scientific reports

Check for updates

OPEN

Effect of dietary *Dendrobium nobile* Lindl. On production performance, immune function and caecal microbiota of Chishui black-bone hens

Jinlin Yang[2], Youbo Chen[2], Xingzhou Tian[2], Huan Yu[2], Yushi Shi[2], Depeng Zhao[2], Xia Long[2], Qisong Tan[2] & Hui Li[1,2]✉

The objective of this study was to investigate the effects of dietary supplementation of *Dendrobium nobile* Lindl. (DNL) on production performance, immune function, and caecal microbiota of Chishui black-bone hens. A total of one hundred and eighty 600-day-old Chishui black-bone hens were randomly allocated to two experimental groups, with six replicates of 15 chickens per group. The control group was fed a basal diet, and the experimental group was supplemented with 2100 mg/kg DNL in the basal diet. The results demonstrated that compared with the control group, the addition of 2100 mg/kg DNL to the diet significantly ($P < 0.05$) increased the average egg production rate of Chishui black-bone hens from 38.97 to 44.95%. Interferon-gamma (IFN-γ) levels were significantly lower in the experimental group than in the control group, whereas serum immunoglobulin A (IgA) levels were significantly higher ($P < 0.05$). However, adding DNL exerted no significant effect on egg quality. Asp, Ser, Gly, Leu, Phe, His, Pro, TAA, EAA, and NEAA levels in the eggs of the experimental group were significantly higher ($P < 0.05$) than in those in the control group. Additionally, the EAA/TAA in the control and experimental groups were 40.38% and 40.76%, respectively, and the EAA/NEAA was 76.62% and 74.72%, both of which were higher than the 40% and 60% thresholds specified in the WHO/HAO ideal protein guidelines. The results also indicated that eggs in the experimental group had significantly higher contents of palmitic acid (C16:0), stearic acid (C18:0), palmitic acid (C16:1), oleic acid (C18:ln9c), behenolic acid (C22:0), palmitic acid (C16:), oleic acid (C18:ln9c), saturated fatty acids (SFA), and monounsaturated fatty acids (MUFA) ($P < 0.05$). In contrast, the contents of hexadecenoic acid (C15:0), heptadecanoic acid (C17:0), α-linolenic acid (C18:3n3), arachidonic acid (C20:0), C10 heptadecanoic acid (C17:1), linoleic acid (C18:2n6c), α-linolenic acid (C18:3n3), eicosapentaenoic acid (C20:2), Docosapentaenoic Acid (DPA), polyunsaturated fatty acid (PUFA), and n-6PUFA were significantly reduced ($P < 0.05$). Moreover, when 2100 mg/kg DNL was added to the diet of Chishui black-bone hens, the composition of the intestinal flora was altered. Specifically, the relative abundances of *Bacteroides*, *Lactobacillus*, *Subdoligranulum*, and *Parabacteroides* increased, whereas those of *Desulfovibrio*, *Lachnoclostridium*, *Ruminococcaceae*, *Fournierella*, *Faecalibacterium*, and *Oribacterium* decreased. In conclusion, adding 2100 mg/kg DNL to the feed can increase serum IgA and reduce IFN-γ content in Chishui black-bone hens during the late laying period. Furthermore, it can enhance the laying rate and types of amino acids in eggs, as well as the ratio of ideal proteins. Simultaneously, it improved the caecal microbial community of Chishui black-bone hens and increased the relative abundance of beneficial bacteria.

Keywords *Dendrobium nobile* Lindl., Egg production rate, Immune function, Fatty acid, Amino acid, Caecal microbiota

[1]College of Animal Science, Guizhou University, Guiyang 550025, Guizhou, China. [2]Key Laboratory of Plateau Mountain Animal Genetics Breeding and Reproduction of Ministry of Education, Guizhou Provincial Key Laboratory of Animal Genetics Breeding and Reproduction, Guizhou University, Guiyang 550025, Guizhou, China. ✉email: ellenlihui@sina.cn

Abbreviations

DNL	Dendrobium nobile Lindl
DM	Dry matter
EM	Metabolic energy
CP	Crude protein
TP	Total phosphorus
AP	Available Phosphorus
Lys	Lysine
Met	Methionine
Cys	Cysteine
ADFI	Average Daily Feed Intake
FCR	Feed Conversion Ratio
TP	Total Protein
IgA	Immunoglobulin A
IgM	Immunoglobulin M
IFN-γ	Interferon-gamma
ALB	Albumin
IL-12	Interleukin-12
SOD	Superoxide dismutase
BUN	Blood urea nitrogen
AST	Aspartate Aminotransferase
T- AOC	Total antioxidant capacit
ALT	Alanine Aminotransferase
CRE	Creatinine
TAA	The total amino acid
EAA	Essential amino acid
NEAA	Nonessential amino acid
SFA	Saturated fatty acid
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
DPA	Docosapentaenoic acid

Dendrobium nobile Lindl. (DNL) is a perennial epiphytic herb belonging to the family Orchidaceae. It is one of the main *Dendrobium* species recorded in the Chinese pharmacopoeia and has ornamental and medicinal value. There are approximately 1500 species of *Dendrobium* worldwide, with 74 species and two varieties in China. Among these species, more than 50 are of medicinal value[1]. The use of medicinal *Dendrobium* can be traced back 1500 years in China, as recorded in Shen Nong Ben Cao Jing[2,3]. Clinical treatment in Chinese medicine considers *Dendrobium* to be beneficial to the stomach and intestine[4]. Dozens of macromolecular compounds have been identified in plants of the genus *Dendrobium*, mainly polysaccharides, amino acids, terpenoids, alkaloids, polyphenols, and flavonoids[5,6]. These compounds play various important roles. In contrast to other natural feed additives which typically contain only one or a few active compounds, DNL contains various, abundant macromolecular compounds. These substances offer distinct advantages for animal production. *Dendrobium* polysaccharides boost immune cell proliferation, thereby enhancing immune regulation. This property makes it a potential candidate for cancer prevention and treatment as it can inhibit the proliferation of tumour cells and scavenge free radicals to achieve anti-ageing effects [7; 8]. In a rat model of polycystic ovarian syndrome induced through letrozole, DNL polysaccharides have been shown to exert protective effects on the ovaries[9]. Further, alkaloids isolated from *Dendrobium* exhibit neuroprotective, anti-inflammatory, and antitumor activities[10]. Polyphenols regulate clearance of reactive oxygen species (ROS) either directly or by enhancing glutathione peroxidase activity, thereby regulating the expression of inflammatory genes in macrophages and lung epithelial cells[11]. As the organism ages, inflammation occurs[12]. Intestinal inflammation may reduce egg production in chickens by disrupting yolk precursor production associated with liver inflammation[13]. However, some studies have found that in mice, phenols and polysaccharides of *Dendrobium* can alleviate intestinal inflammation[14,15]. Flavonoids can be used to prevent cardiovascular disease and provide liver protection, while many also exhibit antibacterial, anti-free radical, and antioxidant properties[16]. Flavonoids have the same effect as phytoestrogens[17]. The use of flavonoids can significantly improve animal production performance, increase resistance to disease, and improve immune function[18–20]. For example, total flavonoids Rhizoma Drynaria (TFRD) attenuates the effects of aflatoxin B1 on breast muscle meat quality and improves meat quality in male broilers[21]. *Dendrobium* polysaccharides can increase the diversity of beneficial microorganisms in the gut and reduce the abundance of harmful bacterial groups by increasing the production, transportation, and utilisation of short-chain fatty acids (SCFAs) in the intestine[22,23]. Recent studies revealed the dietary effects of *Dendrobium* leaves on chicken meat quality, fatty acid composition, and volatile compound profiles[24]. Supplementation of the diets with appropriate dosages of *Dendrobium* leaves may enhance the growth performance, antioxidant capacity, immune response, and intestinal health by promoting intestinal integrity and modulating the caecal microbiota of broiler chickens[25]. Based on the various beneficial effects observed in related studies on different aspects of *Dendrobium* species and their compounds, we hypothesised that DNL may exert similar positive effects.

Egg production efficiency is an important economic characteristic in the global poultry industry which depends on the number of ovulated or atretic follicles and the potential of the oviduct to convert fertilized egg into hard-shelled eggs[26]. In the later stages of egg production, hens experience oxidative stress, disease, and low levels of reproductive hormones, resulting in a reduction in the number of follicles and slow follicular

development, which in turn leads to reduced egg production and quality[27]. Amino acids and fatty acids are important indicators of egg quality. The composition and structure of various amino acids in eggs are very similar to those in human proteins and are easily absorbed and utilised by the body, making eggs an important source of high-quality proteins for human intake. Amino acids in food play an essential role in maintaining normal physiological, biochemical, and immune functions as well as growth, development, metabolism, and other vital activities[28]. The fatty acid content of eggs is closely associated with human health. Addition of plant additives to hen feed can alter the composition and content of amino and fatty acids in eggs, which can positively impact human dietary health[29]. The diet supplemented with 6 g of cottonseed protein hydrolysate (CPH) per kilogram can significantly enhance the production performance, carcass characteristics, intestinal microbial health, antioxidant capacity, and immunity of broiler chickens[30]. In addition, the United States, the European Union, and China have explicitly banned the use of antibiotics in feed, and considering the growing trend of such bans, there is an urgent need to identify and develop alternatives to antibiotics[31,32].

Medical research has shown that *Dendrobium* can exert antioxidant effects, enhance cellular immunity, promote follicle development, and regulate the intestinal flora; however, there are no studies on the use of *Dendrobium* as an additive in animal husbandry. Therefore, we hypothesised that adding *Dendrobium* to chicken diets may enhance antioxidant capacity, strengthen immunity, and improve production performance, and we evaluated the feasibility and reliability of *Dendrobium* as a feed additive based on the test results. By providing an alternative to traditional feed additives, DNL may help reduce reliance on antibiotics and improve the sustainability and safety of animal production. This is an advantage over existing natural feed additives, which may have limitations in terms of cost, availability, and effectiveness.

Materials and methods

Ethics statement

This study was approved by the Animal Care and Use committee of Guizhou University (Approval number: EAE-GZU-2022-T144). All methods were carried out in accordance with the ARRIVE guidelines (https://arriveguidelines.org/) and were performed following the relevant guidelines and regulations.

Experiment material

DNL (in powdered form) was purchased from DNL Industrial Development Co., Ltd. (Chishui City, Zunyi, Guizhou province, China). The dendrobine content was determined by gas chromatography with methanol as the extractant and naphthalene as the internal standard. The gas chromatograph (7820 A VL) was purchased from Agilent Technologies, Inc. (Beijing, China). *Dendrobium* polysaccharides were determined using the onionone-sulphuric acid method and the control was glucose[33]. The content of dendrobine was 0.41%, that of *Dendrobium* polysaccharides was 9.47%, and that of total flavonoids ranged from 0.03 to 0.05% per gram in DNL.

Animals

The feeding experiments were conducted at Guizhou Zhuxiang Chicken Breeding Co., Ltd. (Zunyi, Guizhou, China). A total of 180 healthy, 600-day-old, Chishui black-boned hens weighing 2.12 ± 0.03 kg were used. Chickens were considered healthy as per the following criteria: they had smooth feathers and vivid, alert eyes, with no damage or abnormal colouration in their plumage; they were highly active and vigilant, displaying normal feeding and drinking behaviours, as well as stable walking; the rectal temperature was within the normal range, respiration was even and regular, appetite was normal, and faeces exhibited a normal appearance[34]. The chickens were randomly assigned to two groups with six replicates per group and 15 chickens per replicate. Animals had free access to feed and water during the rearing period. In the chicken house, the temperature was strictly maintained at 25–30 °C using thermostatic heaters and fans. Humidity was kept between 60% and 65% using dehumidifiers and humidifiers, as necessary. Chickens were exposed to 16 h of light and 8 h of dark per day using energy-efficient light-emitting diodes. In addition, each hen was housed in an individual cage (with a space of approximately 0.18 square metres). The experiment included a 15-day pre-feeding period followed by 90 days of normal feeding. The pre-feeding phase was implemented to help the chickens acclimate to the new environment and stabilize their physiological conditions. Ninety days of normal feeding was chosen to ensure sufficient time for observing long-term effects and changes. The control group was fed a basal diet, and the experimental group was fed a basal diet supplemented with 2100 mg/kg DNL powder. The DNL dosage was determined based on the 2020 edition of the Pharmacopoeia of the People's Republic of China and previous research by our team. The amount added was calculated based on the weight of the chickens and their feed intake, and then incorporated into the chicken diet. The daily DNL consumption per chicken was calculated using the following Eq. (1):

$$Y = (X / 1000) \times 2100 \quad (1)$$

where Y is the amount of DNL per chicken per day obtained from feed, and X is the amount of feed consumed per chicken per day (in mg).

The nutritional requirements of the chickens were obtained from the NRC 1994 (1994, Nutrient Requirement of Poultry, America). Nutrients in the diets were tested using the AOAC method (1990, Association of Official Analytical Chemists, America). The feed formulation and nutrient levels are shown in Table 1, and the major components of DNL are shown in Table 2.

Ingredients	Content (%)	Nutrient levels[2]	Content (%)
Corn	61.5	DM	92.88
Soybean meal	26.19	ME/(kcal/kg)[3]	2.93
Soybean oil	1.05	CP	15.99
Limestone	7.86	Ca	3.38
Fishmeal	0.1	TP	0.48
NaCl	0.3	AP	0.19
Premix[1]	3	Lys	0.86
Total	100	Met	0.42
		Met + Cys	0.71

Table 1. Composition and nutrient levels of the basal diets (DM basis). [1] 3.0% Premix applied during the egg production period: Cu = 10.2 mg, Fe = 60 mg, Mn = 81 mg, Zn = 81 mg, Se = 0.36 mg, VE = 25.5 IU, VA = 9900 IU, VD3 = 4005 IU, VK3 = 2.55 mg, VB3 = 36 mg, VB5 = 10.5 mg, VK3 = 2.55 mg, VB1 = 2.1 mg, VB2 = 6 mg, VB6 = 4.05 mg, VB12 = 0.024 mg, VH = 0.27 mg, choline chloride = 360 mg, water = 10%, methionine = 4.0%. [2] Nutrient level is the measured value, and [3]ME (metabolic energy) is the calculated value.

Ingredients	Content (g/100 g)
Cellulose	72.41 ± 2.21
Dendrobine	0.41 ± 0.01
Dendrobium polysaccharide	9.47 ± 0.16
Crude protein	1.62 ± 0.04
Crude fat	2.12 ± 0.12

Table 2. Composition and content of the main components in DNL. The polysaccharide of Dendrobium was determined by onion-sulfuric acid method with glucose as the control.

Determination of main components in *Dendrobium nobile* Lindl

Determination of dendrobine content

Determination of Dendrobine Content by Gas Chromatography (General Chap. 0521). The stems of Dendrobium nobile were dried in an oven, pulverised using a grinder, and passed through a 60-mesh sieve. The resulting powder was stored in a dry beaker until further use. Approximately 0.25 g of the prepared powder (passed through a No. 3 sieve) was carefully transferred into a round-bottom flask. A 25 mL methanol solution containing 10.05% formic acid was added, and the total weight was recorded (denoted as M_1). The mixture was heated under reflux for 3 h. After cooling to ambient temperature, the flask was reweighed (M_2), and the lost solvent mass (M_1 - M_2) was replenished with the methanol-formic acid solution to restore the original weight. The mixture was thoroughly shaken and filtered through neutral filter paper. A 2 mL aliquot of the filtrate was pipetted into a 5 mL volumetric flask. Subsequently, 1 mL of an internal standard solution was accurately added, and the volume was adjusted to the mark with methanol. The solution was homogenised, and 1 µL was injected into a gas chromatograph for analysis. Dendrobine content was quantified based on peak area ratios and calculated on a dry weight basis.

This analysis employed a DB-1 capillary column with a 100% dimethylpolysiloxane stationary phase (30 m length × 0.25 mm internal diameter, 0.25 µm film thickness). A standard calibration curve was constructed by plotting the peak area ratios of dendrobine reference solutions at varying concentrations. Aliquots of dendrobine reference solution (0.1, 0.2, 0.4, 0.8, 1.6, and 2.0 mL) were transferred into separate 5 mL volumetric flasks. To each flask, 1 mL of internal standard solution was added, followed by dilution to the mark with methanol. After homogenisation, the calibration curve was generated by regressing concentration (y-axis) against the peak area ratio (x-axis). The linear regression equation was determined as $y = 0.8026 \times c - 0.2078$ ($R^2 = 0.999$), confirming excellent linearity. A 2 mL aliquot of dendrobine reference solution was precisely pipetted into a 5 mL volumetric flask. After the addition of 1 mL of naphthalene internal standard solution, the mixture was diluted to volume with methanol and analysed by gas chromatography. The correction factor (f) was calculated using the following formula (2):

$$f = \frac{As/cs}{Ar/mr} \tag{2}$$

In the formula, (As) represents the peak area of the internal standard substance; (Ar) represents the peak area of the reference substance; (cs) represents the concentration of the internal standard substance; and (mr) represents the concentration of the reference substance.

The content of the sample was calculated based on the peak areas of the *Dendrobium nobile* Lindl. solution and the internal standard, as well as their respective concentrations, using the following formula (3):

$$Cx = f\frac{Ax}{As/cs} \tag{3}$$

In the formula, (Ax) denotes the peak area of the Dendrobium nobile solution; (As) denotes the peak area of the internal standard substance; (cs) denotes the concentration of the internal standard substance; and (Cx) denotes the concentration of the *Dendrobium nobile* Lindl. solution.

Determination of polysaccharide content in Dendrobium

Using an electronic balance (accurate to 0.0001 g), weigh out 1 g of anhydrous glucose reference standard. Transfer the anhydrous glucose reference standard into a previously tared crucible. Place the crucible containing the reference standard in an oven at 105 °C and dry to constant weight. Precisely weigh 0.033 g of the dried reference standard and slowly transfer it into a 100 mL volumetric flask. Add 80 mL of distilled water and shake well to ensure complete dissolution of the reference standard. Dilute to the 100 mL mark with distilled water and mix thoroughly. Prepare six 10 mL centrifuge tubes. Using a pipette, transfer 0.1 mL, 0.2 mL, 0.4 mL, 0.8 mL, 1.6 mL, and 2.0 mL of the reference standard solution into separate 10 mL centrifuge tubes. Add distilled water to each tube to reach the 2.0 mL mark and mix well. Place all centrifuge tubes in an ice-water bath. Slowly add 0.2% anthrone-sulfuric acid solution to the 10 mL mark, mix thoroughly, and allow to cool. After cooling, place the tubes in a water bath and maintain the temperature for 10 min. Remove the tubes and immediately place them in an ice-water bath for an additional 10 min. Use distilled water as a blank. Measure the absorbance at 582 nm using ultraviolet-visible spectrophotometry (General Chap. 0401). Plot the standard curve with absorbance on the y-axis and concentration on the x-axis. The standard curve equation is $y = 0.0631 \times c - 0.0018$ ($R^2 = 0.9866$).

Dendrobium nobile Lindl. was pulverised using a high-speed grinder and passed through a 40-mesh sieve. Precisely weigh 0.25 g of the sieved *Dendrobium nobile* Lindl. powder and transfer it slowly into a 1,000 mL round-bottom flask. Add 150 mL of 80% ethanol to the flask, then place the flask in a 90 °C water bath for reflux heating for 1 h. Filter the mixture while hot using neutral filter paper. Wash the residue with 10 mL of hot 80% ethanol, repeating this step three times. Transfer the residue and filter paper back into the flask, add 150 mL of distilled water, and heat under reflux in a 100 °C boiling water bath for 1 h. Filter the mixture while hot using neutral filter paper and collect the filtrate in a beaker. Wash the residue and flask with 10 mL of hot distilled water four times. Combine the filtrate and washings, and allow the mixture to cool to room temperature. Transfer the cooled filtrate and washings into a 250 mL volumetric flask, dilute to the mark with distilled water, and mix thoroughly. Using a pipette, accurately transfer 1 mL of the diluted solution into a dry 10 mL test tube with a rubber stopper. Following the method described for preparing the standard curve, starting from the addition of water to 2.0 mL, measure the absorbance according to General Chap. 0521. Determine the weight of anhydrous glucose (mg) in the test solution from the standard curve and calculate accordingly. The content of *Dendrobium* polysaccharides, calculated as anhydrous glucose ($C_6H_{12}O_6$), is expressed on a dried basis.

Sample collection and index determination

Production performance

At 6.30 am each day, the feed remaining in the trough was collected in a special feed recycling bucket using a fine brush and weighed on an electronic balance. The amount of feed remaining each day was recorded, and the daily feed intake was calculated from the amount fed and the amount remaining. The stock was counted, eggs were collected at 7 pm each day, and the total egg weight of each replicate was measured on an electronic balance. The date, number of eggs laid, egg weight, and feed consumption were recorded daily in the production logbook. [ADFI (g/d) = cumulative feed intake/ (number of hens × number of days)], average egg weight (AEW = total daily egg mass/laying number), laying rate [LR (%) = (laying number/layer number) × 100], and feed conversion ratio [FCR = total feed intake/total egg weight] of the laying hens.

Serum biochemical indices

At the end of the feeding experiment, a total of 24 chickens (12 randomly selected from each group) were used for subsequent analysis. When collecting blood samples, measures are taken to reduce stress. First, allow the laying hens to acclimatise to the sampling environment in advance. Then, the personnel should approach the hens gently and slowly, seize them with care, and utilise the manual lateral recumbency restraint method. Next, perform rapid blood collection from the wing vein. After blood collection, promptly press the wound for haemostasis and disinfect it. Blood samples (5 mL) were collected from the subwing vein of each chicken using a common non-anticoagulant tube and immediately placed in an ice pack. Blood was centrifuged for 15 min at 3000 r/min in a centrifuge (Heraeus Multifuge XIR; Thermo, Germany). The serum was collected and dispensed into sterile centrifuge tubes. The samples were stored in an airtight refrigerator at -20 ℃. The levels of immunoglobulin A (IgA; H108-1-1), immunoglobulin M (IgM; H109-1-1), interferon-gamma (IFN-γ; H025-1-1), and interleukin-12 (IL-12; H010-1-1) in the serum were determined using enzyme-linked immunosorbent assays (Nanjing Jianxeng Bioengineering Institute). Total protein (A045-3-2), total antioxidant capacity (A015-1-2), albumin (A028-1-1), creatinine (C011-2-1), urea nitrogen (C013-1-1), and superoxide dismutase (A001-3-1) in serum were determined using colourimetric kits (Nanjing Jienjian Institute of Bioengineering). The kits were used according to the manufacturer's instructions. The above indices were measured using a PowerWave XS full-wavelength microplate reader (Bio-tek Instruments Inc., USA) according to the manufacturer's instructions.

Egg quality index

Three eggs were collected from each replicate, and 18 eggs were randomly collected from each group for a total of 36 eggs per treatment group. Egg quality was measured within 24 h in accordance with Chinese standard NY/T 823–2004 (2004, Performance Ferms and Measurement for Poultry, China). An egg quality tester (model

EA-01, Orka, Israel) was used to determine the weight, yolk colour, albumen height, and Haugh unit of each egg. An eggshell strength tester (model EFA-01, Orka) was used to measure the strength of the eggshells in N/m^2, and the yolk weight was measured using an electric balance (model BSA224S, Sartorius Scientific Instruments Co., Ltd., Beijing, China) accurate to 0.01 g. The eggshell thickness and lateral and longitudinal diameters of the three eggshell zones (apical, equatorial, and basal) were measured using a digital Vernier calliper (model MNT-150T; Shanghai Minet Industrial Co., Ltd.) accurate to 0.01 mm. Eggshell thickness was calculated as the average thickness of the three eggshell zones. The egg shape index was calculated as lateral or longitudinal diameter.

Detection of amino acids and fatty acids in eggs

Three eggs were collected per replicate, from each group of 18 eggs; the eggs were broken, egg white and yolk were mixed, weighed, and placed in a freeze dryer. Water content was calculated after 72 h, and the trituration sample was passed through a 1 mm sieve and stored at -80 °C until analysis of amino acid and fatty acid content. Amino acids in whole eggs were analysed according to the National Standard for Food Safety GB/T5009.124-2016 (2016, National Food Safety Standard. Determination of Amino Acids in Food, China). The fatty acids in whole eggs were analysed according to GB5009.168-2016 (2016, National Food Safety Standard. Determination of Fatty Acids in Food, China). The specific assay was consistent with that described previously[29].

Sequencing of the caecal microorganisms and analyses

By day 90 of the trial, six hens per group were selected, and the hens were subjected to electrical stunning at approximately 70 volts. Subsequently, they were humanely euthanized by cervical dislocation. The hens were then dissected by severing the jugular vein with a scalpel and allowing bleeding for 3 to 5 min to collect the contents of the cecum. When sampling the cecal contents of chickens, measures are taken to prevent contamination. These include isolating the chickens in advance, strictly disinfecting the sampling instruments and the environment, disinfecting the chicken bodies during sampling, carefully separating the ceca and collecting the contents, preventing cross - contamination, properly storing and transporting the samples, and promptly cleaning the sampling instruments and the environment. Microbial community sequencing and diversity analyses were performed by Shanghai Magi Biomedical Technology Co., Ltd. First, DNA was extracted from the samples using a QIAamPFast DNA Stool Mini Kit (QIAGEN, Germany). Genomic DNA was detected by electrophoresis on a 1% agarose gel. The SYBR Safe staining method was used to identify DNA, and the reagents were purchased from Thermo Fisher Scientific (USA). Qualified sample DNA was PCR-amplified, and three replicates were performed per sample. PCR products of each sample were mixed and analysed using 2% agarose gel electrophoresis. PCR products were recovered by gel excision using an AxyPrep DNA Gel Recovery Kit (AXYGEN) and eluted with Tris HCl. TransGen AP221-02: TransStart FastPfu DNA polymerase was used for the PCR assay on an ABI GeneAmp® (Model 9700). PCR products were quantified using a QuantiFluor™-ST Blue Fluorescence Quantification System (Promega, USA). After completing the above steps, library construction and Illumina MiSeq sequencing were performed. Paired-end reads were first spliced according to the overlap. At the same time, the sequence quality was controlled, reads were filtered, and the samples were distinguished by operational taxonomic unit (out) clustering analysis and species taxonomy analysis.

Statistical analysis.

Excel 2010 (Microsoft, Redmond, WA, USA) was used to record and process experimental data. SPSS (version 22.0; SPSS software for Windows; SPSS Inc., Chicago, IL, USA) was used to analyse the data. Specifically, an independent samples *t*-test was used for all comparisons. The results are presented as means and standard errors.

OTU analysis of caecal microorganisms was performed using the Uparse software platform (version 7.0.1090; http://drive5.com/uparse/). Caecal microorganisms were clustered using the USearch 7-UPARSE algorithm. OTU sequence similarity was 0.97. The species classification database silva138/16s_bacteria (classification confidence: 0.7, QIIME 1.9.1) was used to generate each taxonomic water abundance table. R software (version 3.3.1) was used to create a Venn map, dilution curve, community heatmap, and principal component analysis (PCA) map. Bacterial and archaeal 16 S rRNA sequences were obtained from the Silva database (Release138 http://www.arb-silva.de), functional genes were obtained from GenBank (Release7.3 http://fungene.cme.msu.edu/), and PICRUSt function predictions were performed using the EggNOG and KEGG databases[35].

Results and discussion

Production performance

The results are presented in Table 3. Compared with the control group, the average laying rate of the experimental group supplemented with 2100 mg/kg DNL was significantly increased ($P<0.05$), although no significant differences ($P>0.05$) were observed in the average daily feed intake, feed-to-egg ratio, or average egg weight.

Serum biochemical indices

IFN-γ levels were significantly lower ($P<0.05$) in the experimental group than in the control group, but serum IgA levels were significantly higher ($P<0.05$). However, dietary supplementation did not significantly affect the serum levels of TP, IgM, ALB, IL-12, SOD, BUN, T-AOC, or CRE. (Table 4).

Egg quality

The results are presented in Table 5. No significant differences between the two groups were observed in egg quality indices, such as egg weight, yolk colour, eggshell thickness, egg shape index, protein height, Haugh unit, and eggshell strength.

Items	Dietary treatment[1]		SEM-value[2]
	CON	Treatment	
ADFI/g	92.5	93.69	1.151
FCR	5	4.49	0.368
Average egg weight (g)	54.13	53.28	0.442
Average egg production rate (%)	38.97b	44.95a	0.019

Table 3. Effect of adding DNL to the dietary ration on the egg production performance of Chishui black-bone hens. Data were the mean of 90 replicates per treatment. [1] Dietary treatments: CON, the control group, fed with the basal diet; Treatment, the trial group, fed with the basal diet supplemented with 2100 mg/kg DNL. [2] SEM = Standard error of the mean.a – b Values in the same row not sharing a common superscript mean a significant difference ($P < 0.05$).

Items	Dietary treatment[2]		SEM-value[3]
	CON	Treatment	
TP (mg/mL)	44.45	42.22	2.413
IgA (mg/gprot)	1.20b	1.23a	0.002
IgM (mg/gprot)	12.19	11.79	1.3
IFN-γ (mg/gprot)	1.74a	1.71b	0.005
ALB (g/L)	21.41	24.11	3.711
IL-12 (g/L)	1.96	1.96	0.004
SOD (U/mL)	130.26	121.74	12.85
BUN (mmol/L)	53.84	55.76	6.848
AST (U/gprot)	14.1	14.38	1.92
T-AOC (U/mL)	6.27	7.23	0.706
ALT (U/gprot)	30.1	36.41	3.51
CRE (μmol/L)	31.27	32.12	1.594

Table 4. Effect of DNL on the serum biochemical indicesof Chishui black-bone hens[1]. [1]Data were the mean of 6 replicates per treatment. [2] Dietary treatments: CON, the control group, fed with the basal diet; Treatment, the trial group, fed with the basal diet supplemented with 2100 mg/kg DNL. [3] SEM = Standard error of the mean. a – b Values in the same row not sharing a common superscript mean a significant difference ($P < 0.05$).

Items	Dietary treatment[2]		SEM-value[3]
	CON	Treatment	
Egg weight (g)	57.13	55.81	1.328
Egg white height (mm)	4.36	4.25	0.316
Egg yolk weight (g)	16.95	17.41	0.573
Shell thickness (mm)	0.27	0.38	0.078
Egg shape index	1.33	1.31	0.032
Yolk colour	6	5.83	0.575
Haugh unit (hu)	62.43	62.73	3.299
Eggshell strength (kg/cm²)	44.82	41.33	5.522

Table 5. Effect of adding DNL to the feed dietary on the egg quality of Chishui black-bone hen[1]. Note: [1] data were the mean of 18 replicates per treatment. [2] dietary treatments: CON, the control group, fed with the basal diet; treatment, the trial group, fed with the basal diet supplemented with 2100 mg/kg DNL. [3] sem = standard error of the mean.

Amino acids in eggs

As shown in Table 6, Asp, Ser, Gly, Leu, Phe, His, Pro, TAA, EAA, and NEAA contents in the eggs of the experimental group were significantly higher ($P < 0.05$) than those in eggs of the control group. In addition, the EAA/TAA in the control and experimental groups was 40.38% and 40.76%, respectively, and the EAA/NEAA

Items	Dietary treatment[2]		SEM-value[3]
	CON	Treatment	
Asp	4.43b	4.81a	0.111
Thr	2.27	2.4	0.049
Ser	3.32b	3.60a	0.089
Glu	5.33	6.24	0.311
Gly	1.48b	1.60a	0.029
Ala	2.43b	2.68a	0.067
Val	2.93	3.08	0.062
Met	1.37	1.52	0.073
Ile	2.33	2.45	0.058
Leu	3.81b	4.10a	0.083
Tyr	1.99	2.09	0.042
Phe	2.85b	3.17a	0.075
His	1.04b	1.13a	0.032
Lys	3.28	3.43	0.072
Arg	2.93	3.08	0.065
Pro	1.65b	1.76a	0.036
TAA	43.44b	47.13a	1.112
EAA	18.85b	20.15a	0.438
NEAA	24.59b	26.98a	0.706
EAA/TAA(%)	43.38	42.76	0.308
EAA/NEAA(%)	76.62	74.72	0.945

Table 6. DNL to the dietary on amino acids in eggs of Chishui black-bone hens[1] (%).

was 76.62% and 74.72%, respectively, and both ratios exceeded the 40% and 60% standards of the WHO/HAO protein guidelines.

[1] Data were the mean of 3 replicates per treatment. [2] Dietary treatments: CON, the control group, fed with the basal diet; Treatment, the trial group, fed with the basal diet supplemented with 2100 mg/kg DNL. [3] SEM = Standard error of the mean. a – b Values in the same row not sharing a common superscript mean a significant difference ($P < 0.05$).

Fatty acids in eggs

As shown in Table 7, the palmitic acid (C16:0), stearic acid (C18:0), palmitic acid (C16:1), oleic acid (C18:ln9c), behenic acid (C22:0), palmitic acid (C16:) oleic acid (C18:ln9c), saturated fatty acid (SFA), and monounsaturated fatty acid (MUFA) contents were significantly higher in eggs of the experimental group ($P < 0.05$) than in the control group. The eggs in the experimental group contained hexadecenoic acid (C15:0), heptadecanoic acid (C17:0), α-linolenic acid (C18:3n3), arachidonic acid (C20:0), c10-heptadecanoic acid (C17: l), linoleic acid (C18:2n6c), α-linolenic acid (C18:3n3), and the eicosapentaenoic acid (C20:2), DPA, PUFA, and n-6PUFA contents were significantly lower ($P < 0.05$) compared with the control group.

Caecum flora analysis

OTU statistics and alpha diversity analysis

Twelve caecal content samples from the two groups were subjected to 16 S rRNA sequencing and the number of reads per sample was determined. The sequencing depth is shown in Table S1. As shown in Table 8, the number of effective sequences in both groups was high, ranging from 32,298 to 35,033. No significant differences ($P > 0.05$) were observed in sequence numbers between the control and experimental groups. The sequence similarity was greater than 97%. The coverage value of the generated OTUs was greater than 99.7%, indicating sufficient sequencing depth. There were no significant differences in Shannon, Simpson, and Chaol indices between the control and experimental groups ($P > 0.05$). The abundance-based coverage estimator (ACE) index of the experimental group was significantly higher than that of the control group ($P < 0.05$). According to the dilution curve (Fig. 1, the Shannon diversity index curve of caecal content gradually flattened with an increase in the number of reads. The results showed that sequencing covered the information of all samples in the two groups, the amount of sequencing data was reasonable, the sequencing depth adhered to the requirements, and the sequence information of the caecal flora in the samples was accurately reflected.

Venn diagram and PCA analysis of caecal flora

As shown in Fig. 2A, there were 906 OTUs in the caecal microbiota of the control group. Among them, 101 OTUs were unique. The experimental group had 942 OTUs. Of these, 137 OTUs were unique, and 805 OTUs were shared by the two groups. The total numbers of OTUs and unique OTUs were higher in the experimental group than in the control group. As shown in Fig. 2B, the PCA analysis revealed that the PC1 of the appendix

Items	Dietary treatment[2]		SEM-value[3]
	CON	Treatment	
Saturated fatty acids(SFA)			
C14:0	0.456	0.477	0.0119
C15:0	0.051a	0.045b	0.0005
C16:0	26.576b	27.172a	0.0409
C17:0	0.143a	0.131b	0.0009
C18:0	7.308b	7.599a	0.0933
C20:0	0.031	0.028	0.0031
C21:0	0.02	0.02	0.002
C22:0	0.068b	0.086a	0.0008
C24:0	0.019	0.014	0.0043
Monounsaturated fatty acids (MUFA)			
C14:l	0.123	0.133	0.0064
C16:l	4.124b	4.343a	0.0717
C17:l	0.108a	0.102b	0.0007
C18:ln9t	0.17	0.159	0.0201
C18:ln9c	45.582b	46.304a	0.1313
C20:ln9	0.013	0.014	0.0009
C22:ln9	0.015	0.015	0.001
Polyunsaturated fatty acids(PUFA)			
C18:2n6c	13.273a	11.653b	0.057
C18:3n6	0.059	0.062	0.0022
C18:3n3	0.310a	0.257b	0.0036
C20:2	0.124a	0.105b	0.0013
C20:3n6	0.12	0.12	0.0011
C20:4n6	0.941	0.939	0.0135
DPA	0.064	0.05	0.0011
C22:6n3	0.114	0.156	0.0419
SFA	34.662b	35.567a	0.1201
MUFA	50.135b	51.070a	0.0633
PUFA	15.031a	13.349b	0.0824
n-3PUFA:	0.488	0.464	0.043
n-6PUFA	14.420a	12.781b	0.0494

Table 7. Effects of adding DNL to the feed dietary on the fatty acid content in eggs of Chishui black-bone hens[1] (%). [1] Data were the mean of 3 replicates per treatment. [2] Dietary treatments: CON, the control group, fed with the basal diet; Treatment, the trial group, fed with the basal diet supplemented with 2100 mg/kg DNL diet. [3] SEM = Standard error of the mean. a – b Values in the same row not sharing a common superscript mean a significant difference ($P < 0.05$).

control and Test III groups was 17.45% and the PC2 was 13.24%. Samples from the control and test groups did not cross-cluster at the OTU level, indicating low species similarity and diversity among the treatment groups. This indicates that the bacterial community in this experiment was significantly altered by the addition of *Dendrobium chrysogenum* to the diet.

Analysis of caecal flora species

In total, 55 phyla and 798 genera were identified. Figure 3 presents the relative abundance of community species at the phylum level of caecal microbes in the samples. Compared with the control group, *Bacteroidetes* increased by 10.12%, *Firmicutes* decreased by 8.36%, and *Desulfobacterota* decreased by 1.68% in the test group. In addition, *Fusobacteria* decreased by 1.14% and *Actinobacteria* increased by 0.97% compared with the control group. The abundance of other phyla also increased in the experimental group compared to that in the control group.

Figure 3 (B, D) presents information on the microbial species in the caecal samples that were more than 1% abundant at the genus level. Compared with the control group, the abundances of Bacteroides, unclassified_f_Rikenellaceae, unclassified_o_Bacteroides, *Lactobacillus*, norank_f_noranko_Clostridia, UCG-014, Christensenellaceae_R-7_group, *Subdoligranulum*, norank_f_Prevotellaceae, UCG-005, and *Parabacteroides* increased in the experimental group. The abundances of Ruminococcustorques_group, unclassified_f_Lachnospiraceae, *Desulfovibrio*, *Lachnoclostridium*, *Ruminococcaceae*, *Fournierella*, *Faecalibacterium*, and *Oribacterium* decreased in the experimental group.

Items	Dietary treatment[2]		SEM-value[4]
	CON	Treatment	
OTUs	926	954	
Coverage (%)	99.7	99.7	
Valid sequences[3]	32510.33	32,298	1871.2
Shannon index	4.49	4.59	0.106
Simpson index	0.03	0.03	0.008
Ace index	678.58b	730.41a	27.713
Chao index	692.39	743.21	20.28

Table 8. Alpha diversity indexes[1]. [1] Data were the mean of 6 replicates per treatment. [2] Dietary treatments: CON, the control group, fed with the basal diet; Treatment, the trial group, fed with the basal diet supplemented with 2100 mg/kg DNL diet. [3] Average number of effective sequences. [4] SEM = Standard error of the mean. a – b Values in the same row not sharing a common superscript mean a significant difference ($P < 0.05$).

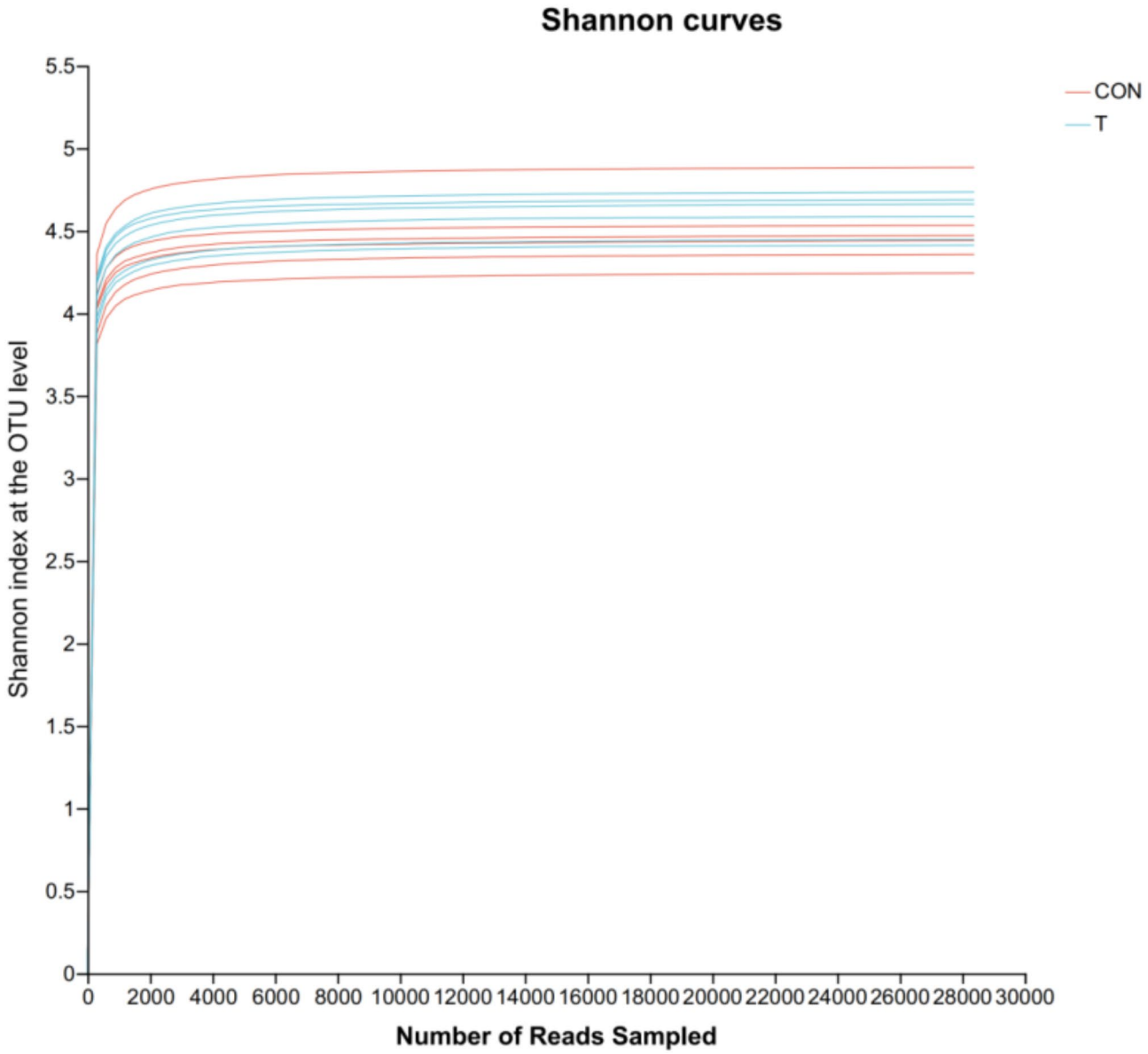


Fig. 1. Dilution curve. CON. control group; T. Treatment; The indexe reflecting the community richness.

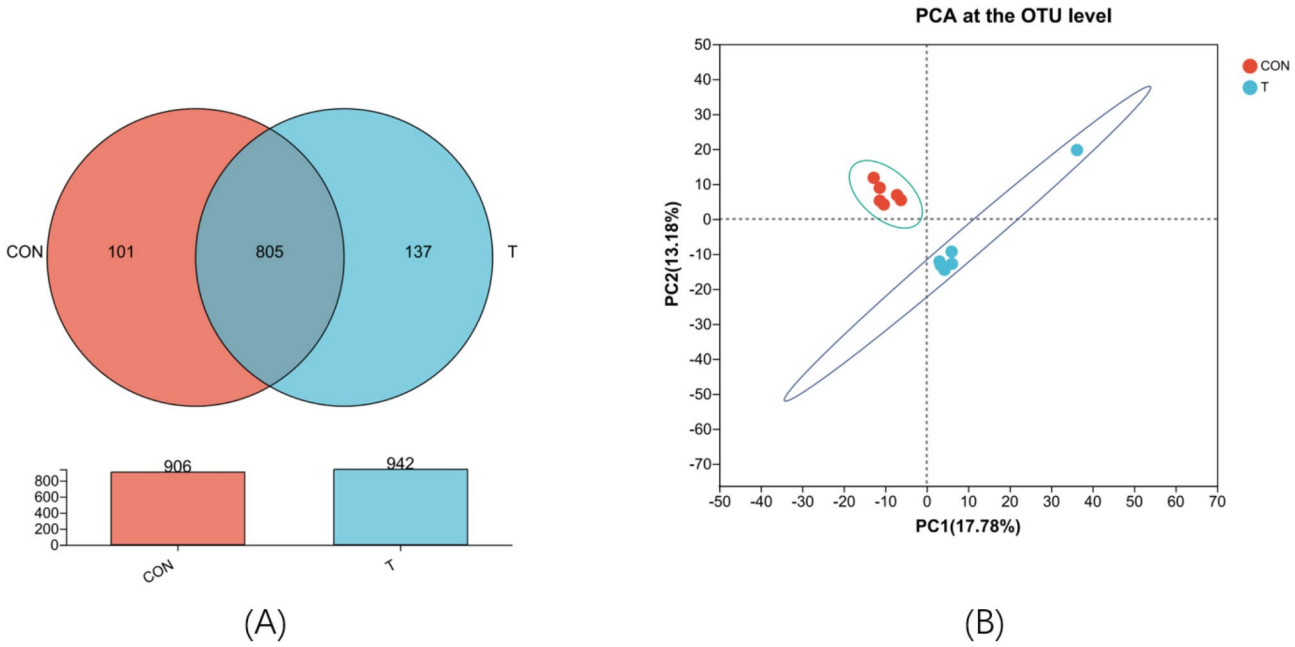


Fig. 2. Venn diagram and PCA analysis of cecum flora. Blue represents control group; red represents Treatment group; The X axis and the Y axis represent the two selected principal component axes, and the percentage indicates the interpretation value of the principal component on the sample composition difference; the scale of the X axis and the Y axis is the relative distance with no practical significance.

Analysis of the variability in the composition of caecal flora

Metastats analysis was performed to compare significant differences between the experimental group and the control group at both the phylum and genus levels. As shown in Fig. 4, at the phylum level, the abundances of *Desulfobacterota*, *Synergistota*, and *WPS-2* in the control group were significantly higher ($P < 0.05$) than those in the experimental group. *Spirochaetota* was significantly higher ($P < 0.05$) in the experimental group than in the control group.

At the genus level Fig. 4 (B), *Desulfovibrio*, *unclassified_f_Lachnospiraceae*, *Faecalibacterium*, *Colidextribacter*, *Negativibacillus*, and *Sellimonas* were significantly ($P < 0.05$) more abundant in the control group than in the experimental group. In addition, *norank_f_norank_o_Clostridia_vadinBB60_group*, *Christensenellaceae_R-7_group*, *unclassified_f_Rikenellaceae*, and *Enterococcus* were significantly ($P < 0.05$) more abundant in the experimental group than in the control group.

COG functional analysis of caecum flora

COG functional annotation of OTUs was performed using PICRUSt to obtain the annotation information of OTUs at the COG functional level (Fig. 5 (A), control group; Fig. 5 (B), experimental group). The results showed that the COG functional compositions of the two groups were similar and that the enriched functions mainly included carbohydrate transport and metabolism, transcription, amino acid transport and metabolism, and unknown functions.

Discussion

The number of ovulated or atretic follicles and egg-producing capacity of the oviduct are key to hen productivity. Oxidative stress, disease, and lower reproductive hormone levels can reduce follicle numbers and decelerate growth, thereby potentially decreasing egg production. Some studies have shown that dietary antioxidants, such as inulin, quercetin, and vitamin E improve egg production rates and increase serum IgA levels[36,37]. In this study, *Dendrobium chrysogenum* also improved egg production rate and serum IgA levels. Astaxanthin improves serum reproductive hormone levels, increases follicle numbers, reduces atresia, and boosts egg production[38]. Soybean flavonoids improve egg production performance and shell quality[39], also indicating that flavonoids exert a positive effect on the egg production performance of laying hens. However, *Dendrobium cinchona* contains a variety of flavonoid compounds that are structurally similar to endogenous oestradiol and can bind to the oestrogen steroid receptor and exert varying degrees of oestrogen-like effects, resulting in increased oestrogen levels[40]. The increased egg production in this study may also be related to the estrogenic effects of the flavonoids and flavonoid substances in DNL supplementation with Comfrey polysaccharides which increases the relative abundance of certain bacterial families and boosts egg production[41]. The same results were found in this study, suggesting that *Dendrobium* polysaccharides may lead to increased egg production by altering the microbial community in the caecum, resulting in increased digestibility of nutrients. In contrast to the results of Yao et al.[42], our results showed no significant effects on egg quality. This difference may be due to different chicken breeds, dosages of additives, or rearing environments used in the two studies. In poultry farming, egg quality

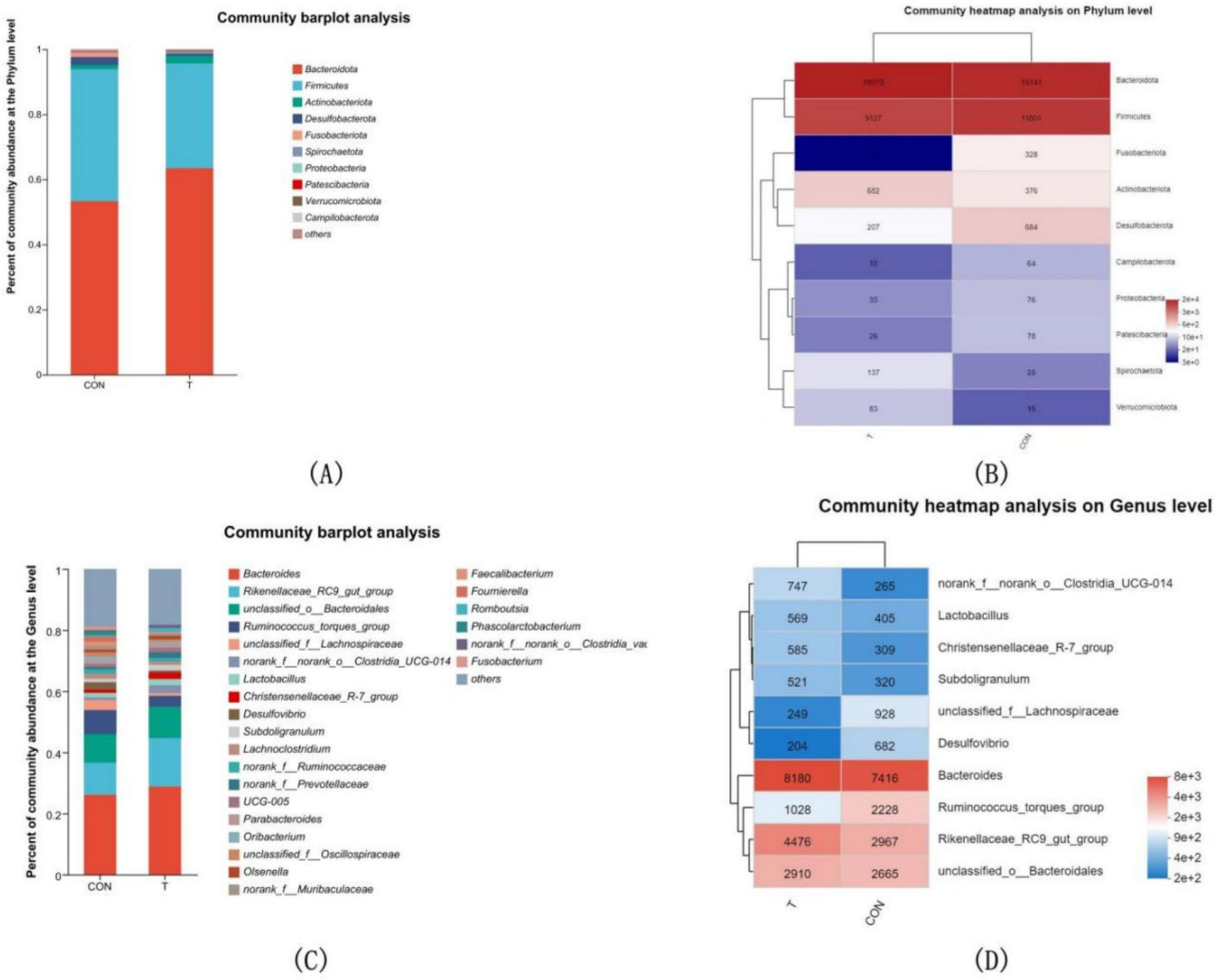
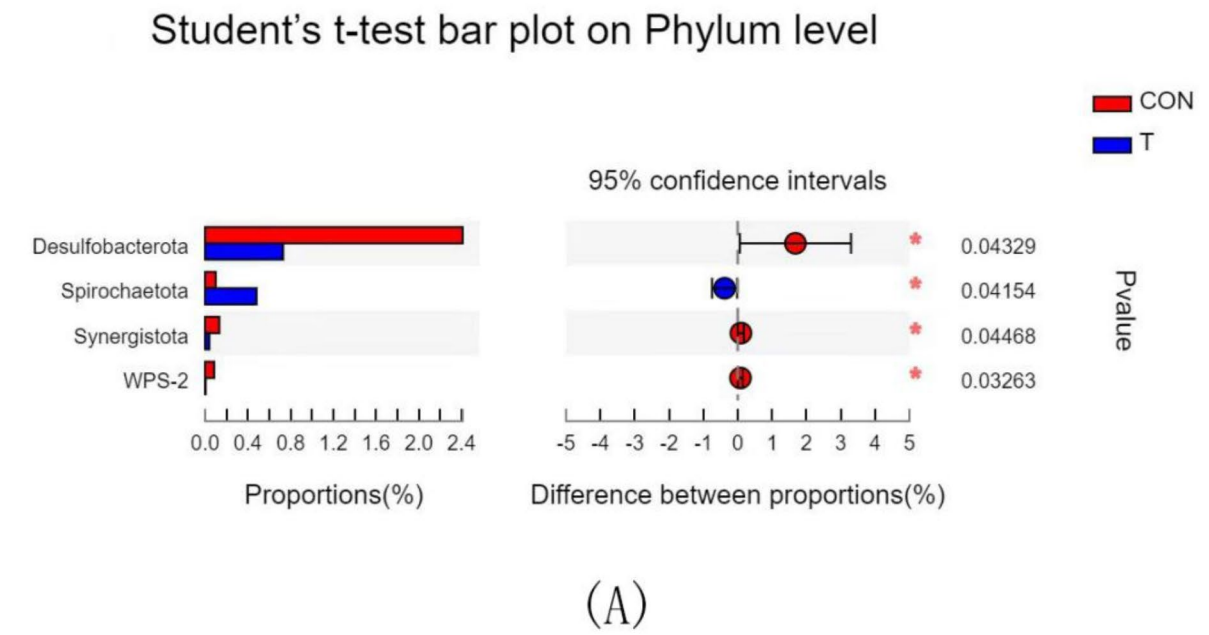


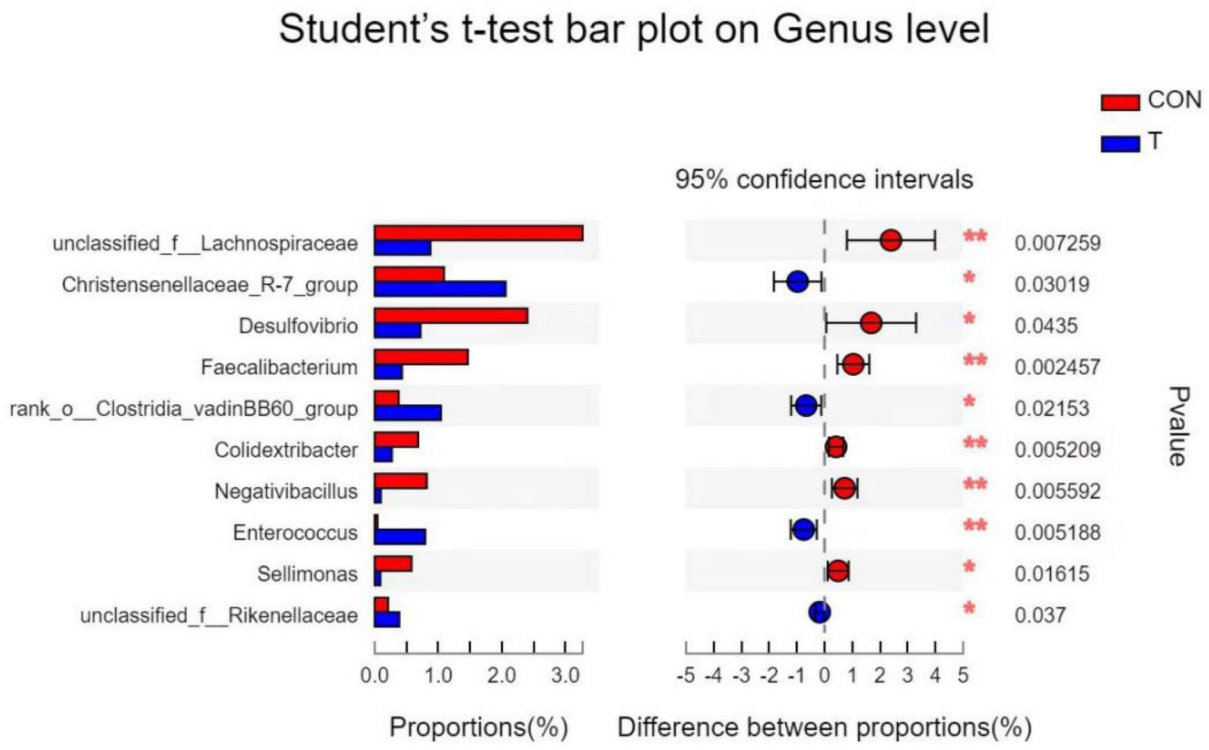
Fig. 3. Graph of relative abundance analysis of cecum flora species. Figure 3 (**A**) and (**C**), the abscissa is the grouping, the ordinate is the proportion of the species in the sample, the columns of different colours represent different species, and the length of the columns represents the size of the proportion of the species. Figure 3 (**B**) and (**D**), the abscissa is the sample name (or group name), and the ordinate is the species name. The change in the abundance of different species in the sample is displayed by the colour block colour gradient. The colour gradient is shown on the right-hand side of the figure.

stability is crucial for production management and market sales. The insignificant differences in egg quality indices between the experimental and control groups indicate that breeders do not need to be concerned about changes in egg quality that affect the selling price or market acceptance when DNL is added.

IgA is secreted by B lymphocytes, and their proliferation increases IgA levels[43]. *Dendrobium* stimulates the proliferation of B lymphocytes in vitro[44], which may be related to the increased serum IgA levels in the experimental group of the present study. Interestingly, IgA can reduce expression of pro-inflammatory genes and oral administration of IgA can inhibit chemically induced colitis[45,46]. IFN-γ is an immunoreactive substance involved in the immunoinflammatory process of the glomerulus. The level of IFN-γ is significantly increased in the glomerulus of inflamed mice. However, *Dendrobium* polysaccharide can significantly reduce the level of IFN-γ cytokines in the serum of mice with nephritis[47,48], indicating that *Dendrobium* can exert a therapeutic effect on nephritis in mice. In summary, an increase in IgA helps enhance mucosal immune defense, form and remove immune complexes, and regulate the balance of intestinal flora, thereby reducing the risk of inflammation. A reduction in IFN-γ can inhibit the activation and chemotaxis of inflammatory cells, reduce the secretion of inflammatory factors, and promote inflammation resolution. These two mechanisms work synergistically to balance the types of inflammatory responses and create favourable conditions for tissue repair. In this study, adding DNL to chicken diets significantly increased IgA content and decreased IFN-γ cytokine levels in chicken serum, showing that adding DNL to the diet had a positive effect on serum immune indices. These findings suggest that DNL supplementation can enhance poultry health by improving immune function and reducing inflammation, which may lead to better disease resistance and overall production performance in commercial poultry farming. Specifically, the increased IgA levels could help protect chickens against mucosal pathogens,



(A)



(B)

Fig. 4. Analysis of the variability in the composition of cecum flora. The X axis represents different groups, different colored bins represent different groups, and the Y axis represents the average relative abundance of a species in different groups.

while the reduced IFN-γ levels may mitigate chronic inflammation, thereby improving growth efficiency and reducing mortality rates. However, the levels of TP, IgM, ALB, IL-12, SOD, BUN, T-AOC, and CRE did not differ significantly from those of the control group, indicating that under the experimental conditions of this study, DNL had an insignificant impact on these specific serum indicators. This suggests that while DNL has certain effects on the production performance, immune function, and intestinal flora of chickens, its influence

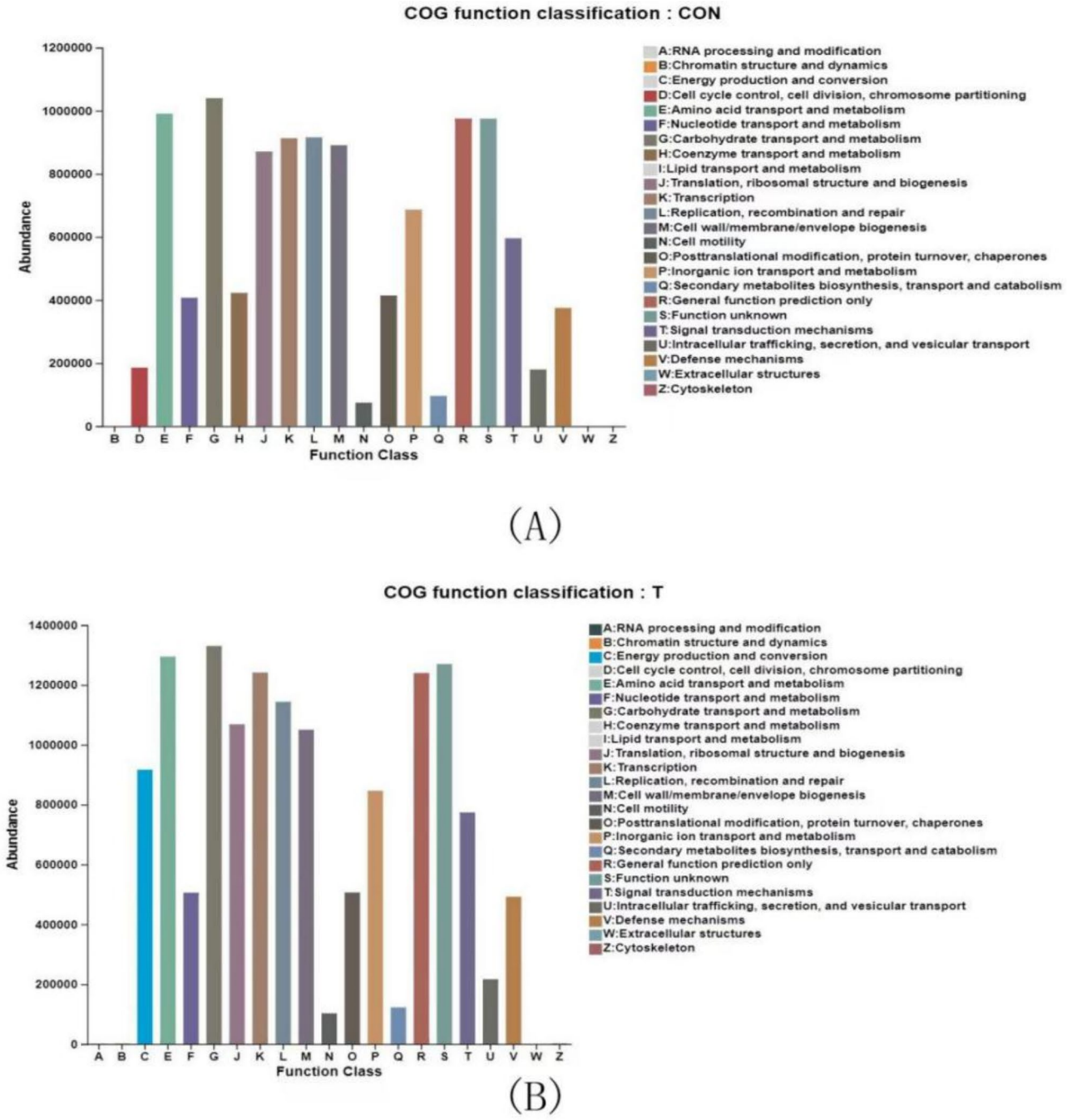


Fig. 5. Functional analysis of cecum flora COG. Samples represents the sample or grouping, and Abundance represents the functional relative abundance.

may be relatively specific and primarily concentrated in certain aspects, such as the regulation of IgA and IFN-γ and changes in the intestinal flora, with a relatively small impact on other physiological indicators. These non-significant results also help eliminate potential confounding factors, as significant changes in these indicators might have complicated the interpretation of the observed changes in production performance and immune function. The robustness of these indicators confirms that the observed improvements in production performance and immune function were primarily driven by DNL's influence on specific physiological processes. Future studies should focus on evaluating the long-term effects of DNL supplementation under field conditions to validate its practical benefits for poultry health and productivity. This study provides crucial information for a more accurate understanding of the physiological effects of DNL in chickens, highlighting its targeted impact on specific aspects of health and performance.

Amino acids and their salts have various flavours. Glutamic acid is the main component of monosodium glutamate and is responsible for its umami taste[49]. Studies have shown that intestinal microbiota play an

important role in amino acid synthesis and metabolism. In humans, 1–20% of plasma lysine, urinary lysine, and body protein lysine originate from gut microbes[50]. Firmicutes and Bacteroidetes vary substantially owing to dietary macromolecular content[51]. In the present study, adding DNL to the diet of Chisui black-bone hens increased Bacteroides abundance in the caecum and the amino acid content in eggs, with PICRUSt COG function analysis showing similar results, leading to our speculation that *Dendrobium* may increase the amino acid content of eggs by increasing the abundance of Bacteroides. This discovery has potential applications in the poultry farming industry for producing premium - quality, higher - priced eggs due to their enhanced nutrition and flavor, and in the food processing industry as raw materials for new functional foods like amino - acid - fortified egg - based products. For future research, it is essential to investigate the effect of DNL supplementation under different environmental and management conditions in poultry farms, including different climate zones, stocking densities, and feeding frequencies to find the optimal application scenario. Also, research could focus on the impact of different *Dendrobium* species and their extraction methods on the intestinal microbiota and amino acid content in eggs to identify the most effective *Dendrobium* - related ingredient for improving egg quality. Moreover, in - depth research on the molecular mechanisms underlying the relationship between *Dendrobium*, Bacteroides, and amino acid synthesis in hens, involving gene expression studies in the intestinal microbiota and the hen's own metabolism - related genes, is needed to comprehensively understand this phenomenon.

Linoleic acid (C18:2n6c) is the predominant PUFA consumed in the human diet and serves as a precursor for the biosynthesis of gamma-linolenic and arachidonic acids. Insufficient intake of linoleic acid during infancy can result in cutaneous desquamation, growth retardation, altered plasma fatty acid profiles, and thrombocytopenia[52]. Furthermore, variations in food flavours primarily stem from differences in fatty acid composition among different foods, leading to disparities in their oxidation products. High-temperature oxidation of oleic acid, linoleic acid, linolenic acid, and arachidonic acid results in the formation of volatile carbonyl compounds such as ketones, aldehydes, and acids which enhance the palatability of food items[53]. SFAs have straight-chain structures and show strong intermolecular forces. Increased SFA content in eggs is associated with higher egg stability[54]. We recognise that an increase in the SFA content in eggs may raise concerns regarding human dietary health. Excessive intake of SFA is associated with an increased risk of some chronic diseases, such as cardiovascular diseases[55]. In the present study, although the SFA content in eggs was increased, the magnitude of the increase was relatively small. Based on egg production in this study and possible human consumption patterns, it is estimated that the impact of this increase in SFA content on the overall SFA intake in the human diet may be limited. Additionally, although the SFA content was increased, the content of some PUFAs in the eggs of the experimental group was decreased, with a significant decrease in n-6PUFA content. PUFAs play important roles in maintaining normal physiological functions in the human body. Particularly, the balance between n-3 and n–6-series PUFAs is crucial to human health[56]. Therefore, from the perspective of overall fatty acid composition, it is necessary to comprehensively consider the impact of various fatty acid changes on human dietary health. Future research should explore the long-term impact of changes in egg fatty acid composition on human health under different dietary patterns. Simultaneously, it is necessary to examine whether there are other methods for regulating egg fatty acid composition to meet the needs of human dietary health.

Bacteroides and Firmicutes in the gut can degrade dietary polysaccharides from fibres to acetic, propionic, and succinic acids[57]. Acetate, the precursor for liver synthesis of C16 and C18 fatty acids and their related glycerophosphatidins, promotes fatty acid metabolism in the liver[58]. The levels of palmitic acid (C16:0), stearic acid (C18:0), palmitic acid (C16:1), and oleic acid (C18:ln9c) were significantly higher in eggs of the experimental group than in eggs of the control group. C16:0 and C18:0 are saturated fatty acids whereas C16:1 and C18:ln9c are MUFAs. The increase in their relative content resulted in significantly higher levels of both saturated and MUFAs in the experimental group than in the control group. In addition, the relative content of n-6PUFA in the experimental group was significantly lower than that in the control group, and the content of n-3PUFA also decreased. Both n-6 and 3PUFA are essential for human nutrition. The balance between the intake of n-6 and 3PUFA is fundamental for maintaining health[59]. *Dendrobium* reduces the synthesis of n-6PUFA in eggs, which is beneficial for human health.

Desulphurising *Vibrio* belongs to Proteobacteria and produces H_2S, which is toxic and causes gastrointestinal disease[60]. *Salmonella* is an important zoonotic pathogen that causes infectious diseases[61]. *Salmonella* infection reduces poultry production and contaminates the human food chain[62]. Previously, *Salmonella* diseases were mainly treated with antibiotics; however, these can cause dysfunction of the beneficial gut microbiota and increase resistance[63]. Antibiotics can also be added to diet to improve the intestinal barrier. Biotics include probiotics, prebiotics, synbiotics, and antibiotics. Modulation of microbiota through such biotics can improve the intestinal barrier and prevent chronic diseases[64]. Probiotics produce organic acids that inhibit harmful microorganisms[65]. Studies have shown that adding *Lactobacillus* and *Bacillus* to chicken diets can inhibit *Salmonella* proliferation and that lactic acid bacteria can enhance immunity[66].

DNL contains prebiotics, such as cellulose, polysaccharides, and alkaloids. Adding cellulose to the diet increases the faecal volume and promotes the growth of beneficial bacteria, thus reducing intestinal diseases[67]. In rats, dietary fibre can increase the abundance of microorganisms in the caecum, which is conducive to the growth of *Lactobacillus*[68]. High cellulose intake altered the intestinal microbial community in mice by increasing *Lactobacillus* and *Clostridium*[69]. Polysaccharides can be decomposed by intestinal microorganisms, thus providing nutrients and changing the floral structure[70]. Polysaccharides from *Dendrobium officinum* are degraded into SCFA by large-intestine microorganisms[71]. Moreover, a mixture of *Dendrobium officinale* and *Panax quinaculinum* can be used as a prebiotic preparation to increase the number of SCFA-producing bacteria and prevent intestinal flora imbalance[72]. Polysaccharides from *Dendrobium huoshanense* can change the intestinal state of mice and affect the intestinal flora[73]. In this study, DNL addition to the diet of chickens increased the abundance of *Lactobacillus* and *Bacteroides* and decreased the abundance of *Desulfovibrio* and *Salmonella*, which may be related to DNL containing abundant prebiotics such as cellulose, polysaccharides,

and alkaloids. The ACE index serves as a metric for estimating species richness within microbial communities. By comparing the ACE index between the experimental and control groups, we evaluated the effect of *D. chrysogenum* addition on the richness of the chicken gut microbiota. The ACE index reflects species richness, which is an important aspect of microbial diversity. The higher ACE index in the experimental group may imply higher species richness in the intestinal microbiota, which is a positive indication of an overall increase in diversity, although microbial diversity also encompasses other elements, such as evenness. Similar to the findings of a previous study[74], our study demonstrated an increase in the ACE index, suggesting a comparable trend in changes in microbial diversity. This consistency further validates the potential influence of *Dendrobium chrysogenum* on the intestinal microbiota.

Consequently, the increased species richness of the gut microbiota can exert a significant impact on nutrient metabolism. A greater number of microbial species implies a broader range of metabolic functions and pathways, enabling more efficient breakdown and utilisation of nutrients in the feed. In the present study, the addition of *Dendrobium chrysogenum* to the feed may have modified the structure of the gut microbiota by increasing the number of certain microorganisms with specific nutrient metabolic functions, thereby facilitating nutrient absorption and utilisation. Likewise, the gut microbiota plays a crucial role in the development and regulation of the immune system. It interacts with the immune system in various ways, such as stimulating the development and differentiation of immune cells and regulating cytokine secretion. Diversity in the intestinal microbiota is essential for immune regulation, as it provides a more comprehensive set of immunostimulatory signals that help maintain the balance and stability of the immune system.

In this study, the increase in the ACE index in the experimental group suggests a positive effect on the immune function of the chickens. Higher species richness in the gut microbiota may lead to a more diverse immune-stimulating environment and enhance the ability of chickens to resist disease. This speculation is further supported by the results of our study, in which a significant increase in serum IgA levels in the experimental group indicated enhanced immune function, which may be related to changes in the gut microbiota.

Conclusions and applications

This study suggests that adding 2100 mg/kg of DNL to the feed may increase the content of serum IgA and reduce the levels of IFN-γ in Chishui black-bone chickens during the later laying period. Additionally, it appears to enhance the laying rate of hens, increase the types of amino acids in eggs, and improve the proportion of ideal proteins. Furthermore, the findings indicate that DNL supplementation could be beneficial in improving the caecal microbial community of Chishui black-bone chickens, potentially increasing the relative abundance of beneficial bacteria. Based on these observations, we propose that DNL could be considered as a functional feed additive to potentially prolong the peak laying period of ageing Chishui black-bone chickens. However, further research is needed to confirm these findings and explore the underlying mechanisms.

Data availability

The datasets generated and/or analysed during the current study are available in the Sequence Read Archive (SRA) repository, under the accession number 'PRJNA1224976' (https://dataview.ncbi.nlm.nih.gov/object/PRJNA1224976?reviewer=dpang4o2q6oo8124ehiumsanp6). All other datasets generated during the current study are available from the corresponding authors upon reasonable request.

Received: 5 November 2024; Accepted: 24 February 2025
Published online: 21 March 2025

References

1. Zeng, S. J. Introduction of medicinal Dendrobium species in China. *Flowers Plants* 19–26. (2015).
2. Shao, Y., Wang, Y., Yuan, Y., Xie, Y. A. & Systematic Review On antibiotics misuse in livestock and aquaculture and regulation implications in China. *SCI. TOTAL ENVIRON.* **798**, 149205. https://doi.org/10.1016/j.scitotenv.2021.149205 (2021).
3. Nie, X., Chen, Y., Li, W. & Lu, Y. Anti-Aging properties of Dendrobium nobile Lindl.: from molecular mechanisms to potential treatments. *J. ETHNOPHARMACOL.* **257**, 112839. https://doi.org/10.1016/j.jep.2020.112839 (2020).
4. Cheng, J. et al. An assessment of the Chinese medicinal Dendrobium industry: supply, demand and sustainability. *J. ETHNOPHARMACOL.* **229**, 81–88. https://doi.org/10.1016/j.jep.2018.09.001 (2019).
5. Mou, Z. et al. Identification, biological activities and biosynthetic pathway of Dendrobium alkaloids. *FRONT. PHARMACOL.* **12**, 605994. https://doi.org/10.3389/fphar.2021.605994 (2021).
6. Yuan, Y. et al. Tissue-Specific transcriptome for Dendrobium officinale reveals genes involved in flavonoid biosynthesis. *GENOMICS* **112**, 1781–1794. https://doi.org/10.1016/j.ygeno.2019.10.010 (2020).
7. Huang, K. et al. Purification, Characterization and Biological Activity of Polysaccharides From Dendrobium Officinale. *MOLECULES*. 21, (2016). https://doi.org/10.3390/molecules21060701
8. Zhou, H., Zhou, L., Li, B., Yue, R. & Anti-Cyclooxygenase Anti-Glycation, and Anti-Skin aging effect of Dendrobium officinale flowers' aqueous extract and its phytochemical validation in aging. *FRONT. IMMUNOL.* **14**, 1095848. https://doi.org/10.3389/fimmu.2023.1095848 (2023).
9. Zhang, S. et al. Dendrobium nobile Lindl. Polysaccharides improve follicular development in Pcos rats. *INT. J. BIOL. MACROMOL.* **149**, 826–834. https://doi.org/10.1016/j.ijbiomac.2020.01.196 (2020).
10. Duan, H. et al. Alkaloids from Dendrobium and their biosynthetic pathway, biological activity and total synthesis. *PHYTOMEDICINE* **102**, 154132. https://doi.org/10.1016/j.phymed.2022.154132 (2022).
11. Rossi, L., Mazzitelli, S., Arciello, M., Capo, C. R. & Rotilio, G. Benefits from dietary polyphenols for brain aging and Alzheimer's disease. *NEUROCHEM RES.* **33**, 2390–2400. https://doi.org/10.1007/s11064-008-9696-7 (2008).
12. Stojanovic, S. D., Fiedler, J., Bauersachs, J., Thum, T. & Sedding, D. G. Senescence-Induced inflammation: an important player and key therapeutic target in atherosclerosis. *EUR. HEART J.* **41**, 2983–2996. https://doi.org/10.1093/eurheartj/ehz919 (2020).
13. Nii, T., Bungo, T., Isobe, N. & Yoshimura, Y. Intestinal inflammation induced by dextran sodium sulphate causes liver inflammation and lipid metabolism Disfunction in laying hens. *Poult. SCI.* **99**, 1663–1677. https://doi.org/10.1016/j.psj.2019.11.028 (2020).

14. Wang, P. et al. Phenolics from Dendrobium officinale leaf ameliorate dextran sulfate Sodium-Induced chronic colitis by regulating gut microbiota and intestinal barrier. *J. AGR FOOD CHEM.* **71**, 16630–16646. https://doi.org/10.1021/acs.jafc.3c05339 (2023).
15. Liu, H. et al. Dendrobium officinale polysaccharide alleviates intestinal inflammation by promoting small extracellular vesicle packaging of Mir-433-3P. *J. AGR FOOD CHEM.* **69**, 13510–13523. https://doi.org/10.1021/acs.jafc.1c05134 (2021).
16. Ciumarnean, L. et al. The effects of flavonoids in cardiovascular diseases. *MOLECULES* **25** https://doi.org/10.3390/molecules25184320 (2020).
17. Grippo, A. A., Capps, K., Rougeau, B. & Gurley, B. J. Analysis of flavonoid phytoestrogens in botanical and Ephedra-Containing dietary supplements. *ANN. PHARMACOTHER.* **41**, 1375–1382. https://doi.org/10.1345/aph.1H497 (2007).
18. Hassan, F. U. et al. Potential of Mulberry Leaf Biomass and its Flavonoids to Improve Production and Health in Ruminants: Mechanistic Insights and Prospects. *ANIMALS-BASEL*. 10, (2020). https://doi.org/10.3390/ani10112076
19. Tan, Z., Halter, B., Liu, D., Gilbert, E. R. & Cline, M. A. Dietary flavonoids as modulators of lipid metabolism in poultry. *FRONT. PHYSIOL.* **13**, 863860. https://doi.org/10.3389/fphys.2022.863860 (2022).
20. Goliomytis, M. et al. Influence of Citrus flavonoids on laying Hen performance, inflammatory immune response, egg quality and yolk oxidative stability. *BRIT Poult. SCI.* **60**, 272–278. https://doi.org/10.1080/00071668.2019.1587150 (2019).
21. Yue, K. et al. Novel Insights Into Total Flavonoids of Rhizoma Drynariae Against Meat Quality Deterioration Caused by Dietary Aflatoxin B1 Exposure in Chickens. *ANTIOXIDANTS-BASEL*. 12, (2022). https://doi.org/10.3390/antiox12010083
22. Zhou, W. et al. Dendrobium officinale Xianhu 2 polysaccharide helps forming a healthy gut microbiota and improving host immune system: an in vitro and in vivo study. *FOOD CHEM.* **401**, 134211. https://doi.org/10.1016/j.foodchem.2022.134211 (2023).
23. Li, M. et al. Intestinal microbes derived butyrate is related to the Immunomodulatory activities of Dendrobium officinale polysaccharide. *INT. J. BIOL. MACROMOL.* **149**, 717–723. https://doi.org/10.1016/j.ijbiomac.2020.01.305 (2020).
24. Zhao, W. et al. Dietary effect of Dendrobium officinale leaves on chicken meat quality, fatty acid composition, and volatile compounds profile. *FOOD CHEM. X.* **22**, 101330. https://doi.org/10.1016/j.fochx.2024.101330 (2024).
25. Zhao, W. et al. Dietary supplementation with Dendrobium officinale leaves improves growth, antioxidant status, immune function, and gut health in broilers. *FRONT. MICROBIOL.* **14**, 1255894. https://doi.org/10.3389/fmicb.2023.1255894 (2023).
26. Chen, X. et al. Transcriptome analysis of ovarian follicles reveals potential pivotal genes associated with increased and decreased rates of chicken egg production. *FRONT. GENET.* **12**, 622751. https://doi.org/10.3389/fgene.2021.622751 (2021).
27. Sun, X. et al. Transcriptome comparative analysis of ovarian follicles reveals the key genes and signaling pathways implicated in Hen egg production. *BMC Genom.* **22**, 899. https://doi.org/10.1186/s12864-021-08213-w (2021).
28. Alagawany, M. et al. Nutritional significance of amino acids, vitamins and minerals as nutraceuticals in poultry production and Health - A comprehensive review. *VET. QUART.* **41**, 1–29. https://doi.org/10.1080/01652176.2020.1857887 (2020).
29. Yao, B. N. et al. Effect of sea Buckthorn extract on production performance, serum biochemical indexes, egg quality, and cholesterol deposition of laying ducks. *FRONT. VET. SCI.* **10**, 1127117. https://doi.org/10.3389/fvets.2023.1127117 (2023).
30. Landy, N. & Kheiri, F. Effects of hydrolyzed cottonseed protein on growth performances, carcass traits, immunity, microbial and morphological responses of the small intestine and total antioxidant capacity of serum, and small intestine in broiler chickens. *Iran. J. APPL. ANIM. SCI.* **13**, (2023).
31. Millet, S. & Maertens, L. The European ban on antibiotic growth promoters in animal feed: from challenges to opportunities. *VET. J.* **187**, 143–144. https://doi.org/10.1016/j.tvjl.2010.05.001 (2011).
32. Walsh, T. R. & Wu, Y. China bans colistin as a feed additive for animals. *LANCET INFECT. DIS.* **16**, 1102–1103. https://doi.org/10.1016/S1473-3099(16)30329-2 (2016).
33. Leyva, A. et al. Rapid and sensitive Anthrone-Sulfuric acid assay in microplate format to quantify carbohydrate in biopharmaceutical products: method development and validation. *BIOLOGICALS* **36**, 134–141. https://doi.org/10.1016/j.biologicals.2007.09.001 (2008).
34. Schat, K. A. & Poultry Health A Guide for Professionals. *AVIAN DIS.* 66, (2022). https://doi.org/10.1637/0005-2086-66.1.1
35. Kanehisa, M., Furumichi, M., Sato, Y., Matsuura, Y. & Ishiguro-Watanabe, M. Kegg: biological systems database as a model of the real world. *NUCLEIC ACIDS RES.* **53**, D672–D677. https://doi.org/10.1093/nar/gkae909 (2025).
36. Shang, H. M. et al. In vitro and in vivo antioxidant activities of inulin. *PLOS ONE.* **13**, e192273. https://doi.org/10.1371/journal.pone.0192273 (2018).
37. Amevor, F. K. et al. Synergistic effects of Quercetin and vitamin E on egg production, egg quality, and immunity in aging breeder hens. *Poult. SCI.* **100**, 101481. https://doi.org/10.1016/j.psj.2021.101481 (2021).
38. He, W., Wang, H., Tang, C., Zhao, Q. & Zhang, J. Dietary supplementation with Astaxanthin alleviates ovarian aging in aged laying hens by enhancing antioxidant capacity and increasing reproductive hormones. *Poult. SCI.* **102**, 102258. https://doi.org/10.1016/j.psj.2022.102258 (2023).
39. Ni, Y. et al. Effect of dietary Daidzein on egg production, shell quality, and gene expression of Er-Alpha, Gh-R, and Igf-Ir in shell glands of laying hens. *J. AGR FOOD CHEM.* **55**, 6997–7001. https://doi.org/10.1021/jf071085r (2007).
40. Wang, Y. et al. Black tea and D. Candidum extracts play estrogenic activity via Estrogen receptor Alpha-Dependent signaling pathway. *AM. J. TRANSL RES.* **10**, 114–125 (2018).
41. Zhou, H. et al. Comfrey polysaccharides modulate the gut microbiota and its metabolites Scfas and affect the production performance of laying hens. *INT. J. BIOL. MACROMOL.* **215**, 45–56. https://doi.org/10.1016/j.ijbiomac.2022.06.075 (2022).
42. Yao, W. et al. Effects of garcinol supplementation on the performance, egg quality, and intestinal health of laying hens in the late laying period. *Poult. SCI.* **102**, 102939. https://doi.org/10.1016/j.psj.2023.102939 (2023).
43. Gupta, S., Su, H. & Agrawal, S. Cd8 Treg cells inhibit B-Cell proliferation and Immunoglobulin production. *INT. ARCH. ALLERGY IMM.* **181**, 947–955. https://doi.org/10.1159/000509607 (2020).
44. Zha, X. Q. et al. Immunoregulatory activities of Dendrobium huoshanense polysaccharides in mouse intestine, spleen and liver. *INT. J. BIOL. MACROMOL.* **64**, 377–382. https://doi.org/10.1016/j.ijbiomac.2013.12.032 (2014).
45. Suzuki, K. Diversified Iga-Bacteria interaction in gut homeostasis. *ADV. EXP. MED. BIOL.* **1254**, 105–116. https://doi.org/10.1007/978-981-15-3532-1_9 (2020).
46. Okai, S. et al. High-Affinity monoclonal Iga regulates gut microbiota and prevents colitis in mice. *NAT. MICROBIOL.* **1**, 16103. https://doi.org/10.1038/nmicrobiol.2016.103 (2016).
47. Uhm, W. S. et al. Cytokine balance in kidney tissue from lupus nephritis patients. *RHEUMATOLOGY* **42**, 935–938. https://doi.org/10.1093/rheumatology/keg255 (2003).
48. Wang, Q., Sun, P., Wang, R. & Zhao, X. Therapeutic effect of Dendrobium candidum on lupus nephritis in mice. *PHARMACOGN MAG.* **13**, 129–135. https://doi.org/10.4103/0973-1296.197653 (2017).
49. Ding, Y. Q., Liu, Y. M. & Xiong, S. B. The comparative study on nutritional components between the muscle of Elopichthys Bambusa and Ctenopharyngodon idellus. *Acta Nutrimenta Sinica*. **33**, 196–198 (2011).
50. Metges, C. C. Contribution of microbial amino acids to amino acid homeostasis of the host. *J. NUTR.* **130**, 1857. https://doi.org/10.1093/jn/130.7.1857S (2000). S-1864S.
51. Newsome, S. D. et al. Isotopic and genetic methods reveal the role of the gut Microbiome in mammalian host essential amino acid metabolism. *P ROY SOC. B-BIOL SCI.* **287**, 20192995. https://doi.org/10.1098/rspb.2019.2995 (2020).
52. Dupont, J. W. P. J. Food uses and health effects of corn oil. *J. Am. Coll. Nutr.* **9**, 438–470 (1990).
53. Shahidi, F. & Hossain, A. Role of lipids in food flavor generation. *MOLECULES* **27**, (2022).

54. Toyosaki, T. & Rheological Properties Oxidative stability, and Tocopherol content during storage of fried dough made with silky fowl egg: comparison with Hen egg. *Poult. SCI.* **89**, 1009–1014. https://doi.org/10.3382/ps.2009-00025 (2010).
55. Vandevijvere, S. & Kraak, V. Future directions to prevent obesity within the context of the global syndemic. *OBES. REV.* **20 Suppl 2**, 3–5. https://doi.org/10.1111/obr.12887 (2019).
56. Park, S. et al. Divergent regulation of adipose tissue metabolism by calorie restriction and Inhibition of growth hormone signaling. *EXP. GERONTOL.* **44**, 646–652. https://doi.org/10.1016/j.exger.2009.07.002 (2009).
57. Sun, Y. Z. S. N. Gut firmicutes: relationship with dietary Fiber and role in host homeostasis. *Crit. Rev. Food Sci. Nutr.* **63**, 12073–12088 (2023).
58. Kindt, A. et al. The gut microbiota promotes hepatic fatty acid desaturation and elongation in mice. *NAT. COMMUN.* **9**, 3760. https://doi.org/10.1038/s41467-018-05767-4 (2018).
59. Wijendran, V. & Hayes, K. C. Dietary N-6 and N-3 fatty acid balance and cardiovascular health. *ANNU. REV. NUTR.* **24**, 597–615. https://doi.org/10.1146/annurev.nutr.24.012003.132106 (2004).
60. Singh, S. B. & Lin, H. C. Hydrogen Sulfide in Physiology and Diseases of the Digestive Tract. *MICROORGANISMS*. 3, (2015).
61. Li, Q. Salmonella-Containing vacuole development in avian cells and characteristic of Cigr in Salmonella Enterica serovar pullorum replication within macrophages. *VET. MICROBIOL.* **223**, 65–71 (2018).
62. Sylejmani, D., Musliu, A., Ramadani, N., Sparagano, O. & Hamidi, A. Associations between the level of biosecurity and occurrence of dermanyssus gallinae and Salmonella spp. In layer farms. *AVIAN DIS.* **60**, 454–459. https://doi.org/10.1637/11327-111415-Reg (2016).
63. Gut, A. M., Vasiljevic, T., Yeager, T. & Donkor, O. N. Salmonella Infection - Prevention and treatment by antibiotics and probiotic yeasts: A review. *MICROBIOL-SGM* **164**, 1327–1344. https://doi.org/10.1099/mic.0.000709 (2018).
64. Li, H. Y. et al. Effects and Mechanisms of Probiotics, Prebiotics, Synbiotics, and Postbiotics On Metabolic Diseases Targeting Gut Microbiota: A Narrative Review. *NUTRIENTS*. 13, (2021). https://doi.org/10.3390/nu13093211
65. Ciarlo, E. et al. Impact of the microbial derived short chain fatty acid propionate on host susceptibility to bacterial and fungal infections in vivo. *SCI. REP-UK.* **6**, 37944. https://doi.org/10.1038/srep37944 (2016).
66. Tabashsum, Z. et al. Competitive reduction of Poultry-Borne enteric bacterial pathogens in chicken gut with bioactive Lactobacillus Casei. *SCI. REP-UK.* **10**, 16259. https://doi.org/10.1038/s41598-020-73316-5 (2020).
67. Adebowale, T. O., Yao, K. & Oso, A. O. Major cereal carbohydrates in relation to intestinal health of monogastric animals: A review. *Anim. Nutr.* **5**, 331–339. https://doi.org/10.1016/j.aninu.2019.09.001 (2019).
68. Myint, H. et al. Functional modulation of caecal fermentation and microbiota in rat by feeding bean husk as a dietary fibre supplement. *BENEF MICROBES.* **9**, 963–974. https://doi.org/10.3920/BM2017.0174 (2018).
69. Kim, Y. et al. Dietary cellulose prevents gut inflammation by modulating lipid metabolism and gut microbiota. *GUT MICROBES.* **11**, 944–961. https://doi.org/10.1080/19490976.2020.1730149 (2020).
70. Marcobal, A. et al. Bacteroides in the infant gut consume milk oligosaccharides via Mucus-Utilization pathways. *CELL. HOST MICROBE.* **10**, 507–514. https://doi.org/10.1016/j.chom.2011.10.007 (2011).
71. Li, L. et al. Destiny of Dendrobium officinale polysaccharide after oral administration: indigestible and nonabsorbing, ends in modulating gut microbiota. *J. AGR FOOD CHEM.* **67**, 5968–5977. https://doi.org/10.1021/acs.jafc.9b01489 (2019).
72. Liu, C. Z. et al. Dendrobium officinale Kimura et Migo and American ginseng mixture: A Chinese herbal formulation for gut microbiota modulation. *CHIN. J. NAT. Med.* **18**, 446–459. https://doi.org/10.1016/S1875-5364(20)30052-2 (2020).
73. Xie, S. Z. et al. Dendrobium huoshanense polysaccharide regionally regulates intestinal mucosal barrier function and intestinal microbiota in mice. *CARBOHYD POLYM.* **206**, 149–162. https://doi.org/10.1016/j.carbpol.2018.11.002 (2019).
74. Yang, P. et al. Radix bupleuri aqueous extract attenuates Mk801-Induced Schizophrenia-Like symptoms in mice: participation of intestinal flora. *BIOMED. PHARMACOTHER.* **172**, 116267. https://doi.org/10.1016/j.biopha.2024.116267 (2024).

Acknowledgements

We thank Guizhou University for providing the places and instruments needed for the experiment, Li Hui and Teacher Xingzhou for guiding the paper, Yu Huan for processing the data for the experiment, Editage for providing the article polishing serviceand and all the students who have worked hard for this article.

Author contributions

JLY; YBC: data collation and draft writing, include article revision and editing. HL, X-ZT: Revise the first draft and supervise the completion of the test. HY, YSS, DPZ and XL: assist with feeding trials and writing. All authors have read and approved the final manuscript. provide approval for publication of the content agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding

Guizhou Provincial Department of Science and Technology, Key Projects in Agriculture, Project No.: Qian Kehe Support [2022] Key 034, Project Name: Guizhou Local Chicken Germplasm Resource Excavation and New Breeds (Matching Lines) Selection and Breeding; Guizhou Provincial Department of Agriculture and Rural Development, Project No.: Qian Qian Cai Nong [2024] 80, Guizhou Ecological Broiler Advantageous and Characteristic Industrial Cluster Project.

Declarations

Competing interests

The authors declare no competing interests.

Ethics statement

This work was handled in accordance with the Inspection Form for the Guizhou University, Experimental Animal Ethics (EAE-GZU-2022-T144).

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-91901-4.

Correspondence and requests for materials should be addressed to H.L.