The effect of combined dietary supplementation of herbal additives on carcass traits, meat quality, immunity and intestinal flora composition in Hungarian white geese

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The objective of this study was to evaluate the effects of dietary supplementation of herbal additives on meat quality, slaughter performance and intestinal microbiota of Hungarian white goose . A total of 60 newborn Hungarian white geese were randomly divided into control and herbal complex supplemented (HS) groups. Initially, HS group received the basal diet supplemented with 0.2% of Compound Herbal Additive A (CHAA), which is composed of *Pulsatilla, Gentian* and *Rhizoma coptidis* at the postnatal stage from day 0 to day 42 of age. Then, from day 43 to day 70 of age, HS group was fed the basal diet supplemented with 0.15% of Compound Herbal Additive B (CHAB), which consisted of *Codonopsis pilosula, Atractylodes, Poria cocos* and *Licorice*. The control group was fed the basal diet. The results showed that, compared with the control group, the slaughter rate (SR), half chamber rates (HCR), eviscerated rate (ER) and breast muscle rate (BMR) in the HS group tended to increase slightly (no significance, ns). In addition, the shear force, filtration rate and pH value of breast muscle and thigh muscle in the HS group were slightly enhanced compared to the control group (ns). However, a notable increase in carbohydrate content (P<0.01) and a significant decrease in cholesterol content (P<0.01) was observed in the muscle of the HS group compared to the control group. In addition, the total amino acid (Glu, Lys and Asp) content in the muscle of geese supplemented with the herbal mixture increased compared to the control group (P<0.01). Dietary treatments significantly increased the serum concentrations of IgG (P<0.05) at day 43 and the higher concentrations of IgM, IgG (P<0.01) were observed in the HS group at day 70. Further, 16S rDNA gene sequencing conducted for cecal flora composition found that herbal additives increased the abundance of beneficial bacteria and suppressing harmful bacteria in the cecum of Hungarian white goose. In conclusion, these results provide valuable information that goose diets supplemented with CHAA and CHAB powder could improve the meat

quality, regulate immunity and alter the intestinal flora composition of the Hungarian white geese.

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Abstract

The objective of this study was to evaluate the effects of dietary supplementation of herbal additives on meat quality, slaughter performance and intestinal microbiota of Hungarian white goose. A total of 60 newborn Hungarian white geese were randomly divided into control and herbal complex supplemented (HS) groups. Initially, HS group received the basal diet supplemented with 0.2% of Compound Herbal Additive A (CHAA), which is composed of *Pulsatilla, Gentian* and *Rhizoma coptidis* at the postnatal stage from day 0 to day 42 of age. Then, from day 43 to day 70 of age, HS group was fed the basal diet supplemented with 0.15% of Compound Herbal Additive B (CHAB), which consisted of *Codonopsis pilosula, Atractylodes, Poria cocos* and *Licorice*. The control group was fed the basal diet. The results showed that, compared with the control group, the slaughter rate (SR), half chamber rates (HCR), eviscerated rate (ER) and breast muscle rate (BMR) in the HS group tended to increase slightly (no significance, ns). In addition, the shear force, filtration rate and pH value of breast muscle and thigh muscle in the HS group were slightly enhanced compared to the control group (ns). However, a notable increase in carbohydrate content ($P<0.01$) and a significant decrease in cholesterol content ($P<0.01$) was observed in the muscle of the HS group compared to the control group. In addition, the total amino acid (Glu, Lys and Asp) content in the muscle of geese supplemented with the herbal mixture increased compared to the control group ($P<0.01$). Dietary treatments significantly increased the serum concentrations of IgG ($P<0.05$) at day 43 and the higher concentrations of IgM, IgG ($P<0.01$) were observed in the HS group at day 70. Further, 16S rDNA gene sequencing conducted for cecal flora composition found that herbal additives increased the abundance of beneficial bacteria and suppressing harmful bacteria in the cecum of Hungarian white goose. In conclusion, these results provide valuable information that goose diets supplemented with CHAA and CHAB powder could improve the meat quality, regulate immunity and alter the intestinal flora composition of the Hungarian white geese.

Keywords: Goose, Slaughter performance, Immune, Gut microbiota composition, Compound Chinese herbal medicine additives, Meat quality.

Introduction

Producing healthy livestock and poultry with meat products that are low in fat and high in protein is required to fulfill people's health and nutrition demands. (Li et al. 2019). Goose meat, which is rich in protein and minerals, is considered to be a healthy and highly nutritious food with beneficial effects on human health (Simopoulos 2008). Numerous diseases, including as cardiovascular disease, inflammation and autoimmune disorders, are influenced by the imbalance of n-6 and n-3 polyunsaturated fatty acids in the diet(Qi et al. 2010). The higher concentration of polyunsaturated fatty acids in goose flesh makes it a healthier choice for consumers. In recent years, goose production in China has accounted for approximately 94% of the global total production (Liu and Zhou 2013; Yu et al. 2020).The use of antibiotic growth promoters (AGPs) has been extensively applied in poultry diets to prevent and treat disease in the past few decades to improve geese growth, so that antibiotics for feed utilization could maximize profits and efficiency. However, the misuse of widespread antibiotics has led to an increasing number of drug-resistant strains of bacteria in animal products, posing a serious threat to human and animal health. Therefore, AGPs banned by the European Union in 2006 (Villanueva 2012), by North America in 2017, and by China in 2020. Sustainable special additives that are safe, effective, and consumer-friendly are required to support animal production.

It is reported that over the past few decades, Chinese herbal medicines and their purified components have been extensively studied as growth enhancers and antibiotic alternatives (Seidavi et al. 2021). Chinese herbal medicine has shown great promise in terms of improving immunity, reducing inflammation, and providing antibacterial and antioxidant properties (Hernandez et al. 2004; Acamovic and Brooker 2005; Giannenas et al. 2018). Thus, they can protect the intestinal mucosa of poultry, stimulate digestion and affect the intestinal microflora of poultry, both in quantitative and qualitative ways (Abd El-Hack et al. 2022; Khalaji et al. 2011; Abolfathi et al. 2019). Many studies have shown that Chinese herbal medicine plays a significant role in antioxidant, immunomodulatory functions, intestinal mucosal protection and promoting digestion and absorption in the gastrointestinal tract(Gu, Hao, and Xiao 2022). It is currently confirmed that the following Chinese herbal medicines: *Pulsatilla, Gentian, Rhizoma coptidis, Codonopsis pilosula, Atractylodes, Poria cocos* and *Licorice* also have similar functions. (Zhong et al. 2022; Mirzaee et al. 2017; Wang et al. 2019; Fu et al. 2018; Bailly 2021b; Tian et al. 2019).

With the large-scale production and intensification of animal husbandry gaining steam, the poultry growth rate increased significantly, which greatly shortened the feeding cycle, but generated a severe metabolic burden and reduced immunity, leading to the destruction of meat quality (Xing et al. 2019). Chinese herbal medicine has been shown to have antioxidant activity and previous studies evinced potential beneficial effects on poultry meat quality and immunity (Qaid et al. 2022; Xie et al. 2022). It has been demonstrated that gentian, as a bittering agent, can affect digestion by stimulating bitter receptor cells in rats' gastrointestinal tracts, increasing appetite, and thus improving nutrient absorption and muscle water retention (Mirzaee et al. 2017). Moreover, many reports indicate that the addition of herbs such as *licorice* can improve the quality of meat in broilers and fattening pigs (Ahmed et al. 2016; Qiao et al. 2022). Flavonoids and triterpene saponins are the main active substances of *licorice*, showing antioxidant, immunomodulatory and anti-inflammatory activities that alleviate oxidative damage, thus improving meat quality(Zhai et al. 2020; Abo-Samaha et al. 2022). *Licorice* extract improves the immune response of animals by increasing interferon and serum globulin concentrations(Toson et al. 2022). Moreover, *Poria cocos* polysaccharide has also been shown to have multiple immune effects, by promoting antibody production in B lymphocytes and the spleen, increasing serum antibody IgG levels, and enhancing the phagocytosis of macrophages(Tian et al. 2019; Pu et al. 2019). *Codonopsis pilosula*, an important traditional medicinal plant, has been reported to be used to effectively enhance immunomodulatory effects(Bailly 2021a).

Studies have proven that *Pulsatilla*, *Rhizoma coptidis*, *Codonopsis pilosula* and *Atractylodes* have a role in regulating intestinal flora(Li et al. 2020; Wang et al. 2019; Bailly 2021a, 2021b). *Pulsatilla* chinensis saponins (PRS) is the main active component of *Pulsatilla* and produce antioxidant and immunomodulatory pharmacological activities (Li et al. 2020). In a rat model of dextran sodium sulfate (DSS)-induced ulcerative colitis, the administration of PRS regulated the composition and biodiversity of the rat intestinal flora, significantly improving DSS-induced UC and reducing inflammation(Liu et al. 2021). In addition, *Rhizoma coptidis* has a variety of pharmacological effects consisting mainly of alkaloids such as berberine, coptisine and palmatine, which can exert antioxidant, intestinal flora regulation and anti-viral effects(Lyu et al. 2021; Zhang, Guo, et al. 2021; Wang et al. 2018). Studies have shown that *Codonopsis* polysaccharides can enhance intestinal mucosal immune function by stimulating the secretion of sIgA, while potentially repairing damaged intestinal flora by stimulating the growth of lactic acid bacteria (Fu et al. 2018; Zou et al. 2019). According to previous studies, sesquiterpene lactams and lactones are the main active components of *Atractylodes macrocephala*, which have good anti-inflammatory and antioxidant activities (Bailly 2021b). *Atractylodes* are commonly used in the treatment of gastrointestinal disorders due to their potential role in treating spleen deficiency and regulating intestinal flora (Feng et al. 2020; Wang et al. 2022). It has been demonstrated that there is a synergistic effect between the bioactive components of herbal mixtures, so that mixtures of multiple herbs can show higher biological efficiency than single herbs (Xu et al. 2022). For thousands of years, complex herbal formulas have often been used in the practice of Chinese medicine to treat diseases. Previous studies have shown that the use of herbs with similar therapeutic properties can have a synergistic effect (Zhou et al. 2016; Li et al. 2021). Therefore, the therapeutic effect observed in this experiment may have been achieved by the combined application of several herbal mixtures.

In our study, three herbs (*Pulsatilla, Gentian* and *Rhizoma coptidis*) and four herbs (*Codonopsis pilosula, Atractylodes, Poria cocos* and *Licorice*) were crushed, dried, ground, mixed and steamed to obtain an CHAA and CHAB, respectively. As mentioned above, these herbal mixtures have beneficial effects such as antioxidants, immunological boosting, and improved intestinal health. Currently, most research on herbal combinations has been performed on mammals rather than poultry and especially geese (Li et al. 2016; Huang et al. 2021; Li et al. 2021). Therefore, this study aims to investigate the effects of CHAA and CHAB on carcass traits, meat quality, serum Ig concentrations and intestinal flora diversity of Hungarian white goose.

Materials & Methods

Ethics Statement

All Hungarian white geese were housed and used according to the animal experimental guidelines set by the Institute of Animal Care and Use Committee of Jilin Agricultural University (approval number No. 2020 04 30 001. Date: 12 April 2020). All animals were maintained in pathogen-free conditions and cared for in accordance with the International Association for Assessment and Accreditation of Laboratory Animal Care policies and certification.

Animals

A total of 60 one-day old Hungarian white geese were involved in this research. The geese were purchased from the breeding base of the Goose Research Center of Jilin Agricultural University and the average weights of male and female goslings were about 4300 g and 3600 g, respectively.

Preparation and characterization of Chinese herbal complex

The specific CHAA and CHAB were provided by Changchun General Animal Husbandry Station and used for two feeding experiments at the initial and growth stages of development. To prepare the herbal additives, Chinese herbs were dried, crushed and pulverized into powder, before being screened through an 800-mesh sieve, and then mixed in proportion to create a herbal compound that was stored at room temperature (25 ℃) (Xu et al. 2022). The herb combination CHAA, which contained *Pulsatilla*, *Gentian* and *Rhizoma coptidis* in the ratio of 2:1:1, was used in the initial stage (from days 0 to 42), while CHAB, contained *Codonopsis pilosula*, *Atractylodes*, *Poria cocos* and *Licorice* in the ratio of 3:3:3:2 was supplemented to the basal diet at the growth stage (from days 43 to 70). According to the studies, total saponin compositions in herbal mixtures are primarily derived from *Pulsatilla*, *Codonopsis pilosula*, and *Licorice*, with alkaloids serving as the primary active substances in *Rhizoma coptidis* and *Atractylodes*. The main sources of total flavonoids were *Rhizoma coptidis* and *Licorice*, while the compositions of total polysaccharides in herbal mixtures mainly come from *Codonopsis pilosula*, *Atractylodes* and *Poria cocos* (Fu et al. 2018; Li et al. 2022; Wu et al. 2021). It has been conclusively shown that polysaccharides, flavonoids, saponins and alkaloids are the main antioxidant compounds (Xu et al. 2022).

Experimental Design

The sixty geese were randomly divided into two groups, each with three replicates of 10 geese. The first group, the control group, was fed with the basal diet (corn–soybean) during both the starter (0-42 days of age) and grower periods (43-70 days of ages) (table 1), and the second group, the herbal complex supplemented groups (HS), was fed with a basal diet (corn–soybean) supplemented with 0.2% of CHAA in starter phases and 0.15% of CHAB in grower phases. The diets were provided twice a day. Throughout the experimental period, the geese were under feeding procedure, zoo-hygienic and managerial conditions, as previously reported (Zheng et al. 2022) . The ventilation system was activated for 5-7 h per day in the 7th-15th days of age, with ventilation reduced or eliminated on rainy days. The ambient temperature in an experimental house was maintained at 30 ± 1 °C during the first week, gradually decreased to 24 ± 1 °C in the second week and exposed to natural environmental conditions thereafter. The artificial lighting program was 23 ± 1 h of light followed by 1 h of darkness until day 10, 18 h ± 1 h from day 11 to day 13 and natural light was used to achieve light until day 70.

Serum antibodies detection

From each group, ten Hungarian white geese were randomly selected to collect blood via the goose wing vein and collected into anticoagulant (heparin) tubes on days 42 and 70 of the experimental period, after a 12 h withdrawal period. The serum samples were obtained by centrifuging (3,000 × g for 10 min) at 4°C, and stored at -20 °C until analysis, referring to Farahat et al. (Farahat et al. 2017). Concentrations of immunoglobulin M (IgM), immunoglobulin G (IgG) and immunoglobulin A (IgA) were determined using an ELISA kit (Nanjing Ao qing Biotechnology Co. Ltd., Jiangsu, China), according to the manufacturer's instructions. Each measurement was replicated three times.

Carcass characteristics

At the end of the experiment (at 70 d), 10 geese from each group were randomly selected, weighed, slaughtered and allowed to bleed. The slaughter process was conducted in a commercial slaughterhouse in accordance with standard procedures(Miao et al. 2020). Afterward, the geese were scalded, de-feathered and their carcasses were eviscerated. The liver, heart, gizzard, neck and abdominal fat were excised and weighed to determine carcass weight (CW, g), the slaughter weight (SW, g) was recorded and the slaughter rate (SR) was calculated according to the formula below:

$$\boldsymbol{SR(\%) = \frac{CW}{SW} X\ 100\%}$$

The weights of the heart, liver, lung, kidney, stomach without content, the abdominal fat pad, breast muscle and leg muscle were recorded individually. The half chamber rate (HCR) and the eviscerated rate (ER) were calculated by the following equations:

HCR (%) = Half chamber weight/Live weight ×100%. ER (%) = Eviscerated weight/Live weight × 100%. The ratio of breast muscle and thigh muscle was calculated using the percentage of total chamber weight.

Meat quality indicators

To measure the quality of the meat, breast and thigh meat samples dissected from each of the geese selected for slaughter were used for pH, shear force and filtration rate analysis. The pH value was evaluated using a microprocessor pH meter (ATF-500, Japan Kyoto Electronics Co., Ltd. Japan). The shear force (g) was measured using a digital meat tenderness meter (J C-LM3. MATTHAUS. Germany) and the filtration rate was evaluated using the procedures described by

Miao et al. and Boz et al,. However, a pressure gravimetric analysis was used to determine the filtration rate, applying the following equation: Filtration rate (%) = initial weight-final weight/initial weight ×100%.

Muscle chemical composition detection

Analysis of basic muscle chemical composition was carried out using various methods. The contents of moisture, protein, fat, carbohydrates, energy and cholesterol were determined according to the corresponding methods of the National Food Safety Standard (GB 5009.3-2016) (GB 5009.5-2016) (GB 5009.6-2016) (GB 28050-2011) (GB 28050-2011) (GB5009.128-2016). Additionally, the contents of trace elements (including Zn, Fe, Ca, P, Na and Se) were measured according to the national food safety standards (Zhejiang Jiuan Testing Technology Co., Hangzhou, China).

Amino Acid Composition Analysis

Amino acid levels (including Asp, Thr, Ser, Glu, Gly, Ala, Val, Met, Leu, Iso, Tyr, Phe, His, Lys, Arg and Pro) were measured according to the related procedure described by the National Food Safety Standard (GB5009.124-2016). The amino acid content in muscle was analyzed on an L-8900 automatic amino acid analyzer (Hitachi, Japan).

DNA Extraction and 16S rRNA Gene of intestinal microbiota Sequencing

Caecum contents were aseptically collected from geese and then stored at -80℃ until analysis. Total DNA was extracted from cecal content samples using the Hi Pure Soil DNA Kits (Ovison, Beijing, China) following the manufacturer's instructions. The V3-V4 hypervariable region of the bacterial 16S rRNA139 gene was amplified with the forward primer 341F (CCTACGGGNGGCWGCAG) and the reverse primer 806R (GGACTACHVGGGTATCTAAT). The PCR products were confirmed with 2% agarose gel electrophoresis, purified with the A x y Prep DNA Gel Extraction Kit (A x y gen Biosciences, Union City, CA, USA) following the manufacturer's instructions. Sequencing libraries were generated using the SMR T bell TM Template Prep Kit (PacBio, Menlo Park, CA, USA) according to the manufacturer's protocol. After the library was qualified by an Invitrogen Qubit 3.0 fluorometer (Thermo Fischer Scientific, USA) and a FEMTO pulse system (Agilent Technologies, Santa Clara, CA, USA). The purified amplicons were combined on the Illumina MiSeq platform for equimolar and paired-end sequencing at Ovison Gene Sequencing Biotechnology Co., Ltd (Beijing, China).

According to the Illumina MiSeq sequencing results, the high-quality screened sequences at both ends are paired and joined according to the overlapping bases using the FLASH software. Sequencing data were processed by QIIME software to eliminate suspicious sequences, and the sequence numbers of the above-mentioned effective sequences were calculated to obtain sequences that could be used for subsequent analysis(Edgar 2010). Paired sequences were merged and divided into the Operational Taxonomic Units (OTUs) representative sequences according to the 97% sequence similarity and the sequence with the highest abundance in each OTUs was selected as the representative sequence of OTUs.

Subsequently, all representative sequences were compared using MUSCLE (version 3.8.31) software to construct the phylogenetic relationship, and further, the data of all samples were normalized to perform the *alpha* and *beta* diversity analyses. The final weighted and weighted UniFrac distances were calculated using QTIME (version 1.7.0), to compare microbial structures between different samples. In addition, the samples were clustered based on an unweighted or weighted UniFrac distance matrix using the unweighted pair group method (UPGMA) performed in QIIME (version 1.7.0). Moreover, linear discriminant analysis (LDA) and effect size (LEfSe) software were used to identify the significant differences in microorganisms between groups. Finally, to illustrate differences in microbial composition, LDA scores were calculated and taxonomic cladograms of microbial composition were generated.

Statistical Analysis

The experimental design was a randomized block design with three replicates per treatment. Data on growth performance, meat quality, serum globulin concentration, and intestinal flora diversity were analyzed using the one-way or two-way ANOVA of the SPSS software (version 20) and statistics from this experiment were recorded with WPS2020. The graphs were made with Graph Prism 5.0 software. All data were presented with appropriate standard error, as mean values ± SEM. (ns, no significance; * p<0. 05, ** p<0. 01, *** p<0. 001).

Results

Serum IgM, IgG and IgA concentrations

The effects of the herbal mixtures, CHAA and CHAB, on the serum IgG, IgA and IgM concentrations of geese are presented in Figure 1. The results showed that CHAA and CHAB supplementation in geese diets had no effect on serum concentrations of IgA (ns), but compared with the control group, the level of IgG concentrations on day 43 was significantly higher in the HS group (P<0.05). At day 70, there was a significant increase in serum IgM and IgG concentrations in the HS group fed with HCAB compared with the control group (P<0.01).

Carcass traits

The influence of CHAA and CHAB powders on the carcass traits of geese is shown in Figure 2. The slaughter rate (SR), half chamber rates (HCR), eviscerated rate (ER) and breast muscle rate (BMR) of geese in the HS group were slightly higher than those in the control group at day 70, but no significant (ns) differences were observed.

Meat quality

Characteristics of the quality of the meat (pH, filtration rate and shear force) for the breast and thigh muscle samples of the geese aged 70 days are presented in Table 2. The results clearly showed that no significant difference (ns) was found between the control group and the other treatment group concerning the meat quality indicators of the breast and thigh muscle samples.

Muscle chemical composition

The chemical composition (moisture, protein, fat, carbohydrate, cholesterol and trace elements: Zn, P, Fe, Na, Ca and Se) of the meat were detected to explore the effect of Chinese herb supplementation. The results are shown in Figure 3. There was no significant effect (ns) of dietary treatment on the moisture, protein and fat levels of the muscle samples among the two groups, in contrast, a significant increase in the carbohydrate content ($P<0.01$) was observed and an obvious decrease in cholesterol content ($P<0.01$) was noticed in the HS group compared with the control group (Figure 3a). On the other hand, the energy level in the HS group was significantly higher ($P<0.05$) compared to the control group (Figure 3a). For the contents of Zn, P, Fe, Na, Ca and Se (Figure 3b) in the muscle, a significant decrease ($P<0.01$) was noticed in comparison to the control group.

Effect of herbal mixture on amino acid composition

The amino acid levels in muscle are shown in Figure 3c. Results demonstrated that the total amount of Glu, Lys and Asp in the muscle of the HS group samples showed a significant increase ($P<0.01$) compared with the control group. On the other hand, the contents of Thr, Ser, Ala, Val, Iso, Leu, Tyr, His and Arg in the muscle samples of the HS group were all slightly higher than those of the control group, but no statistical difference was noticed (ns).

Gut microbial diversity and composition

As shown in Figure 4a, we noticed that the Shannon sparsity curve of each sample reached the saturation platform, which indicates that the sample has enough sequencing coverage and reliable data for subsequent analysis. Also, the results shown in figure 4b indicate that the ranking-abundance curves suggest a high abundance and low variation of OTUs in the community, with a uniform distribution and sufficient homogeneity of species. Generally, the Chao1 index, observed-species index and Simpson index were analyzed to investigate the effect of herb additives on the alpha diversity of the goose intestinal flora. There were no significant differences in the Chao1 index, the observed-species index, or the Simpson index between the HS group and the control group (Figure 4d). However, principal coordinates analysis (PCoA) was used to analyze the differences in cecum microbial community structure between the control and HS groups, based on weight UniFrac. As shown in Figure 4c, the microbial composition of the HS and control groups at days 43 and 70 was similar and no notable difference was observed. In contrast, the bacterial communities in the cecal samples of the HS group at day 43 were significantly different compared to those of the HS group at day 70.

Analysis of the Intestinal Flora Structure

To elucidate the effect of herb additives on the structure of goose cecum flora, the study analyzed the relative abundance of the two groups of samples at the phylum and genus levels. The species composition analysis of intestinal flora revealed that no significant changes were observed in the microbial composition between the HS and control groups during the starter period (0-42 d), while during the grower period (43-70 d), those changes were notably marked. At the phylum level the main common bacterial phylum to the HS group were *Firmicutes*, *Proteobacteria* and *Bacteroidetes*. The cecal flora composition of the geese was dominated by the *Firmicutes,* accounting for 52.46%*, Proteobacteria,* accounting for 25.61% and *Bacteroidetes,* accounting for 5.47%, which were higher in the HS group at day 70 compared to the other groups, and those three phyla are the main groups of the animal gut flora (Figure 5a). At the genus level, the taxonomic unit with the highest average abundance is *Lactobacillus* (5.73%) in the HS group at day 70. Specifically, the control group had a significantly higher abundance of *Escherichia-Shigella* (30.89%), *Desulfovibrio* (5.37%) and *Fusobacterium* (6.61%) and a lower abundance of *Paraclostridium* (9.56%) than the HS group at day 70 (Figure 5b). To determine the relative abundance of the specific cecal flora composition, a heatmap was constructed using R language software. The results are illustrated in Figure 6. Initially, the relative abundance of *Lactobacillus* was highest in the HS group at day 70, medium at day 43 in the control group and lowest at day 70 in the control group. Additionally, the highest relative abundance of *Peptostreptococcus, Fusobacterium, Escherichia coli, Shigella* and *Desulfovibrio* was observed in the control group in comparison with the HS group at day 70. Similarly, the highest relative abundance of *Bacteroides, Clostridium sensu stricto type 1* and *Enterococcus* was noticed in the HS group at day 70 compared to the HS group at day 43.

Linear Discriminant Analysis Effect Size (LEfSe) Analysis

To identify the specific bacterial taxa that may be responsible for the observed differences in community structure associated with CHAA and CHAB, a LEfSe was conducted on the caecum community. There were 41 biomarkers with statistical differences in the linear discriminant analysis distribution histogram. Among these, the biomarker bacterial taxa were enriched in the HS group at day 43, including *Lactobacillus sharpeae*, *Streptococcus_parauberis*, *Corynebacteriales*. The bacterial taxa with the highest average richness corresponding to the HS group on day 70 were *Epulopiscium*, *Lachnospiraceae bacterium mt14*, *Bacteria*, and *Paraclostridium*. Also, *Actinobacteria*, *Alphaproteobacteria*, *Micrococcales*, *Bifidobacteriales*, *Bifidobacteriaceae*, *Bifidobacterium*, *Lactococcus hircilactis* et al. were shown the highest average richness corresponding to the control group on day 43. The control group at day 70 had the highest average richness of bacterial taxa, which included *Fusobacterium*, *Fusobacteriaceae*, *Bacteroides caecigallinarum*, *Lawsonia* et al. (Figure 7).

Discussion

In recent years, the development of antibiotic resistance and antibiotic residues in food have become serious problems, therefore, antibiotics are gradually being banned as growth promoters in animal husbandry. Hence, the discovery of alternatives to antibodies and the use of herbal feed additives have become more common and significant in the poultry industry(Gao et al. 2022). It has been widely reported that Chinese herbal medicines have been shown to have pro-oxidant, anti-inflammatory, feed utilization, immunomodulatory, and intestinal flora balance properties (Khan et al. 2022; Huang et al. 2021). Furthermore, herbal medicine as an alternative to antibiotic feed additives also improved the quality of poultry meat(Bellucci et al. 2022; Yu et al. 2021). Several studies have revealed that combined herbal mixture have been shown to be more effective in improving animal health than single herbal extracts (Xu et al. 2022). However, the effect of Chinese herbal medicine mixtures on poultry production at different growth stages is not yet known. The present study aims to investigate the effects of CHAA (containing, *Pulsatilla, Gentian,* and *Rhizoma coptidis*), used at the starter phase and CHAB (containing, *Codonopsis pilosula, Atractylodes, Poria cocos* and *Licorice*), used at the growth phase, on the meat quality, immunity and intestinal flora of Hungarian white geese.

Since the gosling has a short digestive tract, poor digestive gland function and weak immunity during the starter phase period (0-42), it is crucial to enhance digestion and enhance the resistance against pathogens. HCAA contains *pulsatilla, gentian* and *Rhizoma coptidis,* which are widely known to have significant functions in antioxidants, immune regulation, the balance of intestinal flora, and the promotion of gastric juice secretion. Therefore, utilizing CHAA can significantly boost geese's low resistance and weak digestive system in the early stages (Lyu et al. 2021; Zhang, Guo, et al. 2021; Wang et al. 2018; Mirzaee et al. 2017).The main characteristics of geese in the growth stage are improved digestion and resistance, but since bones, muscles, and feathers develop faster, it is crucial to improve spleen and stomach functions in order to improve the diet of geese at this stage to ensure their growth and development. There is increasing evidence that *Codonopsis pilosula*, *Atractylodes* and *Poria cocos* can regulate spleen and stomach function and these organs are the main source according to the Qi-blood theory of Chinese medicine (Gong and Hu 2022; Cheng et al. 2009; Zhang, Luo, et al. 2021; Yan et al. 2021). As a result, CHAB was consequently added to the basal diet during the grower phase (43 -70). In addition, our previous study has revealed that adding 0.2% CHAA and 0.15% CHAB to the diet has a better effect on Zi geese.

Immunoglobulins are important indicators of the immune response status of animals and play a very important role in the immune system (Mikocziova, Greiff, and Sollid 2021). Studies have shown that plant polysaccharides can improve the immune function of poultry (Long et al. 2021; Yu et al. 2022), activate macrophages to exert immunomodulatory effects by recognizing and binding to specific receptors (Zhang, Liu, et al. 2021). Polysaccharides is rich in *Codonopsis pilosula*, *Atractylodes* and *Poria cocos.* In addition to polysaccharides, some other chemical components in herbal medicines also showed immune-enhancing effects, such as flavonoids in *Rhizoma coptidis* and glycyrrhizic acid in *Licorice* (Zhang, Liu, et al. 2021). Previous research showed that *Codonopsis pilosula*, *Poria cocos*, and *Licorice* supplementation can improve the serum immune performance of animal, especially serum concentration of IgG and IgM (Zhan, Yang, and Xiao 2015; Xie et al. 2013; Yin et al. 2022). This study found that supplementation of

CHAA and CHAB enhanced humoral immune responses by increasing serum IgG and IgM concentrations, which was in line with the research findings of the above studies.

Studies have shown that meat quality is closely related to immune function. Meat quality traits can be evaluated by several indicators, such as pH value, filtration rate, shear force, fat content and flavor . Meat pH is one of the most important indicators affecting meat quality and it has been widely reported that lactic acid accumulation caused by anaerobic respiration after slaughter can affect water holding capacity . In this study, we found that the addition of Chinese herbal medicine had no effect on the meat pH value of Hungarian white goose. Similarly, several studies have argued that Anacardium occidentale leaf and Moringa oleifera leaf supplementation had no influence on meat pH. On the other hand, shear force was reported to be an important sensory characteristic for assessing meat quality, in which the tender taste improves with decreasing shear force. Regarding the shear force and the filtration rate of breast and thigh muscles, a slight increase was observed with the pre-administration of CHAA and CHAB in Hungarian white goose, which indicates that the dietary supplementation of these herbals had no effect on the meat quality. This was in accordance with the previous research (Yu et al. 2020), which reported that the addition of cottonseed meal to the feed had no effect on the meat quality of the geese. In contrast, some studies have found that the addition of Astragalus and Glycyrrhiza complex polysaccharides could improve broiler meat quality by reducing muscle water loss and shear force (Qiao et al. 2022). The composition and content of amino acids are important factors in meat quality, which are frequently used to predict the nutritional value and flavor of meat . It has been well known that the composition and content of flavor amino acids, including Gly, Ala, Asp, Glu, Phe and Tyr, affect directly the freshness and flavor of meat . The diet with CHAA and CHAB significantly increased the total amino acid levels, especially Glu, Lys and Asp levels, in the muscle of Hungarian white geese. As a result, these findings proved that CHAA and CHAB supplementation could improve the quality of goose meat by increasing the concentration of flavor amino acids and total amino acids. Similar, it is reported that the addition of resveratrol increases the concentration of flavor-enhancing AA content in Pekin ducks .Cardiovascular disorders are mostly caused by the level and type of cholesterol found in foods derived from muscle . Clinical studies have shown that natural antioxidants (flavonoids, saponins, alkaloids, etc.) have the ability to reduce the risk of cardiovascular disease . In this study, we observed that cholesterol concentrations in geese fed herbs were significantly lower than those in geese fed a basal diet. Moreover, Markina (Markina et al. 2022) reported that *Licorice* has been shown to have an anti-atherosclerotic effect. Also, it has been suggested that the correlation between anti-atherosclerotic activity and low concentrations of cholesterol is positive , which may explain the decrease in cholesterol after adding CHAB, which contains *licorice* in our study. However, the meat has more nutritional value when its water content is lower and its carbohydrate and protein levels are higher. Furthermore, it has been widely known that a certain amount of moisture contributes to the juiciness and tenderness of the meat and fat affects the flavor, protein content, as well as tenderness of the meat .This study indicated that moisture, protein, and fat were not affected by dietary treatment of herbs.Carcass traits can directly reflect the growth and development of poultry and are important indicators for evaluating the growth performance of animal meat . The results showed no difference in the percentage of carcass traits tested or carcass weight for all treatments. These findings are consistent with previous studies . This may be related to the fact that during avian development, carcass traits grow at a similar rate to bodyweight . It is also possible that the CHAA and CHAB used in our study did not increase the content of protein and energy in the feed, resulting in similar carcass weights between the two groups.

The intestine is not only the main place for food digestion and nutrient absorption but also an important immune organ to improve body resistance to disease and promote the healthy development of animals . In this study, we analyzed the microbiota of the cecum contents of Hungarian white geese by high-throughput sequencing of 16S rRNA amplicons. The results revealed that the predominant phyla were *Firmicutes*, followed by *Proteobacteria* and *Bacteroidetes*, which was in accordance with previous studies (Li et al. 2017; Liu, Luo, et al. 2018). The *Firmicutes*/*Bacteroidetes* ratio (F/B) was significantly correlated with the capacity to obtain energy(Kasai et al. 2015). In the present study, we found that dietary CHAA and CHAB supplementation increased this ratio. Moreover, *Lactobacillus*, as a probiotic, can improve poultry production performance and immunity by regulating gut microbiota (Bian et al. 2016). The results showed that the addition of Chinese herbs increased the levels of *Lactobacillus*.

Further, *Bacteroides* and *Desulfovibrio* have been reported to be major producers of LPS(Diling et al. 2017). At the genus level, we found that the abundance of *Bacteroides* and *Desulfovibrio* in the HS group decreased at day 70 of age, indicating that CHAB may inhibit the proliferation of these bacteria, reduce the secretion of LPS and reduce the inflammatory response. *Escherichia coli/Shigella* is a pathogenic bacterium that causes the bloody diarrheal diseases of bacillary dysentery and hemorrhagic colitis . Studies have shown that flavonoids and saponins can inhibit the growth of pathogens such as intestinal *Escherichia coli* and *Pseudomonas* . Polysaccharides inhibited the number of pathogenic bacteria by promoting the production of short-chain fatty acids and organic acids in the intestine , significantly increasing the abundance of dominant bacteria, thereby regulating the function of the intestinal flora and maintaining the balance of the intestinal flora .In this experiment, the abundance of *Escherichia-Shigella* in the HS group decreased at day 70 of age which was in line with the results reported by (Chen et al. 2020). As a conclusion, CHAA and CHAB can regulate the balance of intestinal flora by increasing the level of beneficial bacteria and reducing the level of pathogenic bacteria.

Conclusion

In conclusion, the results of this study indicate that CHAA and CHAB supplementation had no effect on filtration rate, shear force, pH value, moisture content, fat content and carcass traits of the goose meat, but significantly improved the meat quality and flavor by improving the total amino acid content (including Glu, Lys and Asp) and reducing the cholesterol content in the goose. Additionally, dietary supplementation with CHAA and CHAB significantly stimulates and improves humoral immunity by increasing the serum content of IgG and IgM. It can also be concluded that dietary supplementation with either CHAA or CHAB showed an influence on increasing the beneficial flora content and decreasing the pathogenic flora content of the cecum. Generally, these results provide valuable information that goose diets supplemented with CHAA and CHAB could improve the meat quality, regulate immunity and alter the intestinal flora composition of the Hungarian white geese.

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Figure 1

Effects of herbal additives on serum Ig parameters in Hungarian white geese at different ages.

HS= herbal complex supplement group. Addition of CHAA during the initial stage (from days 0 to 42) of geese feeding (HS 42d). Addition of CHAB during the growth stage (from days 43 to 70) of geese feeding (HS 70d). * Significant difference compared to the Control group (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). All data was presented with appropriate standard error, as mean values ± SEM.

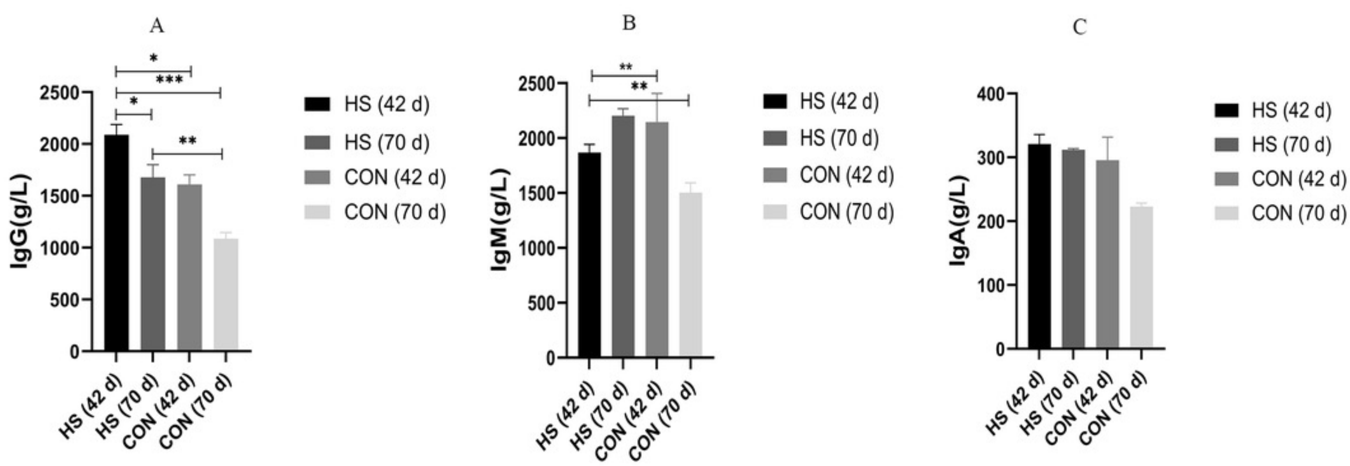

Figure 2

Effects of herbal mixture on carcase traits of Hungarian white geese.

SR=slaughter rate; HCR=half carcass rate; ER= eviscerated rate; BMR= breast t muscle rate.

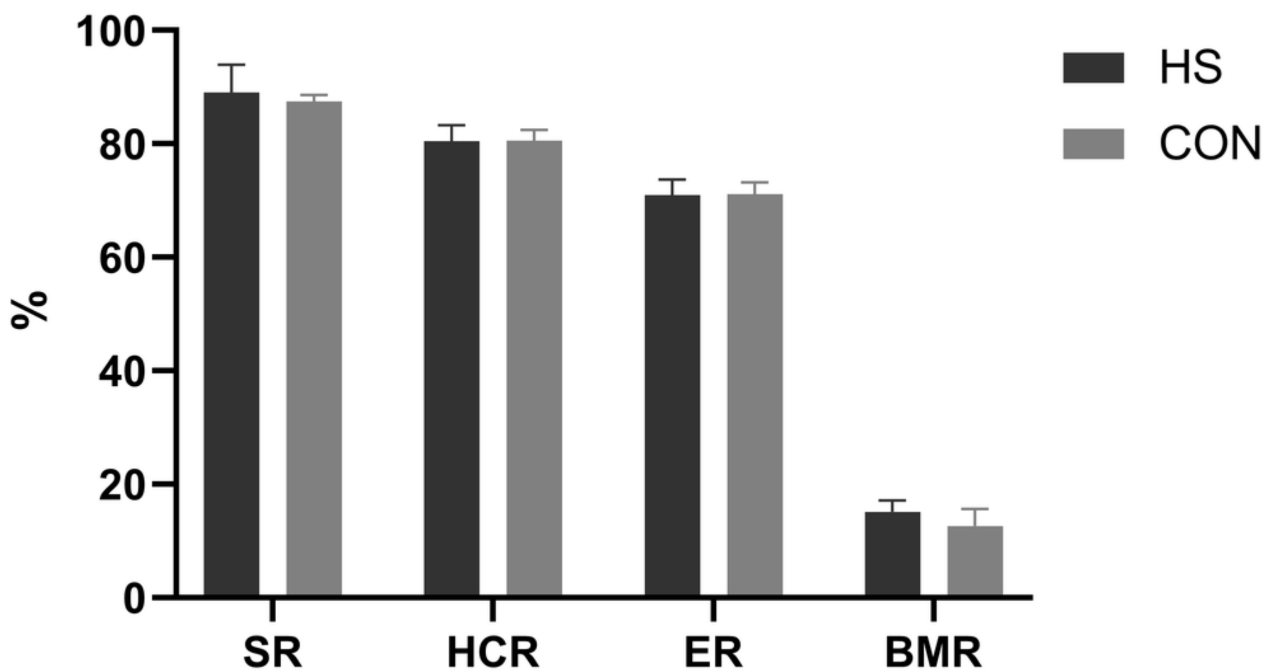

Figure 3

Effect of herbal additives on the multi-component index of Hungarian white geese muscle.

A The concentrations of carbohydrate, energy, moisture, cholesterol, protein, and fat in Hungarian white goose. (B) The concentrations of Zn, p, Fe, Na, Ca and Se in Hungarian white goose. (C) The concentrations of various amino acids in Hungarian white goose. * Significant difference compared to the Control group (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). Data are the mean of 3 replicates of 2 samples each. All data was presented as mean values ± SEM, with appropriate standard error.

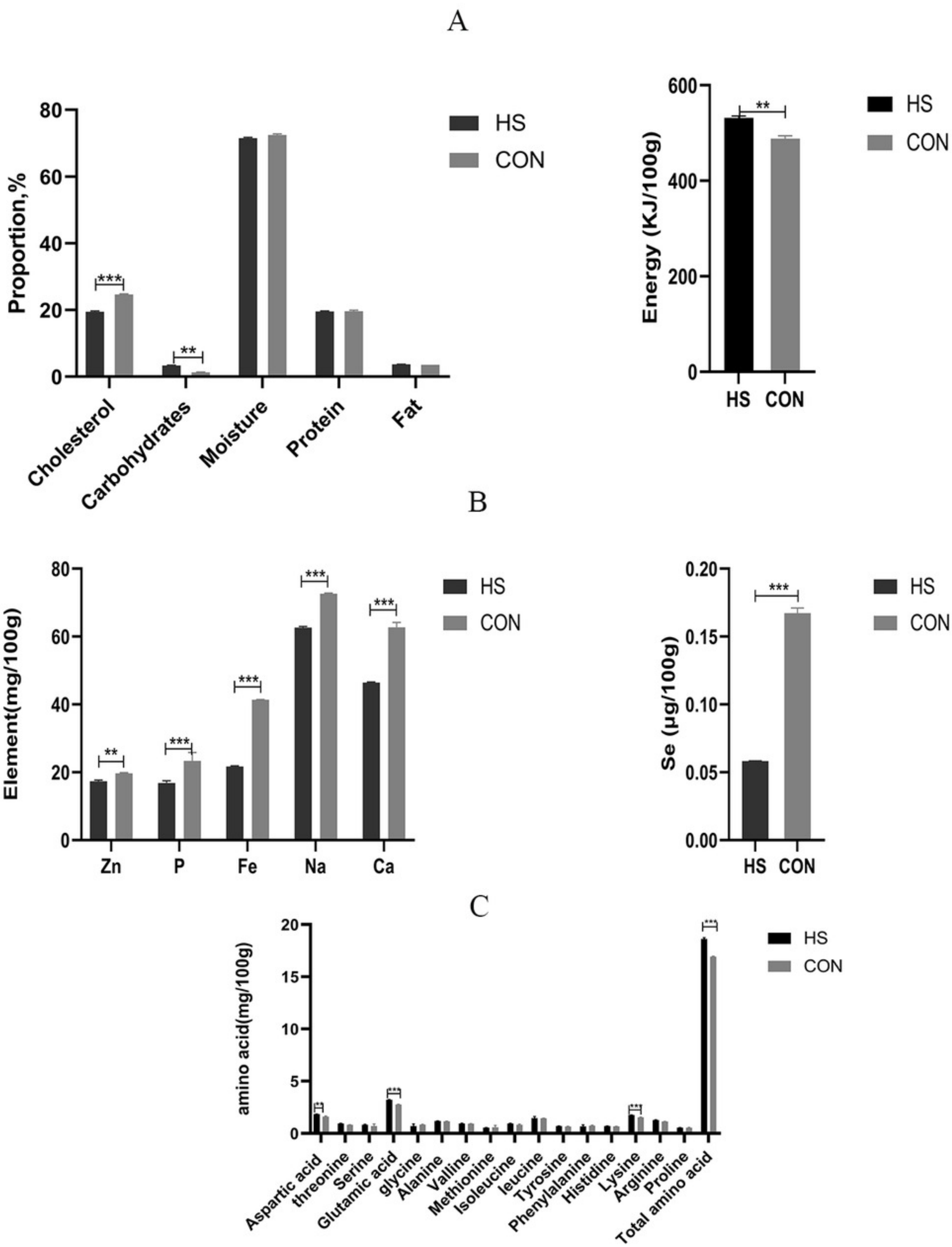
A
Proportion,%
80
60
40
20
0
Cholesterol
Carbohydrates
Moisture
Protein
Fat
***
**
HS
CON
Energy (KJ/100g)
600
400
200
0
HS
CON
**
HS
CON
B
Element(mg/100g)
80
60
40
20
0
Zn
P
Fe
Na
Ca
**
***
***
***
***
HS
CON
Se (µg/100g)
0.20
0.15
0.10
0.05
0.00
HS
CON
***
HS
CON
C
amino acid(mg/100g)
20
15
10
5
0
Aspartic acid
threonine
Serine
Glutamic acid
glycine
Alanine
Valline
Methionine
Isoleucine
leucine
Tyrosine
Phenylalanine
Histidine
Lysine
Arginine
Proline
Total amino acid
HS
CON

Figure 4

Effects of herbal mixture on the intestinal microbiota diversity in Hungarian white geese.

(A) Sample Shannon sparse curve. The horizontal coordinate indicates the number of sequences and the vertical coordinate indicates the Shannon value. When the curve tends to flatten, it indicates that the amount of sequencing data is large enough to reflect the vast majority of microbial information in the sample. (B) Rank Abundance Curve. The greater the abundance of species, the greater the range of the curve on the horizontal axis, the flatter the curve, the more evenly distributed the species. (C) Principal Coordinates Analysis (PCoA) based on weighted Unifrac metrics. (D) Alpha diversity analysis of four experimental groups. The horizontal coordinate is the group name and the vertical coordinate is the Alpha index. Chao1 and shannon were used as richness estimates. obeserved-species index was used to indicate the number of OTUs actually observed with increasing sequencing depth. The obeserved-species index was used to calculate the number of OTUs actually observed as sequencing depth increased. A= HS group (P 42), B= HS group (P 70), C=Control group (P 42), D= Control group (P 70).

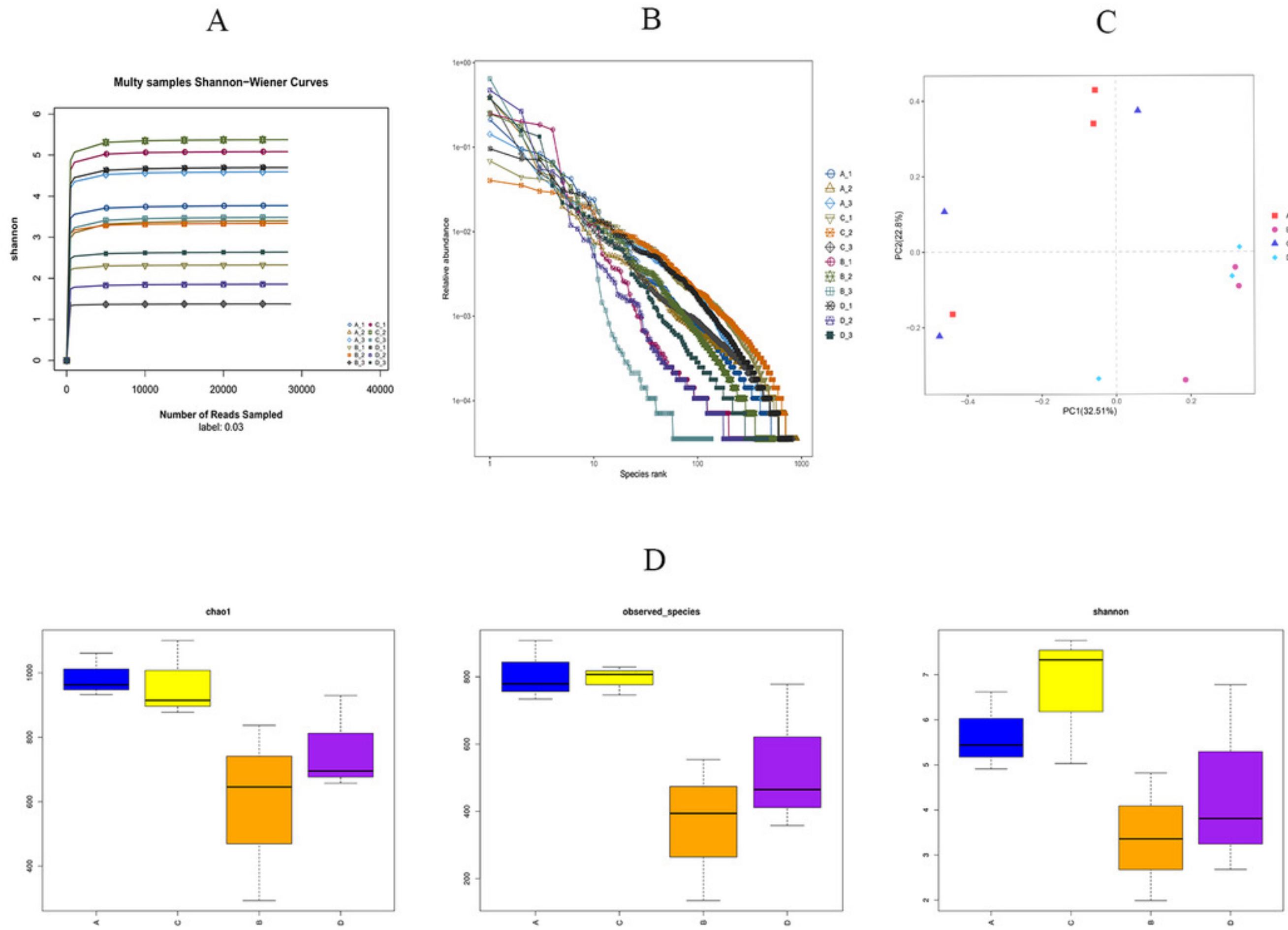
A
B
C
D
Multy samples Shannon−Wiener Curves
shannon
Number of Reads Sampled
label: 0.03
Relative abundance
Species rank
PC1(32.51%)
PC2(22.8%)
chao1
observed_species
shannon

Figure 5

Effect of herbal additives on intestinal microbiota compositions of goose cecum flora at the phylum (A) and genus (B) levels.

The horizontal coordinates in the figure are the group names and the vertical coordinates are the relative abundance of each taxonomic unit at a given taxonomic level, the longer the column, the higher the relative abundance of taxonomic units in the corresponding sample.A= HS group (P 42), B= HS group (P 70), C=Control group (P 42), D= Control group (P 70).

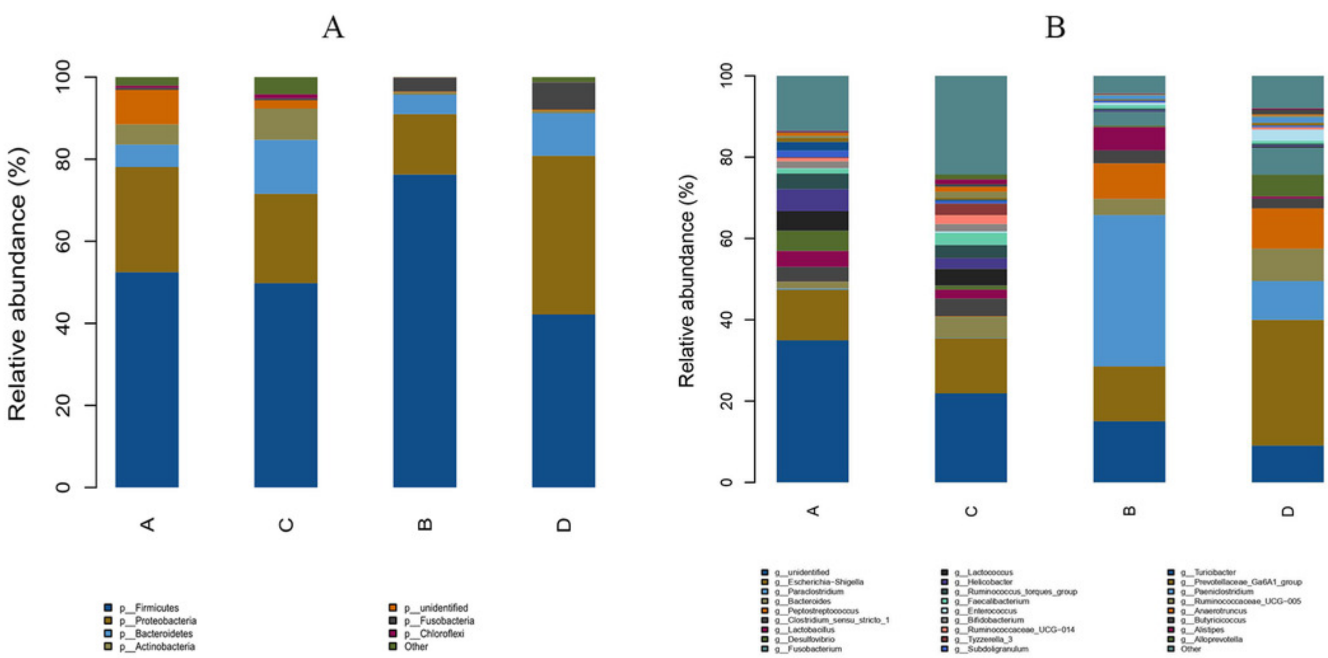

Figure 6

Heatmap depicting the relative abundance of each group of bacterial genera.

Group names are plotted on the x-axis and the y-axis represents each bacterial genus. A= HS group (P 42), B= HS group (P 70), C=Control group (P 42), D= Control group (P 70).

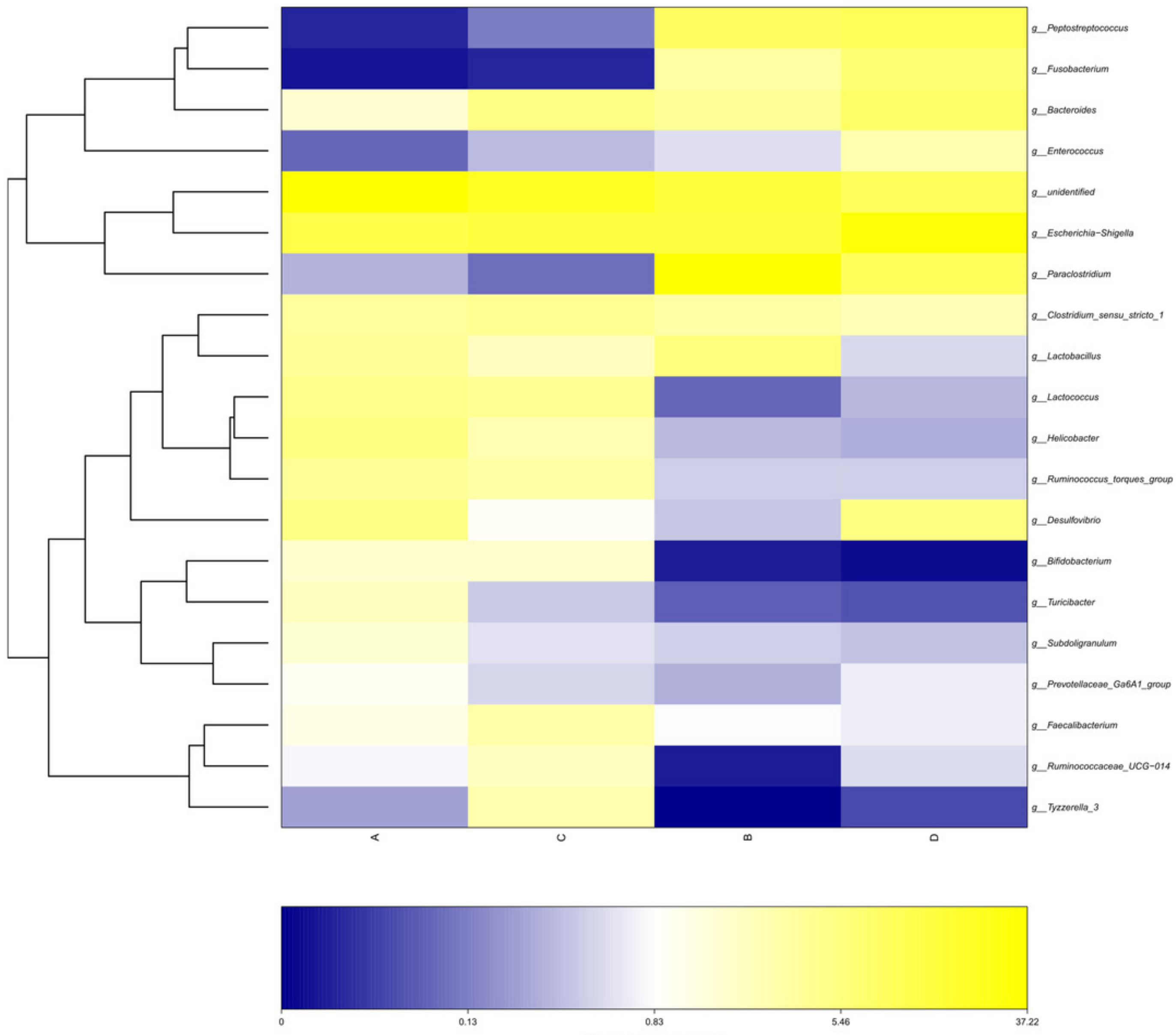

Figure 7

LDA scores obtained from the LEfSe analysis of the gut microbiota in different groups.

Species with signifcantly diferent abundances in diferent groups are shown, and the length of the bar graph represents the efect size of the signifcantly diferent species. phylum to genus: p, phylum; c, class; o, order; f, family; g, genus. A= HS group (P 42), B= HS group (P 70), C=Control group (P 42), D= Control group (P 70).

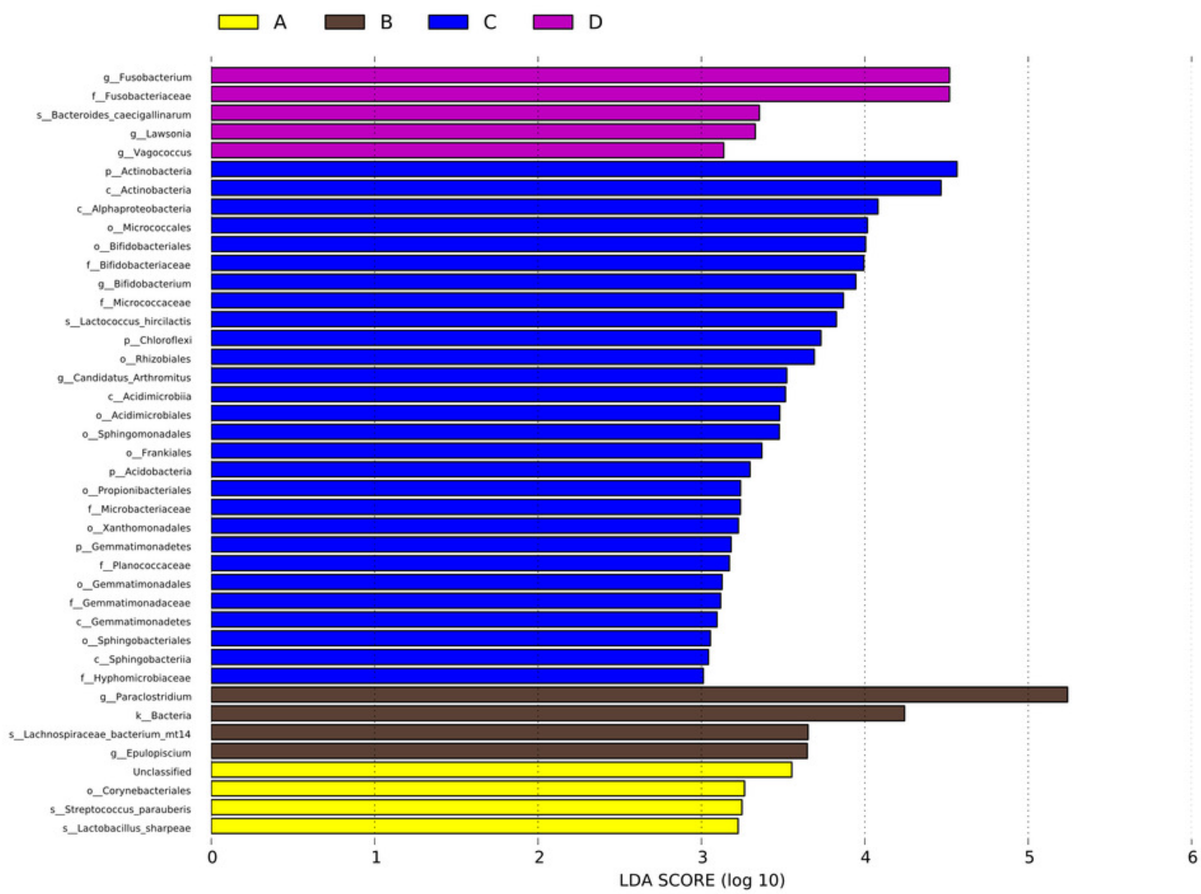

Table 1(on next page)

Ingredients and composition of basal diets (DM basis) %.

ME=Metabolizable energy, CP = crude protein, CF = crude fiber, Ca = calcium, P = phosphorus, LYS: lysine, Met: Methionine.

Table.1Ingredients and composition of basal diets (DM basis) %.

Items	Starter 0 to 42 d	Grower 43 to 70 d
Ingredients, %		
Corn	52.00	53.00
Soybean meal	22.00	14.00
Wheat bran	8.00	15.00
Fish meal	4.00	1.00
Corn gluten meal	4.00	2.00
Stone meal	6.00	6.00
Calcium hydroxide	1.50	1.80
Soybean oil	1.20	1.50
Salt	0.30	0.30
Rice bran	0.00	4.00
Additives	1.00	1.00
Total	100	100
Chemical composition, %		
CP	19.68	14.82
CF	3.00	6.88
MET	0.67	0.55
LYS	1.34	1,03
THR	0.87	0.66
Ca	0.65	0.59
P	0.48	0.41
ME, kcal/kg	13.02	12.75

ME=Metabolizable energy, CP = crude protein, CF = crude fiber, Ca = calcium, P = phosphorus, LYS: lysine, Met: Methionine.

Table 2(on next page)

Effects of herbs supplementation on on goose meat quality.

Data are presented as the mean ± SEM.

Table.2 Effects of herbs supplementation on on goose meat quality.

muscle	Group	Shear force (g)	Filtration rate (%)	pH value
Breast muscles	HS	102.19±11.13	24.21±9.6	5.58±0.05
	Control	82.00±10.83	24.00±7.8	5.50±0.02
Thigh muscles	HS	71.39±25.69	19.64±5.92	6.22±0.12
	Control	59.70±13.91	16.08±6.43	6.05±0.10

Data are presented as the mean ± SEM.