperature boiled technology on fatty acid content

Fatty acid is an important nutritional index to evaluate meat and meat products. The change of FA after processing directly affects the nutritional quality of meat [202]. Sun Chengfeng et al. [203] studied the changes of FA in pork during high temperature boiling, and found that the content of FA increased after high temperature boiling. In

addition, the contents of SFA, MUFA and PUFA in meat increased significantly after different heat treatments, which may be related to the decrease of water content during processing [204]. Previously, it was reported that the high temperature water cooking method had different effects on the FA of rabbit meat, and the cooking loss rate was greater than that of baking [205]. Liu Meng et al. [206] studied the effects of different heating methods on the antioxidation of beef products, and found that when heated at 100 ℃, the antioxidation was the best, and the FA retention was the highest. In this experiment, the content of MUFA and PUFA of chicken fed with *Astragalus* extract and licorice extract increased after boiling and heating, which further showed that the consumption of AE and GE could improve the meat flavor and meat quality of broilers.

SECTION 5 CONCLUSIONS, INNOVATION POINTS, RESEARCH OUTLOOK

Conclusion

1. Adding *Astragalus* extract and *Glycyrrhiza* extract alone or in combination can improve the production performance of broilers and increase the apparent metabolic rate of dietary energy for broilers. Adding *Glycyrrhiza* extract alone can increase the apparent metabolic rate of dietary crude protein.

2. *Astragalus* extract and *Glycyrrhiza* extract alone or in combination can increase the content of unsaturated fatty acids in muscles. The low-dose combined addition group reduces muscle shear force and drip loss, and improves meat quality.

3. *Astragalus* extract and *Glycyrrhiza* extract alone or in combination can improve the structure of small intestine villi of broilers and improve the permeability of the intestine and trachea.

4. *Astragalus* extract and *Glycyrrhiza* extract alone or in combination can improve the antioxidant performance of broiler chickens, and have the same effect on the trachea, improve the immune function of broilers, inhibit the occurrence of inflammatory factors, and have the same effect on the lungs.

5. *Astragalus* extract and *Glycyrrhiza* extract alone or in combination can increase the cecal microbial diversity and flora abundance of broilers, and improve the structure of intestinal flora. It is speculated that AE and GE can affect production performance, serum antioxidant performance, immune function, inflammatory factors, intestinal

mucosal morphology and intestinal permeability by improving the structure of intestinal flora.

6. Adding *Astragalus* extract and *Glycyrrhiza* extract can achieve the feeding effect of adding Terramycin calcium, and the effect of adding 150 mg/kg *Astragalus* extract + 75 mg/kg *Glycyrrhiza* extract is better.

7. Adding *Astragalus* extract, *Glycyrrhiza* extract, high-dose *Astragalus*-*Glycyrrhiza* combination, and low-dose *Astragalus*-*Glycyrrhiza* combination to the diet increased the income of each chicken by $0.078, $0.120, $0.044, and $0.113, respectively. Adding *Glycyrrhiza* extract alone has the best economic benefits.

8. High-temperature boiled technology can improve meat quality of broilers fed with *Astragalus* extract and *Glycyrrhiza* extract

Innovation points

1. For the first time, the effects of *Astragalus* extract and *Glycyrrhiza* extract and their combination on broiler performance, meat quality, antioxidant function, immune function and intestinal barrier function were systematically studied.

2. Through the analysis of intestinal microbial diversity, the possible mechanism of *Astragalus* extract and *Glycyrrhiza* extract by regulating intestinal microbes on broilers was studied.

Research Outlook

1. Further study the mechanism of action of *Astragalus* extract, *Glycyrrhiza* extract and their combination on the respiratory barrier function of broilers

2. Further study the metabolic pathway mechanism of the influence of *Astragalus* extract, *Glycyrrhiza* extract and their combination on the intestinal microbes of broilers.

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APPENDIX

Fatty acid content determination

1. Component list:

(1) Chromatographic column Model: HP-88 Specification: L: 100m (length) D: 0.250mn (inner diameter) F: 0.20um (coating thickness)

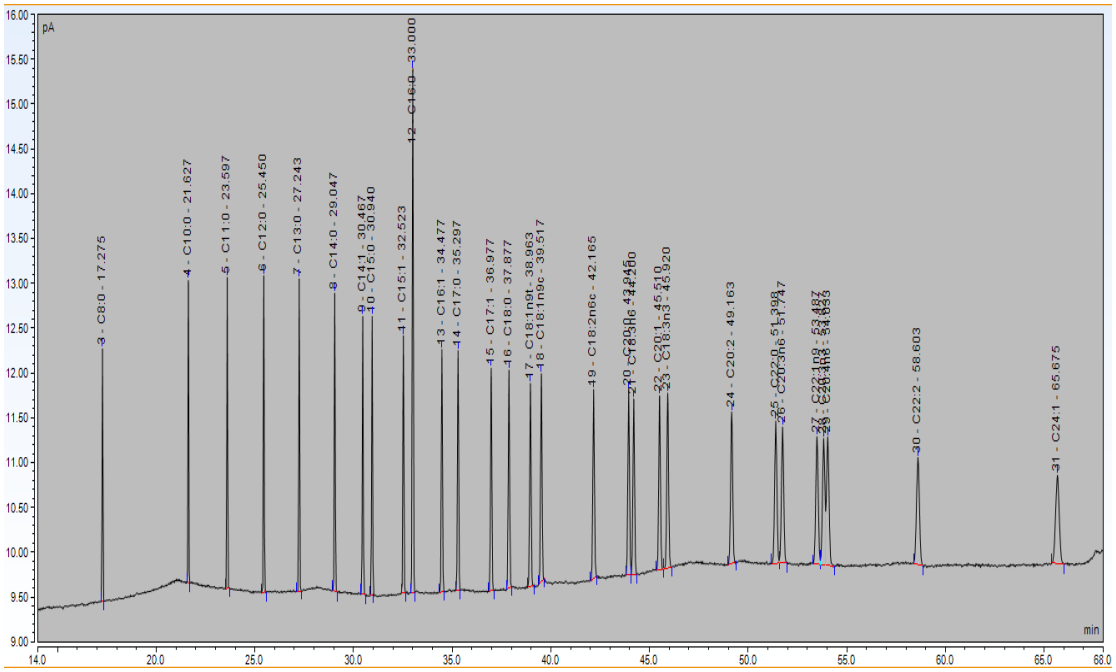
(2) Instrument manufacturer: Thermo Scientific TRACE detector FID (Hydrogen Flame Ionization Detector)

(3) Inlet temperature: 270 ℃, detector temperature 280 ℃, column flow rate: 1.0ml/min, split ratio: 100:1, programmed temperature: 100 ℃ (hold for 13min), 10 ℃/min, increase to 180 ℃ (hold for 6min) , 1 ℃/min to 200 ℃ (hold for 20min), 4 ℃/min to 230 ℃ (hold for 10.5min). Carrier gas: high purity nitrogen injection volume: 1.0ul air flow: 350ml/min, hydrogen flow: 35ml/min, makeup flow: 40ml/min.

2. Sample pretreatment:

Weigh 5g of meat sample in a centrifuge tube, add 100mg of pyrogallic acid, add 6ml of 95% ethanol and vortex, add 4ml of water, add 10ml of hydrochloric acid solution (8.3mol/L) and mix well, and put it in a 70-80℃ water bath Hydrolyze for 40 minutes, shake once every 10 minutes, and cool to room temperature. Add 10ml of 95% ethanol and transfer to a glass tube, add 40ml of diethyl ether and petroleum ether mixture (1:1), extract three times and combine into a 250ml Erlenmeyer flask and spin-evaporate to dryness. Add 8ml of 2% methanol solution of sodium hydroxide, reflux at 80±1℃ for 10min, add 7ml of 15% boron trivertide methanol solution, continue to reflux for 2min, take out and cool to room temperature immediately. Add

10ml of n-hexane and shake for 30s, add 5-6 drops of saturated sodium chloride, cover, take the supernatant, and pass over anhydrous sodium sulfate for inspection.



Fatty Acid Separation Reference Chromatogram