INTERPLAY OF NUTRITION AND GENOMICS: POTENTIAL FOR IMPROVING PERFORMANCE AND HEALTH OF POULTRY

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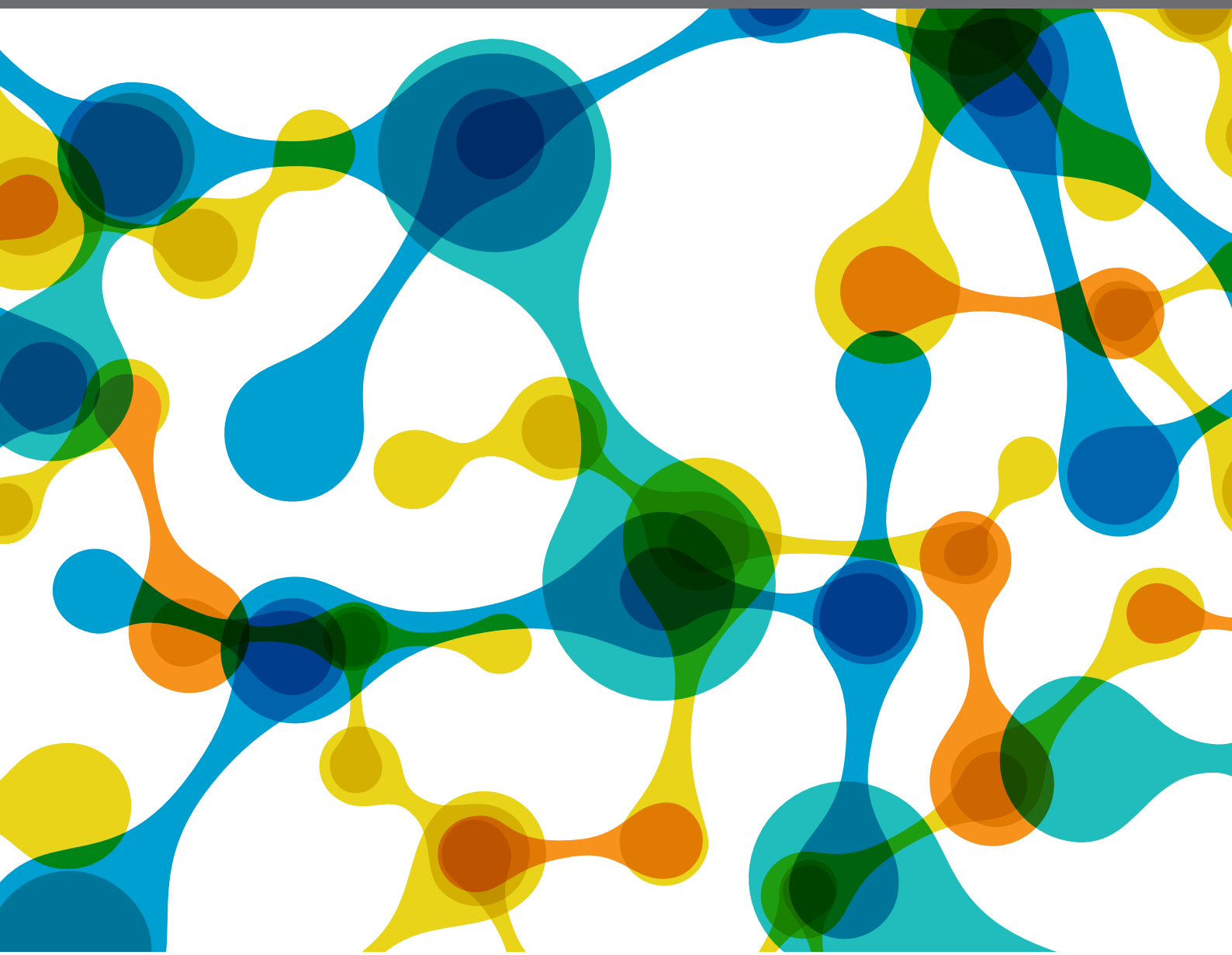

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INTERPLAY OF NUTRITION AND GENOMICS: POTENTIAL FOR IMPROVING PERFORMANCE AND HEALTH OF POULTRY

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Editorial: Interplay of nutrition and genomics: Potential for improving performance and health of poultry

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Editorial on the Research Topic
Interplay of nutrition and genomics: Potential for improving performance and health of poultry

Nutrigenomics is one of the most rapidly developing scientific fields that holds great promise for improving the health and performance of food-producing animals. Nutrigenomics begins a new era of working with nutrition and genetics, and gives us an insight into how nutrients interfere with an organism's genetics and the resulting phenotypic response. It focuses on elucidating the effect of dietary nutrients on expression patterns of different genes and epigenetic modifications, such as DNA methylation and histone modifications (Fenech et al., 2011). The use of nutrients and nutraceuticals in poultry diets can improve expression of different genes related to immunity, metabolism, health, growth and antioxidant capacity (Alagawany et al., 2022). Nutritional programming during prenatal and postnatal life may have long-lasting consequences on the growth and health of birds and animals. Particularly during prenatal life, nutrients not only influence the developing embryo, but also affect the primary germ cells responsible for the next generation (Harrison, 2020). Therefore, exploring the effect of nutrients on genomic functions holds great promise to enhance the production performance and health of food animals.

Elucidation of epigenetic modifications controlled by nutrients is required to better understand the diet-gene interactions in avian species. Therefore, comprehensive studies are required to provide mechanistic insights into the nutritional regulation of gene expression through DNA methylation, histone modifications, and noncoding RNA interactions (Hassan et al., 2019). In addition, recent advances in high throughput techniques, such as whole-genome bisulfite sequencing, chromatin immune-precipitation sequencing, and global RNA-Sequencing, must be utilized for nutrigenomic studies in avian species.

This Research Topic covers the recent advances in nutritional and nutrigenomic interventions focused on improving health and performance, focusing on the discovery of diet-gene interactions in avian species. Various nutrients including phytonutrients have shown to affect different metabolic pathways in birds to modulate performance, immune response, and egg/meat quality. Phytochemicals in different herbs possess significant biological activities owing to their potential antioxidant and antimicrobial activities. These active compounds not only affect gut health but also enhance metabolic activity leading to enhanced nutrient digestibility and utilization (Hassan et al., 2020). Owing to their excellent biological activities of phytochemicals, their use as feed additives in poultry is increasing day by day to potentially modulate growth performance, gut health, immune response, and product quality. The biological activities of these phytochemicals mainly stem from their antioxidant and antimicrobial effects. Excellent antioxidant activities make them potential compounds to be used as therapeutic agents to scavenge free radicals (ROS) in the cell to mediate adverse effects of oxidative stress, which is a major challenge for bird's health under extreme weather conditions. This is mainly attributed to the fact that higher production of ROS challenges cellular homeostasis and antioxidant defense leading to oxidative stress that subsequently affects vital physiological functions of the body (Mishra and Jha, 2019). The disruption of oxidative balance adversely affects the production performance and immune functions in birds since oxidative stress and inflammatory damage are multi-stage processes. The oxidative stress induced by higher ROS (H_2O_2) levels has shown to drastically affect the meat quality of broiler thigh muscle through mediating apoptosis, autophagy, and ROS/NF-κB signaling pathway (Yan et al., 2022). Furthermore, studies have shown that phytochemicals can also modulate key transcription factors involved in oxidative stress and inflammation, including nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and nuclear factor kappa B (NF-κB) to regulate many downstream metabolic and signal transduction pathways (Lee et al., 2019). Regulation of different vital metabolic pathways through dietary supplementation of phytochemicals opens the horizon for nutrigenomic interventions to improve performance and health in poultry birds.

Dietary supplementation of herbs with rich antioxidant phytochemicals not only protects against ROS damage but also enhances cellular antioxidant defense, which can enhance the quality and shelf life of poultry meat. Herbal adaptogen consisting of well-known immune-boosting herbs *Ocimum sanctum*, *Withania somnifera*, and *Emblica officinalis* showed positive effects on growth performance and feed conversion ratio (FCR) in heat-stressed broilers (Greene et al.). Supplementation of this combination of herbs also modulated the amino acid profile, pH, color, and quality of breast meat. This indicates that potent antioxidant activities of phytochemicals of these herbs alleviated adverse effects of ROS in birds while improving immune functions, meat yield and quality (Lee et al., 2019). Moreover, these phytochemicals can also be used to potentially enrich the meat with antioxidants with subsequent enhancement in shelf life and health-promoting effects. Dietary supplementation of different levels of garlic straw powder showed potential improvement in meat quality and antioxidant capacity of yellow-feathered broilers without affecting growth performance and intestinal mucosal morphology (Liao et al.).

The major stress alleviating effect of dietary phytochemicals is mediated by increasing the endogenous antioxidant enzymes while decreasing oxidant enzymes. For example, Alfalfa (Medicago sativa Linn)-mixed silage fermentation material enhanced the glutathione content in chest and leg muscles and serum superoxide dismutase (SOD) activity while reducing the muscle malondialdehyde content in Lande geese (Li et al.). In addition, it also substantially increased the serum concentrations of triglycerides, total cholesterol, urea, and aspartate aminotransferase, consistent with good liver and kidney function. This is mainly mediated by enhancing the expression of genes meant for producing antioxidant proteins through multifaceted pathways. For example, dietary supplementation of 6% ramie (*Boehmeria nivea*) powder promoted the antioxidative capacity of the ducks by increasing the serum activities of SOD and glutathione as well as the mRNA expressions of glutathione peroxidase (GSH-Px) in the breast meat and SOD in the leg meat (Lin et al.). Similarly, dietary inclusion of 3% ramie significantly increased the activities of liver SOD and GSH-Px in laying hens. However, the addition of 3%~6% ramie powder significantly increased the villus height of jejunum and villus height/crypt depth of ileum, revealing desirable effect on intestinal development of layers (Wang et al.).

Phytogenic feed additives have been largely exploited in poultry feeds to modulate gastrointestinal functions and health, and their implications on the birds' systemic health and welfare, the production efficiency of flocks, food safety, and environmental impact (Abdelli et al., 2021). Keeping in view of the potential of phytochemicals to modulate gastrointestinal functions and gut health in birds (Biagini et al., 2022), many feed ingredients with functional compounds have been evaluated in poultry feed to exploit the synergistic effects of nutrients and phytochemicals. For example, dietary inclusion of ramie (*Boehmeria nivea*) powder at various levels has shown to affect growth and health status in Linwu ducks, indicating 6% as an optimum inclusion level for better growth performance (Lin et al.). Similarly, dietary inclusion of ramie powder in the diet of laying hens also affected the egg composition, as the addition of 6% ramie significantly increased total omega-3 polyunsaturated fatty acids and phenylalanine in egg yolk (Wang et al.).

Inclusion of *Artemia argyi* (1%) in poultry feed enhanced the antioxidant capacity of laying hens through increasing T-SOD

and CAT activities, as well as GSH-Px contents in the liver. However, the dietary supplementation of 3% A. argyi substantially increased the serum and liver MDA contents and adversely affected the intestinal morphology by increasing duodenal crypt depth (Chen et al.).

In addition to antioxidant activity and immunogenic effects, phytochemicals have also shown to increase nutrient digestibility and metabolism in poultry birds. In order to formulate high density diets, high fat levels are used which can put burden on liver and might lead to oxidative stress and fatty liver syndrome. Phytochemicals can also help in these conditions as certain compounds like phospholipids can improve liver function and alleviate oxidative stress and liver damage. Soy lecithin is a phospholipid and, being the major component of cell membranes, plays a key role in cell repair and liver health. Long-term dietary supplementation of soy lecithin reduced the MDA (product of lipid peroxidation in the liver) content while increasing the antioxidant capacity (Total antioxidant capacity, SOD, and GSH-Px contents) of the liver in laying hens (Hu et al.). These findings indicated that long-term dietary lecithin supplementation can enhance the blood and liver lipid contents in laying hens and also improve the antioxidant capacity of the liver ensuring liver health. Overall this research topic has provided inisghts about the modulation of growth, metabolism, antioxidant status and immune response using different dietary nutrients and phytochemicals in poultry. It is clearly evident that nutrigenomics will serve as a new tool for nutritional research in addressing the issues related with poultry production particularly bird's health, oxidative stress and growth performance. In future, innovations in nutritional interventions with aid of various molecular technologies will help to better understand the nutrient gene interactions ultimately leading to find more refined and sustainable methods for managing poultry production. Nutritional manipulation for the targeted modulation of the specific genes seems quite possible in near future to get the desired performance in terms of better health and performance (Zhao et al., 1995; Salami et al., 2015).

Author contributions

F-uH wrote the draft and RJ and MA revised the manuscript.

Conflict of interest

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

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Effects of Herbal Adaptogen Feed-Additive on Growth Performance, Carcass Parameters, and Muscle Amino Acid Profile in Heat-Stressed Modern Broilers

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Heat stress has strong adverse effects on poultry production and, thereby, threats its sustainability, which energized scientists to search for innovative and effective solutions. Here, we undertook this study to evaluate the effects of in-feed herbal adaptogen (stress response modifier) supplementation on growth performances, meat quality, and breast amino acid profile in chronic cyclic heat-stressed broilers. Day-old male Cobb 500 chicks (n = 720) were randomly assigned, in environmental chambers (n = 12, 24 pens), to three diet-treatments: a three-phase corn-soybean based diet fed as such (Control, C), or supplemented with the herbal adaptogen at 500 g/1000 kg control diet (NR-PHY-500) or at 1 kg/1000 kg control diet (NR-PHY-1000). From d29 to d42, birds from 9 chambers were exposed to cyclic heat stress (HS, 35°C from 9:30 am-5:30 pm), however, the rest of the chamber were maintained at thermoneutral conditions (24°C, TN), which creates 4 experimental groups: C-TN, C-HS, NR-PHY-500HS, and NR-PHY-1000HS (6 pens/group, 168 birds/group). HS altered growth performance via depression of feed intake and body weight. Adaptogen supplementation stimulated feed intake and averaged 65.95 and 83.25 g better body weight and 5 and 10 points better FCR at low and high dose, respectively, compared to heat-stressed birds. This increase in body weight was mirrored in enhanced weights of body parts (breast, tender, wings, and legs). Adaptogen supplementation modulated also breast amino acid profile, pH, color, and quality. Together, these data suggested that adaptogen supplementation could be a promising solution to alleviate heat stress, however further in-depth investigation for its mode of action and its underlying mechanisms are warranted.

Keywords: heat stress, broilers, adaptogen, growth performance, meat quality

INTRODUCTION

Poultry industry supports the livelihoods and food security of billions of people worldwide. Both poultry meat and egg are globally highly regarded as the most efficient protein sources, with high organoleptic quality, relatively inexpensive, and without religious taboos (Barroeta, 2007; Cavani et al., 2009; Marangoni et al., 2015). However, poultry production sustainability is facing several challenges from a steep projected increase in global demand for high animal protein quality and the need to adapt to the pressure on natural resource availability and environmental constraints. In fact and according to the Food and Agriculture Organization (FAO) of the United Nations (Food and Agriculture Organization [FAO], 2018) and to the 2019 World Population Prospect (United Nations, 2019), it is predicted that the world human population will increase by 10% over the next decade, reaching approximately 9.7 billion in 2050. This, in turn, is estimated to drive a significant rise in the demand for food (∼73% in meat and 58% in dairy products), which require greater animal production including poultry that is projected to double by 2050.

Doubling poultry production within "planetary boundaries" to feed the growing global population will be challenging. It is clear that climate changes drive the earth system into a much warmer state (Alley et al., 2005; Chen et al., 2011). Indeed, unusual warm season with widespread and more intense heat waves have increased markedly over the past decades, and are likely to be larger in the future (Mora et al., 2013). Modern broiler chickens are highly thermo-sensitive and cannot cope well with high environmental temperatures because they are covered with feathers, they have high core body temperature (∼40°C) and high metabolic activity, and they lack sweat gland (Settar et al., 1999; Caulfield et al., 2014; Nawab et al., 2018). The strong adverse effect of heat stress-induced by high environmental temperature on broilers are well documented. Heat stress alters bird's well-being by inducing stress (Star et al., 2008; Gu et al., 2012), depressing feed intake (Flees et al., 2017; Rajaei-Sharifabadi et al., 2017), inducing thirst (Belay et al., 1993), causing immunosuppression (Ghazi et al., 2012; Monson et al., 2018), reducing performance (Quinteiro-Filho et al., 2010; Lara and Rostagno, 2013), and in extreme case increasing mortality rate by spiraling hyperthermia (Furlan et al., 1998). For example, the European heat wave of summer 2003 resulted in the death of more than one million chickens in France (Fouillet et al., 2008). Another heat wave in 2015 killed more than 17 million birds in India (Bhadauria et al., 2016). In addition to welfare and performance issue, heat stress is a significant economic burden to the industry (St-Pierre et al., 2003), and it is a major thrust of intense research effort to identify effective strategies to ameliorate heat stress productivity loss.

Seminal works and various nutritional and/or managerial strategies were applied to mitigate heat stress, yet the poultry productivity losses are still high during hot seasons. Although they are known for more than 60 years in traditional medicine (for review see Panossian and Wikman, 2011), the use of in feed-adaptogens in livestock just begins to gain popularity (Selvam et al., 2018). Adaptogens, plant extracts, also known as stress response modifiers, are defined as metabolic regulators, which increase the ability of an organism to adapt to environmental stressors and to avoid damage from such stressors (Wagner et al., 1994; Panossian and Wikman, 2009, 2010). We undertook the present study to assess the effect of the adaptogen NR-PHY-30 (Natural Remedies, Bengaluru, India) on growth performance and carcass quality in broilers exposed to chronic cyclic heat stress.

MATERIALS AND METHODS

Ethics Statement

All animal experiments were approved by the University of Arkansas Institutional Animal Care and Use Committee (IACUC # 21050) and were in accordance with the recommendations in NIH's *Guide for the Care and Use of Laboratory Animals*.

Animals, Experimental Design, and Sampling

A total of 672 one day-old Cobb 500 male broiler chicks were neck tagged, individually weighed, and randomly housed in twelve environmentally controlled chambers in the Poultry Environmental Research Laboratory at the University of Arkansas Research Farm. Each chamber was divided into 2 floor pens covered with fresh shavings and equipped with separate feeders and drinkers. Each pen contains 28 birds. The experiment was conducted in a complete randomized design with three diet treatments: a three-phase corn-soybean based diet (**Table 1**) fed as such (Control, C), or supplemented with the herbal adaptogen NR-PHY-30 (Natural Remedies, Karnataka, India) at 500 g/1,000 kg control diet (NR-PHY-500) or at 1 kg/1000 kg control diet (NR-PHY-1000) according to the manufacturer's recommendation. The adaptogen was added to the diet in the crumble starter (d0–14) and in the pelleted grower (d15–28) and finisher (d29–42) as recommended by the manufacturer. The composition of the herbal adaptogen is proprietary to Natural Remedies (Karnataka, India), but is a polyherbal formulation of pre-standardized and tested herbs containing *Ocimum sanctum*, *Withania somnifera*, and *Emblica officinalis*. Lighting schedule was 24 h light for the first 3 days, reduced to 23 h light:1 h dark d 4–7, and reduced further to 18 h light:6 h dark thereafter. Ambient temperature was maintained as follows: 32°C for the first 3 days, then gradually reduced approximately by 3°C each week until it reached 24°C on d 21. On day 28, the temperature was increased daily in nine chambers to 35°C for 8 h per day (9:30 am to 5:30 pm) to create a cyclic heat stress pattern and mimic United States summer season until day 42. These chambers reached 35°C within 15 min of temperature adjustment. The three remaining chambers (six pens) were maintained at 24°C as a thermoneutral condition (TN). This creates 4 experimental groups (6 pens/group, 168 birds/group): birds fed C diet and maintained at TN (C-TN), birds fed C diet and exposed to heat stress (C-HS), birds fed NR-PHY-500 and exposed to heat stress (NR-PHY-500HS), and birds fed NR-PHY-1000 and exposed to heat stress (NR-PHY-1000HS). Before the onset of heat stress, two birds per pen were randomly selected and equipped

with a Thermochron temperature logger (iButton, Embedded Data Systems, KY) for continuous monitoring of core body temperature as previously described (Rajaei-Sharifabadi et al., 2017). The environmental temperature and relative humidity were also continuously recorded in each chamber, inside and outside the barn. Feed intake and water consumption were recorded daily, and body weights were measured weekly. On day 41, after blood sampling, thermologger-equipped birds were humanely euthanized via cervical dislocation and tissues samples were collected, snap frozen in liquid nitrogen, and kept at –80°C for future use. On d42, the rest of birds were processed at the University of Arkansas Pilot Processing Plant (Fayetteville, AR, United States).

Growth Performance

Birds were weighed individually on a weekly basis, while feed and water intake were measured on daily basis. Average body weight, average daily feed intake (individual and cumulative), and feed conversion ratio (FCR) were calculated for the experimental period (d 0–42).

Mortality

Birds were monitored twice daily. For each dead bird, date, neck tag number, body weight and cause of death were recorded. This procedure continued throughout the study (up to d 42) to record mortality/treatment for each period thus allowing for adjusting performance parameters for daily mortality.

Peripheral (Skin) Temperature

The surface temperature was measured by an Extech Flir I5 thermal imaging infrared camera (Extech Instruments, Long Branch, NJ, United States) in four birds per pen (24 birds/group) on d41 at 12 pm.

Processing Parameters, Carcass Quality, and Meat Yield

Birds (∼150 birds/group) were processed at the University of Arkansas Pilot Processing Plant (Fayetteville, AR, United States) using a commercial inline system and carcass quality traits were determined as previously described (Orlowski et al., 2018). Briefly, birds were electrically stunned (11 V, 11 mA for 11 s), exsanguinated, scaled at 53.8°C for 2 min, and de-feathered using a commercial, inline equipment (Foodcraft Model 3; Baker international, MI, United States). Carcasses were manually eviscerated and rinsed before prechilling at 12°C for 15 min. Carcasses were, then, chilled for 90 min at 1°C in immersion chilling tanks with manual agitation at 15 min regular intervals. Slaughter weight, and prechilled carcasses were recorded, and following a 2 h chill at 4°C, the weight of breasts, tenders, leg quarters, wings, liver, and abdominal fat were recorded.

Color

At time of debone, 24-h postmortem, and following cooking, intact left filets had color recorded with a handheld Minolta colorimeter and data was configured using SpectraMagic NX software (Minolta CM-400, Konica Minolta Sensing Americas Inc., Ramsey, NJ, United States), set with a 2-degree observer, decreasing surface reflectance, and illuminant parameters of D65. Before measuring, the colorimeter was calibrated to CIE specifications using a white calibration tile in agreeance with the procedure provided by the American Meat Science Association (AMSA) (American Meat Science Association [AMSA], 2012). Calibration values were entered according to the Y, x, and y calibration scheme (D65) and entered as 84.8, 0.3203, and 0.3378, respectively. Intact filets were positioned dorsal side up on white storage trays where measurements could be recorded on the left

TABLE 1 | Composition of basal diets (as fed basis, %)[1].

Ingredients (%)	Period (Days)		
	Starter (0–14) Crumble	Grower (15–28) Pellet	Finisher (29–42) Pellet
Corn (7.81% CP)	60.53	60.99	66.52
Soybean meal (48% CP)	32.95	32.55	27.22
Poultry Fat (9000 kcal/kg)	1.80	2.38	2.46
Dicalcium Phosphate (18.5% P, 22% Ca)	2.08	1.85	1.67
Limestone (37% Calcium)	1.10	1.00	0.91
Sodium Chloride	0.38	0.40	0.44
DL-methionine (990 g/kg)[2]	0.38	0.30	0.27
L-lysine Hydrochloride (788 g L-lysine/kg)[3]	0.37	0.22	0.20
L-threonine (985g/kg)[4]	0.16	0.08	0.08
Choline Chloride (60%)	0.10	0.08	0.08
Vitamin/Trace Mineral Premix[5]	0.15	0.15	0.15
Calculated Analysis (% unless specified)			
ME (kCal/kg)	2994	3038	3108
Crude protein	21.71	21.30	19.18
Total phosphorus	0.77	0.71	0.66
Available phosphorus	0.45	0.42	0.38
Calcium	0.90	0.84	0.75
Chlorine	0.33	0.32	0.34
Sodium	0.16	0.17	0.19
Potassium	0.84	0.83	0.74
Methionine	0.67	0.59	0.54
Methionine + Cysteine	0.98	0.89	0.82
Lysine	1.32	1.18	1.04
Threonine	0.86	0.78	0.70
Linoleic acid	1.46	1.47	1.57
Dietary cation-anion balance	192	196	176

[1]Treatments include: C-TN: birds raised under thermoneutral condition (24°C) from days 29 to 42 and had free access to the control diet; C-HS: birds exposed to cyclic high temperatures (8 h/d at 35°C; from 9:30 am to 5:30 pm) from days 29 to 42 and had free access to the control diet; NR-PHY-500HS: birds raised under the same condition as C-HS group, but fed the control diet supplemented with the herbal adaptogen at 500 g/1000 kg of diet, and NR-HPY-1000HS: birds raised under the same conditions as C-HS group, but fed the control diet supplemented with the herbal adaptogen at 1000 g/1000 kg of diet.

[2]Rhodimet® NP9, ADISSEO, GA, United States.

[3]L-lysine HCl, AJINOMOTO HEARTLAND, INC. Eddyville, IA, United States.

[4]FENCHEM Ingredient Technology, Nanjing, China.

[5]Vitamins supplied per kg diet: retinol 3.33 mg, cholecalciferol 0.1 mg, α-tocopherol acetate 23.4 mg, vitamin K3 1.2 mg, vitamin B1 1.6 mg, vitamin B2 9.5 mg, niacin 40 mg, pantothenic acid 9.5 mg, vitamin B6 2 mg, folic acid 1 mg, vitamin B12 0.016 mg, biotin 0.05 mg, choline 556 mg. Minerals supplied per kg diet: Mn 144 mg, Fe 72 mg, Zn 144 mg, Cu 16.2 mg, I 2.1 mg, Se 0.22 mg.

filet. Three separate L*, a*, and b* values were recorded for each filet in the cranial, medial, and caudal locations which were then subsequently averaged.

pH

Immediately after color was recorded, pH of each left filet was measured. Left halves were also evaluated at 24-h postmortem for pH using a spear tip pH probe with automatic temperature compensation (Model 205, Testo instruments, West Chester, PA, United States). Samples were collected by inserting the pH probe near the wing joint area of each filet and allowed to equilibrate until a reading was maintained for 3 s.

Water Holding Capacity/Drip Loss

Following part weight collection, filets were placed on white plastic storage trays, wrapped in plastic overlay liners, and placed in a walk-in cooler held at 4°C until 24-h postmortem. At 24-h postmortem, breast filets were removed from the cooler and reweighed for determination of drip loss. Drip loss percentage was calculated as a percent by weight in relation to deboned weight.

Cook Loss

Deboned butterflies were trimmed of excess fat and any residual skin was removed. Butterflies were excised down the keel line and identification was kept on an individual basis for each left filet. Individual filets were then weighed to determine a precook weight. Eight filets of similar weight were cooked in aluminum foil covered pans (65 × 395 × 290 mm) on elevated baking racks in a commercial convection oven (Model E101-E, Duke Manufacturing Company, St. Louis, MO, United States) set to 176°C. A final end point temperature of 76°C (Model HT1000 thermometer, Cooper Instruments, Concord, ON, Canada) was reached before the filets were removed and allowed to cool at room temperature on white plastic storage trays. After cooling for approximately 1 h at room temperature, filets were reweighed to determine cook loss percentage. Cook loss was calculated as a percent, by weight, in relation to precook weight. Filets were then wrapped individually in aluminum foil sheets and were stored in refrigerated conditions (4°C) for approximately 24 h until instrumental texture analysis could be completed.

Texture Analysis

Using a texture analyzer (Model TA-XT2 Plus, Texture Technologies, Scarsdale, NY, United States), tenderness was indirectly determined using the Meullenet–Owens Razor Shear (MORS), as described by Cavitt et al. (2004), during which MORS force (MORSF, N), MORS energy (MORSE, N.mm), and total peak counts (sums) were recorded. Briefly, a 5-kg load cell using a razor blade with a height of 24 mm and a width of 8.9 mm was set to a penetration depth of 20 mm. Crosshead speed was set at 5 mm/s and was triggered by a 5 g contact force. Data points were collected with an acquisition rate of 200 points per second. Breasts were punctured perpendicular to muscle fibers in four locations and shear energy was calculated as the area under the force deformation curve from the beginning to the end of the test.

Measurement of Amino Acid Profile

Free amino acid assays of breast muscle tissues were carried out by Novus Analytical Service (St. Charles, MO, United States). Briefly, breast tissues were lyophilized for 48 h and milled to 1 mm particle size. Samples (200 mg) were accurately weighed into 50 mL digestion tubes and performic acid (2 mL) is added. The tubes are sealed and kept at 4°C for 16–24 h. Sodium metabisulfite (1.1 mL) is added to each sample, allowed to sit for 15 min followed by the addition of 11.9 mL 6N HCl. Samples were nitrogen purged, recapped, and placed in a 110°C oven for 24 h. The resulting hydrolyzates are neutralized along with internal standard addition. The samples are then filtered and analyzed on a Hitachi Amino Acid Analyzer using anion exchange chromatography followed by ninhydrin derivatization. For tryptophan measurement, samples (200 mg) were accurately weighed into 30 mL digestion tubes. 10 mL of nitrogen purged 4M NaOH was added to each tube along with 0.4 mL of 1M Dithiothreitol. Each tube is then purged with nitrogen, sealed, and placed in a 110°C oven for 22 h. Samples are neutralized with 0.22 M Sodium acetate then filtered for analysis. Tryptophan is separated using reverse phase chromatography and directly detected at 280 nm.

Statistics

Data were analyzed by one-way ANOVA. In case ANOVA showed significant effects, the means were compared by Tukey's multiple range test using Graph Pad Prism software (version 6.00 for Windows, Graph Pad Software, La Jolla, CA, United States). Data are expressed as the mean ± SEM, and means were considered statistically significant at a P-value ≤ 0.05.

RESULTS

The experiment has been conducted from January 21 to March 3, 2021 for 42 days. The weather cast (temperature and RH) is shown in **Figures 1A,B**. The average temperature was approximately 10°C and the RH was about 60–70%, which is typical in Arkansas during that season. The average temperature and the RH inside of the barn (but outside of the chambers) were ∼20°C and 30–40%, respectively (**Figures 1A,B**). The temperature inside of the environmental chambers was accurately manipulated to reach 35°C from 9:30 am to 5:30 pm and return to 24°C during the rest of the day each day from d29 to d42, creating a cyclic heat stress condition as planned (**Figure 1C**). The RH inside the chambers was ∼16–20% until d29 and then increased during heat stress to reach ∼45–60% (**Figure 1D**).

As expected, heat stress significantly increased the broiler body core temperature by ∼0.5°C compared to TN conditions (**Figure 2A**). The supplementation of the herbal adaptogen (NR-PHY-30) increased further the body core temperature of heat-stressed broilers, although the difference was not statistically discernable (**Figure 2A**). Similarly, the infrared thermal imaging showed that the surface temperature was significantly higher in heat-stressed broilers fed with control- and adaptogen-supplemented diets compared to birds maintained

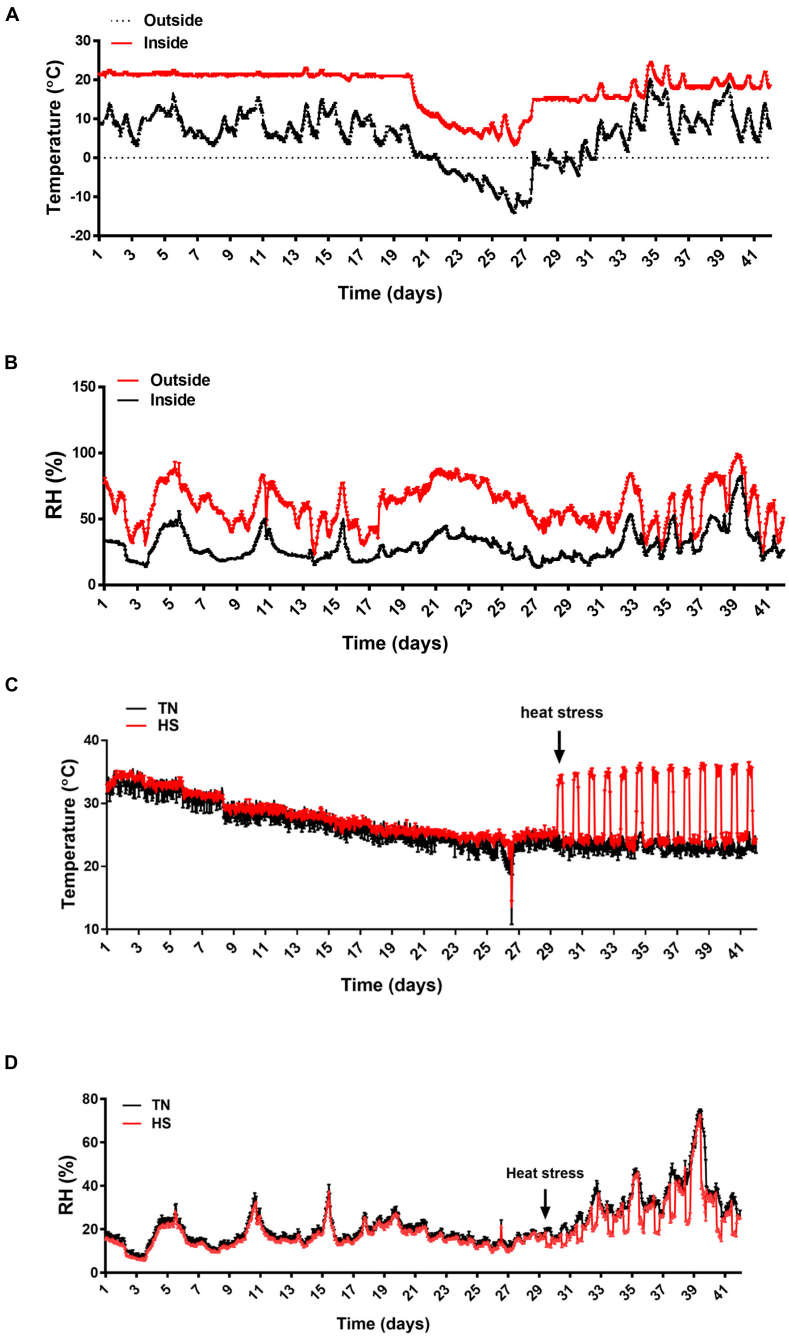

FIGURE 1 | The environmental conditions during the experimental trial. **(A)** Temperature variations outside and inside the barn, **(B)** RH variations outside and inside the barn, **(C)** temperature variation inside the environmental chambers, and **(D)** RH variations inside the environmental chambers. Temperatures and RH were measured using thermologgers. RH, relative humidity.

at TN conditions (**Figures 2B,C**). There was no significant difference in the mortality rate between all groups (7.73, 6.54, 5.95, and 7.14% in C-TN, C-HS, NR-PHY-500HS, and NR-PHY-1000HS, respectively).

As shown in **Figures 3A–C**, heat stress significantly reduced feed intake and body weight compared to TN conditions. Adaptogen supplementation significantly increased feed intake and body weight in a dose-dependent manner in heat-stressed broilers compared to those fed control diet and exposed to heat stress (**Figures 3A,B**). Adaptogen supplementation averaged 65.95 and 83.25 g better SW (**Table 2**) and 5 and 10 points better FCR at low (500 g/1000 kg diet) and high dose (1 kg/1000 kg diet), respectively, compared to heat-stressed birds (**Figure 3D**). Heat stress significantly reduced body part weights (HCW,

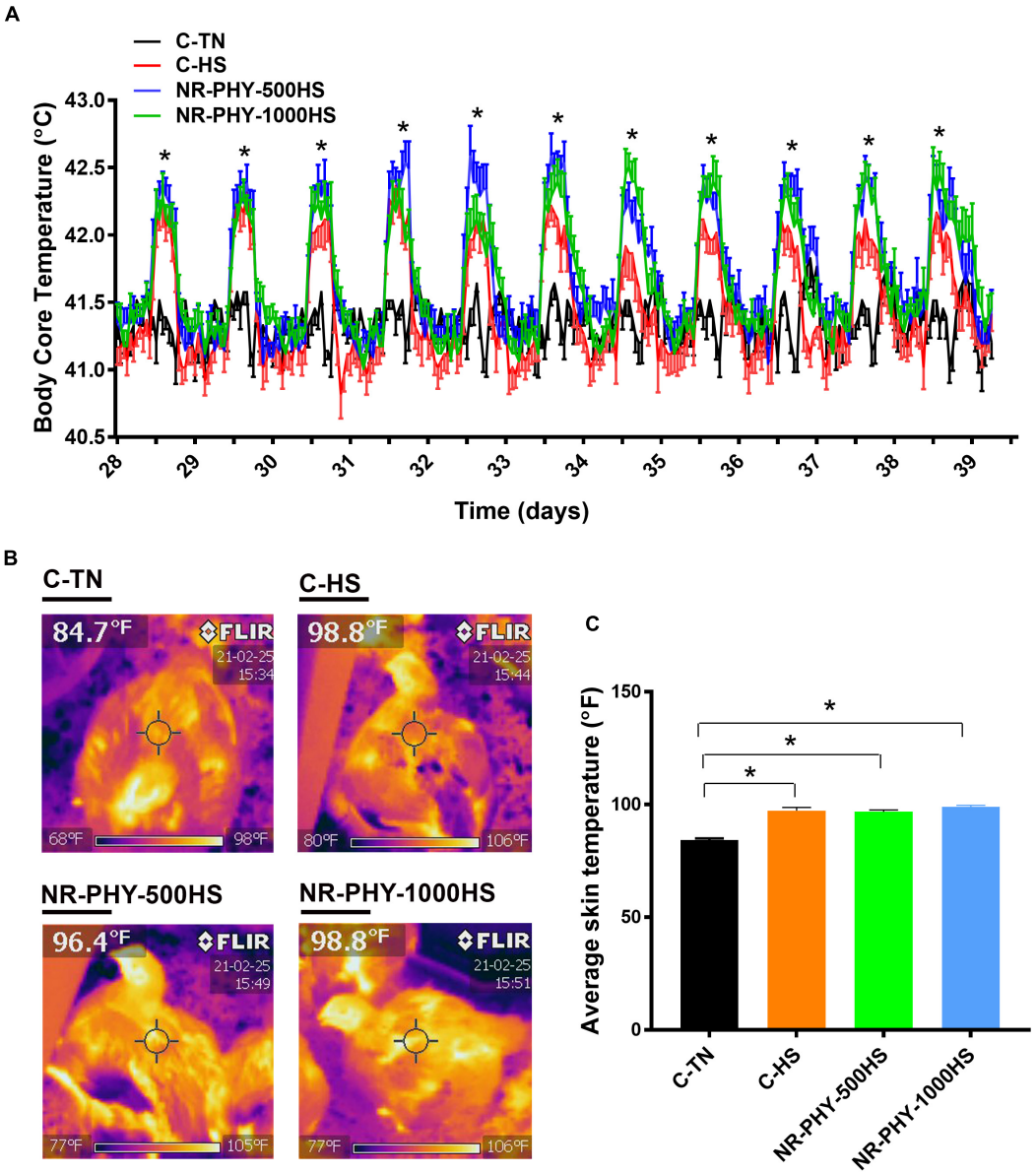

FIGURE 2 | Effects of heat stress and adaptogen supplementation on surface and body core temperature. Core body temperature **(A)** was monitored using Thermochron temperature loggers (iButton, Embedded Data Systems, KY, United States) and surface temperature **(B,C)** was measured using an Extech Flir I5 thermal imaging infrared camera (Extech Instruments, Long Branch, NJ, United States). Data are mean ± SEM (n = 12/group for the body core temperature and n = 24 for the surface temperature). *Indicates a significant difference at $P < 0.05$. C, control diet; HS, heat stress; TN, thermoneutral.

CCWG, breast, tender, wings, and leg) (**Table 2**). Although it was not significant, except for tender, adaptogen supplementation increased, in a dose-dependent manner, breast weight (by ∼20 and 27.28 g), tender weight (by ∼2.66 and 5.18 g), and leg weight (by ∼7.42 and 15.94 g) at low and high doses, respectively, compared to heat-stressed birds fed control diet (**Table 2**).

Heat stress did not affect the pH of breast meat at both processing and 24 h postmortem (**Table 3**). However, adaptogen supplementation at both doses significantly increased pH at processing only, and not at 24 h postmortem (**Table 3**). Heat stress significantly reduced breast meat b* value at processing, and did not affect any other color value (**Table 4**). Breasts from adaptogen-fed birds exhibited significant higher processing L* and b* values compared to those from heat-stressed birds fed a control diet (**Table 4**). Meat color parameters (L*, a*, and b*) did not differ between all groups at 24 h postmortem and after cook (**Table 4**). Cook loss was significantly reduced by heat stress, and increased to normal levels by adaptogen supplementation (**Table 5**). Adaptogen supplementation at high dose (1 kg/1000 kg

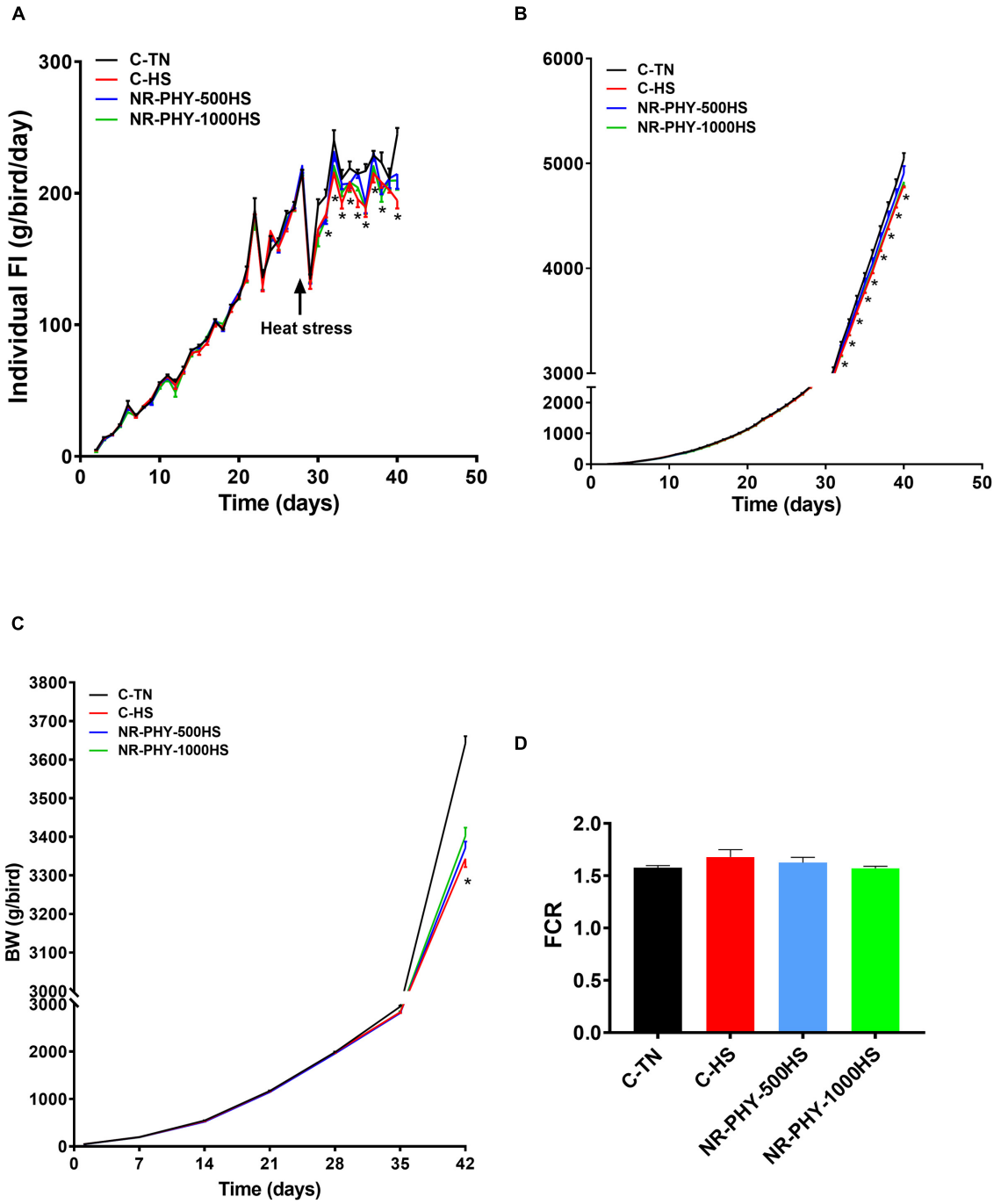

FIGURE 3 | Effects of heat stress and adaptogen supplementation on growth performance. **(A)** Daily individual feed intake, **(B)** cumulative feed intake, **(C)** body weight, and **(D)** FCR. Pen and bird were experimental unit for feed intake and body weight, respectively. Data are mean ± SEM (n = 6/group for the feed intake, and n = 168 for body weight). *$P < 0.05$ compared to the control-TN.

diet) significantly increased MORSE compared to birds fed control diet and maintained under both TN and heat stress conditions (**Table 5**).

Heat stress did not elicit any change to free amino acid profile in breast tissues compared to TN environment in our experimental conditions (**Table 6**). Supplementation of adaptogen at both doses significantly reduced breast cysteine levels compared to heat-stressed birds fed with control diet (**Table 6**). High dose (1 kg/1000 kg diet) of adaptogen significantly decreased the breast levels of glutamic acid, glycine, serine, and threonine compared to heat-stressed birds fed with control diet (**Table 6**).

TABLE 2 | Effects of the herbal adaptogen on carcass and body parts weights and yields of heat-stressed broilers[1].

	C-TN	C-HS	NR-PHY-500HS	NR-PHY-1000HS
SW (g)	3524.37 ± 20.82^{a}	3186.10 ± 25.32^{b}	3252.02 ± 18.91^{b}	3269.35 ± 25.23^{b}
HCW (g)	2610.28 ± 17.21^{a}	2365.98 ± 20.41^{b}	2408.70 ± 15.03^{b}	2426.39 ± 20.50^{b}
CCWG (g)	2674.93 ± 17.37^{a}	2420.82 ± 20.67^{b}	2465.32 ± 15.08^{b}	2485.35 ± 20.98^{b}
Fat (g)	38.67 ± 0.93	36.13 ± 0.88	35.18 ± 0.82	37.00 ± 1.05
Fat (%)	1.49 ± 0.04	1.52 ± 0.04	1.46 ± 0.03	1.52 ± 0.04
Breast (g)	764.36 ± 7.36^{a}	665.94 ± 8.05^{b}	685.92 ± 6.84^{b}	693.22 ± 8.14^{b}
Breast yield (%)	29.23 ± 0.17^{a}	28.07 ± 0.19^{b}	28.43 ± 0.16^{b}	28.52 ± 0.18^{b}
Tender (g)	139.99 ± 1.29^{a}	124.72 ± 1.20^{b}	127.38 ± 1.08^{bc}	129.90 ± 1.42^{c}
Tender yield (%)	5.35 ± 0.03	5.27 ± 0.03	5.29 ± 0.03	5.35 ± 0.03
Wings (g)	270.00 ± 1.78^{a}	249.07 ± 2.15^{b}	251.63 ± 1.68^{b}	249.25 ± 2.11^{b}
Wing yield (%)	10.35 ± 0.05^{a}	10.55 ± 0.05^{b}	10.46 ± 0.04^{ab}	10.29 ± 0.05^{a}
Leg (g)	766.50 ± 5.67^{a}	715.23 ± 7.13^{b}	722.65 ± 4.73^{b}	731.17 ± 6.99^{b}
Leg yield (%)	29.36 ± 0.13^{a}	30.23 ± 0.15^{b}	30.03 ± 0.14^{b}	30.14 ± 0.14^{b}

[1]Data are means ± SEM (n = 150 birds/group). Treatments are: C-TN: birds raised under thermoneutral condition (24°C) from days 29 to 42 and had free access to the control diet; C-HS: birds exposed to cyclic high temperatures (8 h/d at 35°C; from 9:30 am to 5:30 pm) from days 29 to 42 and had free access to the control diet; NR-PHY-500HS: birds raised under the same condition as C-HS group, but fed the control diet supplemented with the herbal adaptogen at 500 g/1000 kg of diet, and NR-HPY-1000HS: birds raised under the same conditions as C-HS group, but fed the control diet supplemented with the herbal adaptogen at 1000 g/1000 kg of diet CCWG, chilled carcass without giblet; HCW, hot carcass without giblet; SW, slaughter weight. Different letters within a row indicate a significant difference at $P < 0.05$.

TABLE 3 | Effects of the herbal adaptogen on breast meat pH of heat-stressed broilers[1].

	C-TN	C-HS	NR-PHY-500HS	NR-PHY-1000HS
At processing	6.10 ± 0.01^{a}	6.13 ± 0.01^{a}	6.19 ± 0.01^{b}	6.19 ± 0.01^{b}
24 h postmortem	5.98 ± 0.02	6.03 ± 0.02	5.99 ± 0.02	6.05 ± 0.02

[1]Data are means ± SEM (n = 150 birds/group). Treatments are: C-TN: birds raised under thermoneutral condition (24°C) from days 29 to 42 and had free access to the control diet; C-HS: birds exposed to cyclic high temperatures (8 h/d at 35°C; from 9:30 am to 5:30 pm) from days 29 to 42 and had free access to the control diet; NR-PHY-500HS: birds raised under the same condition as C-HS group, but fed the control diet supplemented with the herbal adaptogen at 500 g/1000 kg of diet, and NR-HPY-1000HS: birds raised under the same conditions as C-HS group, but fed the control diet supplemented with the herbal adaptogen at 1000 g/1000 kg of diet. Different letters within a row indicate a significant difference at $P < 0.05$.

TABLE 4 | Effects of the herbal adaptogen on breast meat color of heat-stressed broilers[1].

	C-TN	C-HS	NR-PHY-500HS	NR-PHY-1000HS
At processing				
L*	52.75 ± 0.18^{ac}	52.32 ± 0.18^{a}	53.68 ± 0.16^{b}	53.12 ± 0.20^{bc}
a*	3.23 ± 0.10	2.93 ± 0.09	3.13 ± 0.08	3.14 ± 0.10
b*	8.62 ± 0.10^{a}	8.18 ± 0.11^{b}	8.76 ± 0.10^{a}	8.66 ± 0.10^{a}
24 h Postmortem				
L*	54.87 ± 0.49	54.04 ± 0.49	55.09 ± 0.34	55.59 ± 0.36
a*	2.72 ± 0.17	3.04 ± 0.22	2.96 ± 0.17	3.22 ± 0.21
b*	8.25 ± 0.19	8.18 ± 0.24	8.47 ± 0.017	8.83 ± 0.30
Post cook				
L*	77.51 ± 0.67	77.87 ± 0.53	76.87 ± 0.75	76.61 ± 0.39
a*	3.58 ± 0.22	4.00 ± 0.21	4.13 ± 0.26	3.84 ± 0.12
b*	20.32 ± 0.44	19.98 ± 0.62	21.34 ± 0.64	20.86 ± 0.47

[1]Data are means ± SEM (n = 150 birds/group). Treatments are: C-TN: birds raised under thermoneutral condition (24°C) from days 29 to 42 and had free access to the control diet; C-HS: birds exposed to cyclic high temperatures (8 h/d at 35°C; from 9:30 am to 5:30 pm) from days 29 to 42 and had free access to the control diet; NR-PHY-500HS: birds raised under the same condition as C-HS group, but fed the control diet supplemented with the herbal adaptogen at 500 g/1000 kg of diet, and NR-HPY-1000HS: birds raised under the same conditions as C-HS group, but fed the control diet supplemented with the herbal adaptogen at 1000 g/1000 kg of diet. Different letters within a row indicate a significant difference at $P < 0.05$. L, lightness; a*, redness and b*, yellowness.*

DISCUSSION

There is a monumental pressure on farm animals to increase production to feed the future and meet the growing global demand for high-quality animal proteins. Due to various constraints, including extreme environmental conditions, this will be very challenging. Heat load is one of the most challenging stressor to poultry industry worldwide because of its strong

TABLE 5 | Effects of the herbal adaptogen on breast meat drip and cook loss[1].

	C-TN	C-HS	NR-PHY-500HS	NR-PHY-1000HS
Drip Loss (g)	4.13 ± 1.52	4.75 ± 0.71	5.61 ± 0.89	7.54 ± 1.17
Cook Loss (g)	79.21 ± 3.63^{a}	57.54 ± 2.74^{b}	79.13 ± 5.95^{a}	86.13 ± 3.94^{a}
MORSF (N)	12.40 ± 0.39	12.51 ± 0.39	13.39 ± 0.56	13.94 ± 0.43
MORSE (N*mm)	165.34 ± 5.99^{a}	166.27 ± 4.97^{ab}	179.08 ± 7.13^{ab}	187.89 ± 6.32^{b}
Peak Counts	8.02 ± 0.32	8.39 ± 0.40	8.15 ± 0.27	7.72 ± 0.32

[1]Data are means ± SEM (n = 150 birds/group). Treatments are: C-TN: birds raised under thermoneutral condition (24°C) from days 29 to 42 and had free access to the control diet; C-HS: birds exposed to cyclic high temperatures (8 h/d at 35°C; from 9:30 am to 5:30 pm) from days 29 to 42 and had free access to the control diet; NR-PHY-500HS: birds raised under the same condition as C-HS group, but fed the control diet supplemented with the herbal adaptogen at 500 g/1000 kg of diet, and NR-HPY-1000HS: birds raised under the same conditions as C-HS group, but fed the control diet supplemented with the herbal adaptogen at 1000 g/1000 kg of diet. Different letters within a row indicate a significant difference at P < 0.05. MORSF, Meullenet-Owens razor shear force, and MORSE, Meullenet-Owens razor shear energy.

TABLE 6 | Effects of the herbal adaptogen on breast muscle amino acid profile[1].

Amino acids (%)	C-TN	C-HS	NR-PHY-500HS	NR-PHY-1000HS
Alanine	4.536 ± 0.07^{a}	4.536 ± 0.07^{a}	4.408 ± 0.05^{ab}	4.236 ± 0.09^{b}
Arginine	4.926 ± 0.07^{a}	4.918 ± 0.08^{ab}	4.77 ± 0.06^{ab}	4.618 ± 0.09^{b}
Aspartic acid	7.424 ± 0.11	7.428 ± 0.11	7.176 ± 0.10	6.948 ± 0.15
Cysteine	0.976 ± 0.01^{a}	0.966 ± 0.007^{a}	0.928 ± 0.01^{b}	0.904 ± 0.01^{b}
Glutamic acid	11.942 ± 0.12^{a}	11.938 ± 0.12^{a}	11.5 ± 0.18^{ab}	11.086 ± 0.20^{b}
Glycine	3.208 ± 0.01^{a}	3.184 ± 0.03^{a}	3.152 ± 0.02^{a}	3.006 ± 0.05^{b}
Histidine	2.614 ± 0.10	2.704 ± 0.14	2.652 ± 0.06	2.598 ± 0.09
Isoleucine	3.724 ± 0.06	3.746 ± 0.06	3.62 ± 0.05	3.508 ± 0.08
Leucine	6.226 ± 0.09	6.228 ± 0.09	6.018 ± 0.09	5.832 ± 0.12
Lysine	8.114 ± 0.16	8.204 ± 0.18	7.826 ± 0.16	7.632 ± 0.19
Methionine	2.304 ± 0.04	2.33 ± 0.04	2.25 ± 0.03	2.174 ± 0.05
Methionine + Cysteine	3.278 ± 0.05	3.294 ± 0.05	3.178 ± 0.05	3.078 ± 0.07
Phenylalanine	4.926 ± 0.14	5.016 ± 0.20	4.828 ± 0.10	4.75 ± 0.18
Proline	2.062 ± 0.02	2.088 ± 0.04	2.034 ± 0.04	2.01 ± 0.07
Serine	3.124 ± 0.03^{a}	3.112 ± 0.03^{a}	2.994 ± 0.04^{ab}	2.882 ± 0.05^{b}
Threonine	3.518 ± 0.05^{ab}	3.534 ± 0.05^{a}	3.406 ± 0.05^{ab}	3.3 ± 0.07^{b}
Tryptophan	0.802 ± 0.02	0.814 ± 0.01	0.814 ± 0.008	0.762 ± 0.02
Tyrosine	2.042 ± 0.04	2.042 ± 0.03	1.982 ± 0.04	1.938 ± 0.04
Valine	2.552 ± 0.04	2.436 ± 0.09	2.484 ± 0.03	2.422 ± 0.05

[1]Data are means ± SEM (n = 6 birds/group). Treatments are: C-TN: birds raised under thermoneutral condition (24°C) from days 29 to 42 and had free access to the control diet; C-HS: birds exposed to cyclic high temperatures (8 h/d at 35°C; from 9:30 am to 5:30 pm) from days 29 to 42 and had free access to the control diet; NR-PHY-500HS: birds raised under the same condition as C-HS group, but fed the control diet supplemented with the herbal adaptogen at 500 g/1000 kg of diet, and NR-HPY-1000HS: birds raised under the same conditions as C-HS group, but fed the control diet supplemented with the herbal adaptogen at 1000 g/1000 kg of diet. Different letters within a row indicate a significant difference at P < 0.05.

adverse effects on welfare, feed intake, growth, immunity, meat yield, and mortality (Star et al., 2008; Quinteiro-Filho et al., 2010; Ghazi et al., 2012; Lara and Rostagno, 2013; Flees et al., 2017; Rajaei-Sharifabadi et al., 2017; Monson et al., 2018; Liu et al., 2019). Over the past decades, widespread extreme heat waves have occurred repeatedly and caused great losses across the globe (Rotter and Van de Geijn, 1999; St-Pierre et al., 2003; Thornton et al., 2009; Nardone et al., 2010; Reynolds et al., 2010). These heat anomalies are projected to increase in the future and their strong adverse effects will likely take a heavy toll and large amplitude (Meehl and Tebaldi, 2004; Sherwood and Huber, 2010; Liu et al., 2017; Mora et al., 2017). There is, therefore, a critical need to identify new effective strategies (management, nutrition, etc.) to mitigate these adverse effects and ameliorate heat stress productivity losses.

Fueled by consumer preferences for natural products, there is growing interest in using phytobiotic feed additives in the animal food system (American Meat Science Association [AMSA], 2012). Although they are known for more than 60 years in medicine and pharmacosanation (Wagner et al., 1994), and their original plant sources have been used for centuries in traditional medicine (Wagner et al., 1994; Panossian and Wikman, 2011), adaptogens are just recently begin to be used as feed additive in poultry (Selvam et al., 2018; Marimuthu et al., 2020). Herbal adaptogens are plant-derived biologically active substances originally defined as stress-response modifiers and metabolic regulators that increase the ability of an organism to adapt to environmental stressors and, thereby, protect against cellular damage from such stressors (Duraisami et al., 2010; Giri et al., 2011, 2013; Singh et al., 2017). In this study, we

sought to assess the effect of the herbal adaptogen NR-PHY-30, formulated by Natural Remedies Private Limited, Bengaluru, India, on growth performance, carcass quality and breast amino acid profile in heat-stressed broilers.

NR-PHY-30 contains Indian gooseberry (*Emblica officinalis*), holy basil (*Ocimum sanctum*), and winter cherry (*Withania somnifera*) (Natural Remedies, personal communication), and the adaptogenic properties of these plants have been already reported (Wagner et al., 1994; Panossian and Wikman, 2011) as well as their beneficial therapeutic effects (Pattanayak et al., 2010; Malik et al., 2016; Dutta et al., 2019). As expected and in agreement with previous studies, including our own (Flees et al., 2017; Rajaei-Sharifabadi et al., 2017; Liu et al., 2020; Tabler et al., 2020; Wasti et al., 2020), heat stress increased both surface and body core temperatures and reduced feed intake and body weight in modern broilers. Interestingly, although it increased further the surface and body core temperatures, the herbal adaptogen NR-PHY-30 enhanced feed intake and body weight and improved FCR in heat-stressed broilers. This suggests one of the two following potential scenarios: (1) While it is known that the body experiences a temperature-dependent variation in energy needs that should be reflected in feed intake (reduction under heat stress conditions) (Brobeck, 1948; Jakubczak, 1976), the effect of the adaptogen NR-PHY-30 on appetite and feed intake in our experimental conditions was probably independent from the thermostatic mechanism (Brobeck, 1948). For instance, it has been shown that intracerebroventricular administration of IL-1 raised body core temperature without affecting feed intake in rodents indicating that feed intake regulation could be uncoupled from the thermoregulatory mechanisms (McCarthy et al., 1986). (2) Although it was not measured in the present study, as the adaptogen was supplemented from d1 post hatch, it is probable that NR-PHY-30 increased the body core temperature at early age, which mimics a repeated mild exposure and low stress dose that resulted in increased resistance and better adaptation to heat stress (thermo-tolerance) at later age. This second scenario might be supported also by previous thermal conditioning studies showing that temporarily elevated brooding temperatures at early post-hatch or at embryonic age impart long-term resistance to heat stress in broilers, which survive and grow better at higher environmental temperatures (De Basilio et al., 2003; Gunal, 2013; Loyau et al., 2013, 2015; Zaboli et al., 2017).

The increased body weight was accompanied by an enhanced weights of body parts, including breast and tender weights along with a significant reduction in breast levels of free alanine, arginine, cysteine, glutamic acid, glycine, and serine. This indicates that the adapotogen NR-PHY-30 might stimulate amino acid incorporation and use by the muscle for protein synthesis. Previous studies have shown the key role of alanine (Perez-Sala et al., 1987), arginine (Wang et al., 2018), cysteine (Chua et al., 1984), glutamic acid (Brodsky et al., 2017), glycine (Wang et al., 2014), and serine (Galbraith and Buse, 1981) in protein synthesis. What downstream pathways used by the adaptogen to enhance muscle protein synthesis under heat stress condition is not known and merit further in depth investigation.

Although heat stress did not affect the breast meat pH, it reduced the b* (yellowness) value, which corroborated previous studies (McKee and Sams, 1997; Aksit et al., 2006). This might be associated with heat stress-induced disruption of muscle membrane integrity, denaturation of muscle proteins, and in turn increased light scattering (Owens et al., 2000; Sandercock et al., 2001). Interestingly, the adaptogen supplementation increased the pH at processing and increased the L* value, suggesting potential improvement of meat color. Although the underlying mechanisms are not known at this time, it is possible that the adaptogen supplementation improved the antioxidant status of the breast muscle (Arif et al., 2016), which in turn improve meat quality. Intriguingly, heat stress reduced cook loss and the adaptogen supplementation reverse this parameter to the same levels of breast from TN birds fed with control diet. Similarly, MORS total energy (MORSE) was not altered by heat stress, but increased with the adaptogen supplementation, indicating that the adaptogen reduced breast meat tenderness. Although it was not measured here, and in addition to collagen and tissue connective content in the breast, tenderness might be affected by the sarcomere length, muscle fiber diameter, stromal proteins, and collagen solubility (Koohmaraie et al., 2002), all of which might be modified by the adaptogen administration, which warrant further investigations.

CONCLUSION

Supplementation of the herbal adaptogen NR-PHY-30 stimulated appetite and feed intake, and in turn improved growth performance in a dose-dependent manner (average 66–83 g in body weight and 5–10 points better FCR) in cyclic heat-stressed broilers, which make it a promising nutritional strategy. However, further in depth investigation are needed to define its mode of action and its underlying molecular mechanisms.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by the University of Arkansas.

AUTHOR CONTRIBUTIONS

SD designed the experiment and purchased the reagents. EG and SD conducted the trial and processed the animals at the end of trial. EG and CM measured and analyzed the meat quality parameters. SD wrote the manuscript with input from EG, CM, CO, and J-FM. All the authors have read and agreed to the published version of the manuscript.

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Effects of Dietary Ramie Powder at Various Levels on the Production Performance, Serum Biochemical Indices, Antioxidative Capacity, and Intestinal Development of Laying Hens

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The purpose of this study was to investigate the effects of ramie (0, 3, 6, and 9%) included in diets on production performance, antioxidative capacity, serum biochemical indices, and intestinal development of laying hens. A total of 432 Lohmann commercial laying hens were randomly allotted to one of four dietary treatments and fed for 6 weeks. The results showed that the inclusion of ramie had no negative effects on laying performance, and increased (quadratic, $P < 0.05$) the laying rate with the highest value in the 6% ramie group. However, ramie content in the diet up to 9% reduced the apparent metabolic energy, dry matter, and organic matter apparent digestibility of laying hens compared with those in the 3% ramie group. The content of high-density lipoprotein (HDL-C) in serum was increased ($P < 0.05$), but the activity of aspartate aminotransferase (AST) was decreased ($P < 0.05$) by dietary ramie supplementation. As the dietary ramie level increased, the activity of serum glutathione peroxidase (GSH-Px) was increased quadratically ($P < 0.05$). Compared with control, 3% ramie group significantly increased ($P < 0.01$) liver total superoxide dismutase (SOD) activity. Meanwhile, the addition of 3∼6% ramie powder increased ($P < 0.05$) villus height of jejunum and villus height/crypt depth (V/C) of ileum, which reflected the intestinal promotional effect of ramie powder. In conclusion, ramie in a diet of less than 9% might protect the liver and improve the antioxidative capacity with no detrimental impacts on the laying hens. Moreover, it could promote the intestinal mucosal structure and have a positive impact on the intestine health of the laying hens.

Keywords: ramie, laying production performance, serum biochemical indices, antioxidative capacity, intestinal development

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INTRODUCTION

Soybean meal was the main protein source of feed in China, 70% of which were imported from abroad (Yin et al., 2019). The shortage of traditional feed resources and rising prices were important factors that restricted the development of animal husbandry in recent years. Accordingly, new high-quality feed ingredients were in great need to reduce the dependency on imported protein feed, lower the feed cost, and keep or even improve the qualities of livestock and poultry products.

Ramie (*Boehmeria nivea*), also known as "Chinese grass," is a perennial herb of the ramie family in the Urticaceae family Ramie family. With the features of fast moisture absorption and good air permeability, ramie was used as a raw material for the production of textiles (Ni et al., 2018). However, due to the low utilization rate, a large number of ramie by-products were discarded, resulting in a waste of resources (Liu et al., 2013). The nutritional values of ramie tender stems and leaves were similar to that of alfalfa, with high protein content (about 20.00% of dry matter, DM) and moderate neutral detergent fiber (NDF), reasonable amino acid composition (especially lysine, slightly more than 1.00% of DM) (Lee et al., 2009). In addition, the tender stems and leaves of ramie were edible and could be used as medication (Wang et al., 2019). Ramie belongs to the genus Ramie in the Urticaceae family and was a potential vegetable protein feed. Studies showed that feeding mice with nettle plants, which also belonged to the Urticaceae family, could significantly reduce the lipid metabolism and reduce plasma total cholesterol and triglyceride content (Avcı et al., 2006). Using ramie leaf extract could improve the blood lipid status of db/db obese mice and reduce the weight of adipose tissue (Lee et al., 2014). Moreover, previous works of literature indicated that ramie leaves had several flavonoids and polyphenols which possessed antioxidative effects *in vitro* (Chen et al., 2014). However, to our knowledge, no relevant research was conducted to test the influence of ramie treatment on hens. Therefore, the objective of this study was to evaluate the effects of varying levels of dietary ramie powder on the production performance, serum biochemical indicators, antioxidant capacity, and intestinal development of laying hens.

MATERIALS AND METHODS

Ramie Powder Preparation

The ramie powder was prepared as described by Li et al. (2018). Briefly speaking, the leaves and tender tops were cut and collected when the ramie plant (*Boehmeria nivea* cv. Qingsizhu No. 1) grew to about 60 cm, and dried immediately at 60°C for 4 days in a forced-air oven until the water content dropped to 7%. Then the dried ramie materials were pulverized by a milling machine (SRL-Z 500, Zhangjiagang Sevenstars Machinery Co., Ltd., Zhangjiagang, China) to powders with particle size less than 1.5 mm. The processed ramie powders were then packed in seized bags and kept in a light-resistant place for further use.

Animals and Experimental Details

All the experimental procedures were approved by the Animal Care Committee of the Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences, Changsha, China. A total of 432 34-week-old Lohmann Commercial laying hens were randomly divided into four groups, with six replicates of 18 birds each. The diets for the four groups were control diet (corn-soybean meal diets, SBM), 3, 6, and 9% ramie powder substituted SBM, respectively. All the diets were formulated to contain similar levels of CP and to meet recommendations of the National Research Council (NRC) for laying hens (1994) as shown in **Table 1**. The hens were raised in ladder cages with one bird in each cage. After a 2-week adaption, the main experiment started and lasted for 6 weeks. The egg production, body weight, and feed intake of each laying hen were measured on the first day of the experiment, and no statistical differences in the production performance were found among treatments. Free water and feed were provided for all hens. The average temperature was 25 ± 2°C in the house of laying hens during the experimental period. The light time was according to the standard light procedure of commercial laying hens, which was 16 h of light per day, until the end of the experiment.

At the end of the experiment, blood samples were taken from hens *via* the wing vein of hens (two birds/replicate, 12/treatment). One blood sample was collected from the left-wing vein of each hen in vacuum blood collection tubes. The whole blood was coagulated in a tube at room temperature and centrifugated at 3,500 rpm for 15 min. The serum samples were separated and stored at −20°C until it was used for the measurement of antioxidative and biochemical indices.

After blood collection, the hens were euthanized by carbon inhalation. After cervical dislocation, duodenum, jejunum, and ileum tissues were quickly separated from the body in a sterile environment, and about 2 cm of the middle parts of each section were taken, cleaned gently with normal saline, and fixed in 4% paraformaldehyde solution. The liver tissue samples were taken 2 g, kept in the centrifuge tube, and frozen at −20°C for later analysis. The weights of the heart, liver, spleen, small intestine, gizzard, and proventriculus were measured and the corresponding organ indexes were calculated.

Production Performance

During the experiment, eggs of each hen were counted and weighted at 4:30 pm daily. The feed intake for each hen was recorded weekly. Finally, the egg-laying rate was calculated as the number of total eggs produced by each hen divided by experimental days. The feed conversion ratio (FCR) was calculated as grams of total feed intake per hen divided by grams of total egg mass per hen. The egg mass was calculated as the mean egg weight times the egg-laying rate.

Apparent Nutrient Digestibility

An extra of five laying hens were used for the analysis of apparent nutrient digestibility for experimental diets. The excreta samples were collected from each hen twice a day (8:00 am and 5:00 pm) for three consecutive days and added dilute hydrochloric acid, pooled together. Then air-dried samples were prepared for subsequent experimental analysis. At the same time, according to the regulations and requirements of Feed Sampling (China, GB/T 14699.1-2005, General Administration

TABLE 1 | Diet formulation and calculated nutrients (as fed basis).

Items	Control	Ramie power supplementation concentration in diets		
		3%	6%	9%
Ingredients, %				
Corn	51.61	51.34	51.23	51.03
Soybean meal	30.78	29.76	28.66	27.59
Rice husk	3.31	2.21	1.09	0.00
Ramie powder	0.00	3.00	6.00	9.00
Oil	2.85	2.50	2.10	1.73
Limestone	8.45	8.19	7.92	7.65
Premix	3.00	3.00	3.00	3.00
Total	100	100	100	100
Nutrient levels (%)				
ME (Mcal/kg)	2.75	2.75	2.75	2.75
Crude protein (%)	16.98	17.00	17.00	17.01
Crude fiber	4.44	4.44	4.44	4.45
Calcium (%)	3.50	3.50	3.50	3.50
Total phosphorus (%)	0.53	0.53	0.53	0.53
Lysine (%)	0.95	0.95	0.94	0.93
Methionine (%)	0.36	0.35	0.35	0.34

[a] *The premix provided the following (per kilogram of complete diet): vitamin A 6,000 IU, vitamin D3 2,500 IU, vitamin E 25 mg, vitamin K3 2.25 mg, vitamin B1 1.8 mg, vitamin B2 7 mg, vitamin B6 4 mg, vitamin B12 0.2 mg, D-pantothenic acid 12 mg, nicotinic acid 35 mg, biotin 0.14 mg, folic acid 0.8 mg, Cu (as copper sulfate) 11 mg, Zn (as zinc sulfate) 70 mg, Fe (as ferrous sulfate) 60 mg, Mn (as manganese sulfate) 115 mg, Se (as sodium selenite) 0.30 mg, and I (as potassium iodide) 0.4 mg.*
[b] *Nutrient levels are calculated values.*

for Quality Supervision Inspection and Quarantine of China, 2005), the experimental feed samples were collected, about 200 g in each group were collected and packed in the sample bag, and saved to be tested. The apparent metabolic rates were determined by the endogenous indicator method (acid insoluble ash, AIA). Determination of the Hydrochloric acid-insoluble ash concentration in feed and excreta followed the method described by the regulations and requirements of Determination of Hydrochloric acid-insoluble ash in feed (China, GB/T 23742-2009, General Administration for Quality Supervision Inspection and Quarantine of China, 2009). The total tract Dry matter (DM), crude protein (CP) (N × 6.25), ether extract (EE), crude fiber (CF), organic matter (OM), ash (Ash), and calcium (Ca). Phosphorus (P) digestibility and AMEn were calculated using the following Equation 1 (Yu et al., 2021). DM, CP, EE, CF, OM, Ash, Ca, and P contents in feed and excreta were determined using methods developed by the AOAC (Association of Official Analytical Chemists). Gross energy values in feed and excreta were determined using the bomb calorimeter (IKA C1 Compact Bomb Calorimeter, IKA-Werke, Staufen, Germany).

Apparent nutrient digestibility was calculated using the following equation:

$$\text{Apparent nutrient digestibility} = [\,1 - \frac{(\text{excreta nutrient (g/kg)})/\,(\text{excreta AIA (g/kg)})}{(\text{feed nutrient (g/kg)})/\,(\text{feed AIA (g/kg)})}\,] \times 100\%$$

Serum Biochemical Indices

Serum biochemical indices, namely, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total cholesterol (T-CHO), and high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), triglyceride (TG), glucose (GLU), total protein (TP), albumin (ALB), globulin (GLB), and uric acid (UA) were measured using assay kits (BS-200, Shenzhen Mairui Medical International Co., Ltd., China).

Antioxidant Indices Determination

Liver tissues were retrieved from a frozen environment and separately homogenized in PBS *via* a homogenizer (LabGen 850, Cole-Parmer China, Shanghai, China). After centrifuging at 3,000 r/min for 15 min, the supernatants were collected for the examination of liver antioxidant indices levels. Serum and liver antioxidant indices, namely, total antioxidative capacity (T-AOC), total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), catalase (CAT), and malondialdehyde (MAD) were examined using assay kits (H249, Nanjing Jiancheng Bioengineering Institute, Suzhou, China) with an automated fluorescence instrument (MultiskanSkyHigh, Thermo Fisher Scientific, Waltham, MA, United States) following the instructions of the manufacturers.

Determination of Intestinal Mucosa Morphological Structure

The intestinal samples were cleaned and stained with hematoxylin and eosin (HE) and finally made into paraffin sections using a microtome (RM-2235, Leica Microsystems AG Corporation Ltd., Black Forest, Germany). Then, a microscope (Van-Ox S, Olympus Corporation Co., Ltd., Washington, DC, United States) was used to randomly select multiple discontinuous fields of view to observe the slices at 40, 100, and 200 magnification times, and select typical fields of view to take pictures. The intestinal villi heights and crypt depths

of each intestinal segment were analyzed and determined by Motic Images Advanced 3.2 software, then calculated the villus height/crypt depth (V/C) value at the same time.

Statistical Analysis

The data were tested by ANOVA for the control group and experiment groups with Statistical Packages for Social Science 18.0 (SPSS 18.0) software. Orthogonal polynomial contrasts were used to determine linear and quadratic responses of levels of indices associated with production performance, serum biochemical, antioxidative capacity, and intestinal morphology to different level ramie powder. The results were expressed as arithmetic means and SEM. Statistical significance was assigned at $P < 0.05$. The P values between 0.05 and 0.10 were considered as a trend.

RESULTS

Production Performance

The effect of dietary supplementation of ramie powder on the performance of laying hens is presented in **Table 2**. No mortality was found during the 6-week experimental period. The dietary ramie powder supplementation had no effects on FCR, egg weight, feed intake, or egg mass ($P > 0.05$). But it showed a trend of increasing (quadratic, $P = 0.09$) the laying rate with the highest in the 6% ramie group.

Apparent Nutrient Digestibility

As shown in **Table 3**, compared with 3 and 6% ramie groups, 9% ramie group significantly decreased AMEn (quadratic, $P = 0.035$), and extremely significantly decreased OM apparent digestibility (linear and quadratic, $P = 0.008$). Compared with 3% ramie group, 9% ramie group significantly decreased DM apparent digestibility (quadratic, $P = 0.037$).

Organ Indices

As shown in **Table 4**, none of the organ indices (cardiac index, liver index, spleen index, small intestine index, gizzard index, and proventriculus index) were affected by the dietary ramie powder supplementation on laying hens ($P > 0.05$).

Serum Biochemical Parameters

The normal level of ALT, AST, T-CHO, LDL-C, HDL-C, TG, GLU, TP, ALB, GLB, and UA in laying hens ranged from 1 to 15 U/l, 65 to 270 U/l, 2 to 7 mmol/l, 0.2 to 4 mmol/l, 0.3 to 1.5 mmol/L, 7 to 28 mmol/l, 7 to 15 mmol/l, 42 to 85 g/l, 18 to 26 g/l, 23 to 58 g/l, and 177 to 440 μmol/l, respectively (Zhang et al., 2020; Lee et al., 2021; Lu et al., 2021; Tao et al., 2021; Zhu et al., 2021). As shown in **Table 5**, the dietary ramie powder supplementation showed a trend in decreasing (quadratic, $P = 0.095$) the concentration of ALT in serum of laying hens. Compared with the control group (**Table 5**), the serum AST concentration in the 3 and 6% ramie groups was significantly decreased by 22.24 and 14.14% (linear and quadratic, $P = 0.018$), respectively, and the serum HDL-C concentration in the 3% ramie group was significantly increased (linear and quadratic, $P = 0.025$).

Antioxidant Indices

The normal level of T-AOC, T-SOD, GSH-Px, CAT, and MDA in laying hens ranged from 0.6 to 5 U/ml, 50 to 450 U/ml, 180 to 500 U/ml, 3 to 12 U/ml, 0.9 to 6 U/ml, respectively (Li et al., 2019; Yu et al., 2020; Chen et al., 2021; Fu et al., 2021; Gu et al., 2021; Zhu et al., 2021). The antioxidation indices of the serum antioxidant are shown in **Table 6**. Compared with the control group and 9% ramie group, 3% ramie group significantly increased the serum GSH-Px activity of laying hens (quadratic, $P = 0.026$). There were no significant differences in other serum indices with different ramie meal levels ($P > 0.05$). Serum indices (T-AOC, T-SOD, and CAT) in ramie powder groups were slightly higher than those in the control group, MAD was slightly lower than the control group. Compared with other groups (**Table 7**), 6% ramie group significantly increased the activity of T-SOD in the liver (linear and quadratic, $P = 0.003$), but there were no significant differences in other indices ($P > 0.05$).

Intestinal Mucosa Morphological Structure

The morphological structure of the intestinal mucosa of laying hens is shown in **Table 8**. Ramie powder supplement of 3 or 6% increased the villus height of jejunum by 27.76 or 27.36% (linear and quadratic, $P = 0.048$), and V/C value of ileum (linear and quadratic, $P = 0.018$), compared with the control. There was a tendency to increase the value of V/C (quadratic, $P = 0.096$) in the jejunum in different ramie powder levels.

DISCUSSION

The shortage of protein feed is one of the critical factors restricting the development of the livestock industry in China. Due to the harsh weather conditions, it is hard to grow high-quality forages (alfalfa) in south China. However, as an important economic crop, over 90% of the world's ramie is grown in southern China (Kipriotis et al., 2015). The nutritional value of ramie is similar to that of alfalfa (Dai et al., 2019). Furthermore, ramie leaves, which are used for medicinal and edible purposes, are effective in reducing serum cholesterol, improving the meat quality of farmed animals (Avcı et al., 2006; Tang et al., 2021).

Safety was always the top priority to consider when utilizing untraditional resources for animal feed purposes. According to the data of this study, it was the first to report that ramie had no adverse effect on the overall production performance of hens. Many pieces of research showed that egg production has been increased in response to nettle supplementation, such as *Urtica dioica* and *Urtica cannabina* (Loetscher et al., 2013; Zhang et al., 2020). The herb *Boehmeria nivea*, as a perennial dicotyledon of the Urticaceae family, may have the same effect. Similarly, it was reported that egg production remained unchanged (Wang and Jie, 2012) or even tended to increase (Luo et al., 1989) with the supplement of ramie powder. In line with these studies, the supplementation of 3 and 6% ramie powder showed a

TABLE 2 | Effects of dietary ramie supplementation on laying hen production performance.

Items	Control	Ramie supplementation concentration in diets			SEM	*P* value		
		3%	6%	9%		ANOVA	Linear	Quadratic
Egg laying rate (%)	89.05	94.16	94.82	89.34	1.053	0.090	0.651	0.015
FCR	2.20	2.12	2.13	2.21	0.038	0.852	0.990	0.386
Mean egg weight (g)	56.51	56.59	56.91	56.91	0.139	0.640	0.225	0.913
Daily feed intake (g)	114.20	109.86	111.77	113.91	1.596	0.761	0.918	0.315
Egg mass per day (g)	51.97	51.94	52.41	51.48	0.585	0.979	0.828	0.803

[a,b]Means in the same row with different superscript letters indicate differences ($p < 0.05$).
FCR, food conversion ratio.

TABLE 3 | Effects of dietary ramie power supplementation on nutrient apparent metabolic rate of laying hens.

Items	Control	Ramie power supplementation concentration in diets			SEM	*P* value		
		3%	6%	9%		ANOVA	Linear	Quadratic
AMEn (MJ/kg)	12.04^{ab}	12.77^{a}	12.65^{a}	11.86^{b}	0.143	0.035	0.310	0.007
DM (%)	69.80^{ab}	72.02^{a}	70.64^{ab}	67.15^{b}	0.649	0.037	0.062	0.021
Ash (%)	48.51	52.07	52.90	49.64	1.749	0.796	0.584	0.419
OM (%)	73.01^{ab}	75.79^{a}	74.54^{a}	70.08^{b}	0.685	0.008	0.038	0.004
EE (%)	59.48	66.47	66.22	61.18	2.248	0.610	0.610	0.229
CF (%)	31.59	35.41	36.19	31.77	1.574	0.672	0.998	0.236
CP (%)	44.34	52.07	50.76	49.53	1.294	0.135	0.196	0.065
Ca (%)	65.55	66.79	69.22	65.61	0.703	0.332	0.617	0.139
P (%)	31.62	42.18	40.68	35.54	1.795	0.219	0.987	0.051

[a,b]Means in the same row with different superscript letters indicate differences ($p < 0.05$).
AMEn, nitrogen corrected apparent metabolizable energy; DM, dry matter; Ash, ash content; OM, organic compound; EE, ether extract; CF, crude fiber; CP, crude protein.

TABLE 4 | Effects of dietary ramie power supplementation on organ indices of laying hens.

Items	Control	Ramie power supplementation concentration in diets			SEM	*P* value		
		3%	6%	9%		ANOVA	Linear	Quadratic
Heart index (g/kg)	0.40	0.43	0.40	0.40	0.013	0.777	0.818	0.455
Liver index (g/kg)	2.21	2.25	2.39	2.18	0.069	0.736	0.939	0.389
Spleen index (g/kg)	0.09	0.13	0.13	0.12	0.007	0.124	0.067	0.138
Small intestine index (g/kg)	4.12	4.38	4.22	4.17	0.106	0.925	0.964	0.571
Gizzard index (g/kg)	1.90	2.17	2.10	2.23	0.070	0.382	0.146	0.628
Proventriculus index (g/kg)	0.41	0.48	0.44	0.50	0.014	0.101	0.063	0.078

[a,b]Means in the same row with different superscript letters indicate differences ($p < 0.05$).

trend in increasing egg production. Ramie (*Boehmeria nivea*) is rich in cellulose, flavonoids compounds, polyphenol compounds, vitamin C, and minerals. A large number of studies showed that scientific applications of vitamins and trace elements could improve animal performance and feed return (Abd El-Hack et al., 2017; Han et al., 2017). Perhaps, ramie supplemented vitamins and trace elements in the diet, ensured the full price of diet nutrition, thereby improving laying hens production performance. On the other hand, it might be the antioxidant properties of ramie. Recent studies showed that adding flavonoids to the diet could improve the performance of laying hens by improving the body's antioxidant capacity, reducing the occurrence of oxidative stress, and promoting the absorption of nutrients in the intestine (Brisibe et al., 2008; Galal et al., 2008; Seven, 2008; Liu et al., 2014; Zhu et al., 2021).

Ramie has a relatively high crude fiber content, which will affect the digestion and utilization of other nutrients as the dosage increase. Studies showed that the increase of dietary fiber affected the digestibility of CP, EE, and AMEn in varying degrees, and reduced production performance (Tabook et al., 2006; Liu et al., 2010). In our study, dietary substituted with 9% ramie powder significantly decreased AMEn and DM apparent digestibility of laying hens ($p < 0.05$), and extremely significantly decreased the apparent digestibility of OM ($p < 0.01$) compared with 3 and 6% ramie groups. However, the dietary substitution of 3 and 6% ramie had no significant effect on the apparent

TABLE 5 | Effects of dietary ramie power supplementation on serum biochemical indices of laying hens.

Items	Control	Ramie power supplementation concentration in diets			SEM	*P* value		
		3%	6%	9%		ANOVA	Linear	Quadratic
ALT (U/l)	7.91	3.27	4.59	5.48	0.731	0.095	0.229	0.042
AST (U/l)	321.13^{a}	249.72^{b}	258.44^{b}	275.71^{ab}	10.413	0.018	0.030	0.014
T-CHO (mmol/l)	2.82	2.49	2.65	2.76	0.110	0.731	0.823	0.324
LDL-C (mmol/l)	3.40	2.51	3.23	3.14	0.205	0.464	0.744	0.272
HDL-C (mmol/l)	0.46^{b}	0.73^{a}	0.66^{ab}	0.64^{ab}	0.038	0.025	0.017	0.060
TG (mmol/l)	17.75	12.25	17.71	16.51	1.218	0.354	0.750	0.217
GLU (mmol/l)	10.19	11.05	11.46	10.75	3.08	0.955	0.706	0.693
TP (g/l)	74.72	76.52	81.68	74.15	6.66	0.449	0.581	0.258
ALB (g/l)	23.01	23.02	25.66	22.41	1.87	0.112	0.598	0.186
GLB (g/l)	51.71	53.51	56.03	51.75	5.72	0.714	0.638	0.371
UA (μmol/l)	85.42	70.84	59.71	84.56	22.55	0.412	0.520	0.147

^{a,b}Means in the same row with different superscript letters indicate differences ($p < 0.05$).
ALT, glutamic-pyruvic transaminase; AST, glutamic-oxaloacetic transaminase; T-CHO, total cholesterol; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; TG, triglyceride; GLU, glucose; TP, total protein; ALB, albumin; GLB, globulin; UA, uric acid.

TABLE 6 | Effects of dietary ramie power supplementation on serum antioxidant indices of laying hens.

Items	Control	Ramie power supplementation concentration in diets			SEM	*P* value		
		3%	6%	9%		ANOVA	Linear	Quadratic
T-AOC (U/ml)	0.35	0.38	0.39	0.37	0.020	0.921	0.728	0.585
T-SOD (U/ml)	29.69	49.54	49.36	34.81	6.128	0.682	0.923	0.266
GSH-Px (U/ml)	294.36^{b}	531.43^{a}	405.44^{ab}	362.26^{b}	35.763	0.026	0.924	0.011
CAT (U/ml)	10.43	16.64	11.78	11.77	1.258	0.374	0.939	0.254
MDA (nmol/ml)	2.03	0.97	1.56	1.20	0.177	0.183	0.199	0.306

^{a,b}Means in the same row with different superscript letters indicate differences ($p < 0.05$).
T-AOC, total antioxidative capacity; T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; MAD, malondialdehyde.

TABLE 7 | Effects of dietary ramie power supplementation on liver antioxidant of laying hens.

Items	Control	Ramie power supplementation concentration in diets			SEM	*P* value		
		3%	6%	9%		ANOVA	Linear	Quadratic
T-AOC (U/g)	0.51	0.60	0.64	0.55	0.035	0.729	0.830	0.299
T-SOD (U/g)	161.17^{b}	204.67^{b}	296.66^{a}	201.39^{b}	17.245	0.003	0.024	0.002
GSH-Px (U/g)	12.61	14.50	15.54	12.60	0.601	0.182	0.716	0.051
CAT (U/g)	31.62	32.54	34.04	32.50	0.944	0.916	0.784	0.601
MDA (nmol/g)	31.56	31.55	23.25	30.81	3.053	0.830	0.827	0.605

^{a,b}Means in the same row with different superscript letters indicate differences ($p < 0.05$). T-AOC, total antioxidative capacity; T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; MAD, malondialdehyde.

metabolic rate of nutrients. In conclusion, the feed digestion and utilization of laying hens could be promoted if the inclusion of ramie powder was between 3 and 6%, but it might have an adverse effect if the ramie powder substitution rate was too high. This was basically the same for geese (Liu et al., 2010). Although high CP was beneficial to the growth of laying hens, high CF and antinutritional factors in ramie could have negative impacts. Compared with other animals, laying hens had relatively low utilization of fiber forage. The higher CF content was in the diet, the greater the physical shielding effect of the diet was on digestive enzymes, which hindered the full contact of digestive enzymes and nutrients (fat, starch, protein, etc.) in the intestine (Guzmán et al., 2016), and greatly affected the apparent digestibilities of various nutrients in the diet (Knudsen, 2001).

High-density lipoprotein is involved in cholesterol reverse transport and is a key lipoprotein that maintains lipid balance in the cardiovascular system (Pirillo et al., 2019). As a complex of lipids and proteins, HDL-C not only participates in regulating lipid metabolism but also inhibits thrombosis and inflammation and regulates glucose metabolism (Feng et al., 2020). In normal

TABLE 8 | Effects of dietary ramie power supplementation on morphological structure of intestinal mucosa of laying hens.

Items		Control	Ramie power supplementation concentration in diets			SEM	*P* value		
			3%	6%	9%		ANOVA	Linear	Quadratic
Duodenum	Villus height (μm)	581.79	635.02	595.56	612.77	22.504	0.900	0.771	0.739
	Crypt depth (μm)	75.44	75.71	75.58	78.82	2.710	0.976	0.730	0.819
	V/C	7.52	8.31	7.68	7.71	0.300	0.814	0.983	0.517
Jejunum	Villus height (μm)	416.59^{b}	532.25^{a}	530.56^{a}	496.66^{ab}	20.653	0.048	0.037	0.038
	Crypt depth (μm)	72.75	65.27	67.06	72.63	2.027	0.520	0.867	0.169
	V/C	5.42	8.38	7.49	6.84	0.467	0.096	0.277	0.040
Ileum	Villus height (μm)	274.78	309.33	452.70	336.33	29.520	0.122	0.144	0.135
	Crypt depth (μm)	53.75	44.91	57.40	50.41	2.086	0.213	0.983	0.576
	V/C	5.06^{b}	6.68^{a}	7.62^{a}	6.48^{ab}	0.332	0.018	0.033	0.010

^{a,b}Means in the same row with different superscript letters indicate differences ($p < 0.05$).
V/C, villus height/crypt depth.

conditions, two common serum enzymes (AST and ALT) only existed in cells because of the barrier function of cell membranes. When tissues (especially the liver) were damaged, excessive oxygen free radicals were generated and invaded the cell membranes that increasing the permeability of the cell membrane, which in turn led to the escape of AST and ALT into the serum (Ma et al., 2021). Ramie ethanol extract could significantly reduce serum TC and LDL-C levels of mice, and significantly increase HDL-C level ($p < 0.05$) (Lee et al., 2014). In this study, 3 and 6% ramie groups had significantly lower content of AST in serum compared to the control group ($p < 0.05$), indicating that ramie played a protective role. Chlorogenic acid was the main phenolic compound in ramie ethanol extract (Tan et al., 2014), and had a variety of biological effects such as antioxidant, antidiabetic, and antilipid effects (Ong et al., 2013). The serum HDL-C content in the 3% ramie group was significantly increased ($p < 0.05$), which may be due to the reason that polyphenols in ramie leaves (chlorogenic acid, etc.) inhibited glucose 6-phosphate translocase, that modulated hepatic glucose 6-phosphatase system and regulated the homeostasis of blood glucose (Arion et al., 1998).

The animal bodies produced some free radicals in the metabolism process. Among them, oxygen-free radicals could cause lipid peroxidation of polyunsaturated fatty acids in biofilms. With the increase of lipid peroxidation reaction, the capacity of antioxidant decreased, and the original balance between the antioxidant system and the production of pro-oxidants were broken, leading to oxidative stress in the body (Seifried et al., 2007; Lin et al., 2008; Poljsak et al., 2013). Oxidative stress damaged the biological macromolecules (DNA, lipids, proteins, etc.) in the body, affecting animal health and the quality of livestock and poultry products. Antioxidant defense system, included enzymatic reaction system (endogenous antioxidant enzymes, such as SOD, GSH-Px, and CAT) and non-enzymatic reaction system (water and lipid-soluble compounds, such as vitamin C and polyphenols), the latter of which were rich in ramie. Polyphenols and flavonoids in ramie leaves were considered to be the main bioactive components with antioxidant capacity (Lee et al., 2014). Moreover, ramie also promoted antioxidant enzyme activities, decreased lipid peroxidation level, and improved the body's antioxidant capacity (Li et al., 2019). Some oxygenated lipids could regulate the production of antioxidants (Mavangira and Sordillo, 2018). Organic acids accounted for the largest proportion of volatile components in ramie leaves, and the content of polyunsaturated fatty acids was more than 45%. Oxylipid was the abbreviation of various lipid metabolites produced in the oxidative metabolism of PUFA. In this study, 3% ramie group significantly increased the content of GSH-Px in serum ($p < 0.05$), 6% ramie group extremely significantly increased the content of T-SOD in the liver ($p < 0.01$) compared with the control group. It might be because that ramie as a feed source improved the activities of antioxidant enzymes and increased the intake of PUFA and polyphenols, thereby enhancing the antioxidant capacity of laying hens, extending the laying period, and improving the production performance.

Villus height, crypt depth, and the value of villus height/crypt depth (V/C) were critical indicators to evaluate the digestion and absorption function of the intestine. Intestinal villi were the main tissues that absorb nutrients. As the height of the villi increased, the ability of the body to absorb nutrients was also enhanced. The depth of the crypt could reflect the renewal speed of intestinal epithelial cells. The shallower the depth, the slower the differentiated intestinal epithelial cells migrated to the upper part of the villi. It indicated that the higher the maturation rate of intestinal epithelial cells were, the stronger the absorption function they had. The value of V/C comprehensively reflected the functional status of the intestine. The increase in V/C value indicated that the intestinal lining area was larger, the structure of the intestinal mucosa was improved, and the digestion and absorption functions were enhanced (Yason et al., 1987; Lin et al., 2017). Medicinal plants rich in polyphenols could effectively inhibit the growth of harmful bacteria in the gut and improve the composition of the gastrointestinal microbiota. Silage ramie significantly increased ileum villus height and V/C value of geese ($p < 0.05$) (Hou et al., 2018), which was consistent with our study that 3 or 6% ramie powder substitution significantly

increased the villus height of jejunum and the V/C value of ileum. These results indicated that ramie could improve the intestinal mucosal morphology of laying hens. It might be because ramie, a medicinal plant rich in polyphenols, improved the antioxidant capacity of the whole gastrointestinal tract (Bonetti et al., 2016). On the other hand, ramie might inhibit the growth of harmful bacteria in the intestinal tract and improve the composition of gastrointestinal microbiota (Lee et al., 2001).

CONCLUSION

In summary, ramie would not cause adverse effects on the organs of Lohmann commercial laying hens. The substitution rate between 3 and 6% of ramie was beneficial to reduce blood lipids, improve antioxidant capacity and intestinal mucosa structure, and promote egg-laying rate. However, with the substitution rate of ramie exceeding 9%, it could affect the apparent metabolizable energy and the nutrient utilization of dry matter and organic matter of the laying hens. Considering all indicators, the optimum substitution rate of dietary ramie for laying hens was 6%.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The animal study was reviewed and approved by all the experiment procedures were approved by the Animal Care Committee of the Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences, Changsha, China.

AUTHOR CONTRIBUTIONS

QL, S-YZ, H-JQ, and Z-YF designed and conducted the study. XW, H-HZ, G-YD, and Y-MW conducted the animal experiment. C-JL, Y-ZW, T-ML, PH, and Y-HL conducted the detection and analysis works. XW, YL, and QL prepared the manuscript draft. All authors participated in the discussion and editing of the manuscript.

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Effect of Dietary Ramie Powder (*Boehmeria nivea*) at Various Levels on Growth Performance, Carcass and Meat Qualities, Biochemical Indices, and Antioxidative Capacity of *Linwu* Ducks

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Current experiment was designed to check the effect of dietary supplementation of ramie powder on the growth performance, carcass and meat qualities and antioxidative capacity of *Linwu* ducks. A total of 312 ducks at 21-day-age were equally divided into 4 groups, fed with control diet, control diet supplemented of 3, 6, or 12% ramie powder, respectively. The results showed that dietary supplementation of 6 and 12% ramie powder increased the final weight and daily body weight gain ($P < 0.05$), and dietary supplementation of 6% ramie improved the cooking loss of the leg meat 45-mins-postmortem compared with the control group ($P < 0.05$). Moreover, dietary supplementation of 6% ramie powder promoted the antioxidative capacity of the ducks by increasing the serum activities of superoxide dismutase and glutathione ($P < 0.05$), as well as the mRNA expressions of glutathione peroxidase 1 in the breast meat and superoxide dismutase 1 in the leg meat ($P < 0.05$). This experiment demonstrated that dietary supplementation of ramie powder showed beneficial efficacy on the growth performance of *Linwu* ducks. It corroborated the potential of dietary ramie being used as poultry feed ingredient and suggested that 6% was the proper supplementation rate of ramie powder in *Linwu* ducks' feed.

Keywords: ramie, growth performance, meat quality, antioxidative capacity, *Linwu* duck

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INTRODUCTION

In recent years, shortages of feed resources and rising prices restricted the development of animal husbandry. On the other hand, the demand of poultry meat with high quality grew in recent years, which encouraged the poultry industry consistently supplying healthy, safe and tasty poultry meat (Roenigk, 1999). Accordingly, it was imperative to look for new feed ingredients, better with beneficial effect on the meat quality of poultry, to overcome the feed shortage problems.

Ramie (*Boehmeria nivea*), well known as "China grass," is a perennial plant of the *Boehmeria* genus under the Nettle, or *Urticaceae* family, order *Urticales* and class *Magnoliopsida* (Luan et al., 2018). It drew great attention as a type of unconventional feed source for livestock and poultry recently for its relatively low fiber content and high crude protein (about 20.00% of dry matter, DM), amino acids (especially the lysine, slightly > 1.00% of DM), minerals (especially the calcium, about 4.00% of DM) and carotene content in leaves and tender tops (Kipriotis et al., 2015). Ramie contained many biologically active compounds in its roots and leaves, such as flavonoids (Rutin, Rhoifolin, and beta-ionone) and polyphenols compounds (chlorogenic acid, ferulic acid, and caffeic acid) (Wang et al., 2019). Thus, it was practical to evaluate the effects of ramie as a new feed ingredients to the livestock. *Linwu* duck was a major duck breed in China which had strong adaptability to stressful environments (Lin et al., 2016). Therefore, *Linwu* duck was selected as an animal model for the evaluation of ramie powder as a feed ingredient in the present study.

It was commonly known that meat quality was formed by a complex interplay of various factors. The protein degradation in meat was primary caused by degradation effect of various proteases (Kemp and Parr, 2012), and the pH decreasing in meat was mainly because of the metabolism of glycogen in the muscles into lactic acid after the animal's death (Bendall, 1979). Oxidation also partially contributed to the degradation of meat lipids and proteins, and caused the decrease of the pH values and the water holding capacity in meats, which deteriorated the tenderness, flavor, juiciness, and color of meats (Ripoll et al., 2013; Qiao et al., 2017). Previous literatures indicated that ramie leaves had a number of flavonoids and polyphenols which exerted antioxidative activities *in vitro* and cellular (Chen et al., 2014). However, to our knowledge, no relevant research was conducted to test the influence of ramie treatment on ducks' meat quality.

The objective of the present study was to evaluate the effects of varying levels of dietary ramie powder on the growth performance of *Linwu* ducks. Additionally, we tried to verify the hypothesis that ramie treatment could increase the antioxidant status and improve the meat quality of *Linwu* ducks, by examining the activities of antioxidative enzymes and the mRNA expressions of the antioxidative enzymes, as well as the indices associated with the meat qualities.

MATERIALS AND METHODS

All the experimental procedures were conducted in accordance with the Chinese guidelines for animal welfare and approved by the Animal Care and Use Committee, Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences.

Preparation of Ramie Powder

The leaves and tender tops of fresh ramie (*Boehmeria nivea cv.* Qingsizhu No. 1) were cut at about 80 cm height and immediately dried at 60°C for 4 days in a heat drier room. The weight ratio of leaf to stem in dry ramie was 3.37. Then, the dried stems and leaves were crushed to powder using a grinder equipped with a 1.5 mm sieve, and kept in a well-closed and light-resistant room.

Birds, Diets, and Experimental Design

Three hundred and twelve 21-day-old female *Linwu* ducks, free of infectious disease, were obtained from Hunan Shunhua Duck Industrial Development Company, and transferred to the laboratory of the Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences. The ducks were supplied *ad libitum* access to feed and water throughout the trial period. After a 1-week adaptation period fed with the control diet, all the ducks were individually weighed and equally divided into 4 groups, meeting the purpose that the average initial weights among groups were not significant different. The ducks in each group (78 *Linwu* ducks) were further subdivided into six 120 cm × 120 cm cages (13 ducks/cage). Group 1 received the control diet which was based on corn, soybean meal, wheat bran, wheat flour, and rice husk. Group 2, 3, and 4 received a control diet in which corn, soybean meal, wheat bran, wheat flour, and rice husk were partly replaced by ramie powder to reach proportions of 3, 6, or 12%, respectively. All diets were formulated with similar levels of nutrient and to meet recommendations of Nutrient Requirements of Meat-type Duck (China, NY/T 2122-2012), as shown in **Table 1**. The feeding period was 42 days.

Record of Growth Performance

Body weight of *Linwu* duck was individually measured at the beginning (28-day-old) and the end of the trial (70-day-old). Feed intake per cage was recorded daily. The average daily feed intake (ADFI), average daily body weight gain (ADG), and feed/gain ratios (F/G) were calculated for each cage.

Carcass Characteristics and Sample Collection

On the last day of the trial, twelve ducks in each group (two ducks in each cage) with body weights close to the mean were selected after 12-h fasting. Blood samples were collected *via* wing vein with 10-mL heparin-free tubes, and centrifuged at 3,000 × g for 15 min at 4°C. The obtained serum samples were stored at −20°C until analysis. The ducks were then immediately slaughtered by cervical dislocation. The carcasses were bled by hanging for 5 min and scalded in water (65°C) for approximately 1-min for feathers plucking. The weights of carcasses were recorded as dressed weight. After carefully excising the gastrointestinal tract and organs, the weights of half-eviscerated, eviscerated, abdominal fat, leg muscle, breast muscle, and lean meat (total legs and breasts muscle) were recorded. Then, a 1-cm-thick sample of leg muscle and breast muscle was rapidly taken from each duck and frozen in liquid nitrogen and stored at −80°C until RNA extraction. Finally, the dressed percentage (PD), percentage of half-eviscerated yield (PHEY), percentage of eviscerated yield (PEY), percentage of breast muscle (PB), percentage of leg muscle (PL), percentage of lean meat (PLM), and percentage of abdominal fat (PAF) were calculated, respectively, according to the regulations and requirements of Performance Ferms and Measurement for Poultry (China, NY/T 823-2004).

Meat Quality Analysis

The meat qualities of leg and breast muscles were measured as follow: the meat colors (45-min and 24-h postmortem) were determined as the L*, a*, and b*, which were the indicators of lightness, redness and yellowness, respectively, with a colorimeter (CR-400; Minolta Camera Co., Osaka, Japan). The pH values (45-min and 24-h postmortem) were measured with a pH meter (Model 340, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) by the method described previously (Choi et al., 2016). The drip losses at 24-h postmortem, the cooking losses at 45-min and 24-h postmortem were assayed according to the method described previously (Qiao et al., 2017), and the shear forces were further measured with the digital tenderness meter (C-LM3B, Northeast Agricultural University, Harbin, China) after measuring the cooking loss, to evaluate the tenderness of meat (Tang et al., 2009).

Measurement of Serum Antioxidant Biomarkers

The serum levels of glutathione (GSH), malonaldehyde (MDA) and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and total antioxidant capacity (T-AOC) were determined by the commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and an automated fluorescence instrument (Thermo Fisher Scientific, Waltham, MA, United States) following the corresponding procedures. And, the serum level of 8-hydroxy-2′-deoxyguanosine (8-OHdG) was quantified by a specific ELISA kit (Abcam, Cambridge, United Kingdom).

Quantification of mRNA Expression by Real-Time PCR

Total RNA from the leg muscle and breast muscle were isolated using Trizol reagent (TaKaRa, Tokyo, Japan), and then treated with DNase I (Thermo Fisher Scientific Inc., Waltham, MA, United States). The cDNA were synthesized from 1 μg of RNA with a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc., Waltham, MA, United States) according to the manufacturer's instructions. Based on the cloned complete sequences[1] of nuclear factor erythroid-2-related factor 2/erythroid-derived CNC homology factor (*Nrf2/ECH*), catalase (*CAT*), superoxide dismutase-1 (*SOD1*), glutathione peroxidase-1 (*GPX1*), and β-actin from *Anas platyrhynchos*, primer pairs were designed with Primer 5.0 for quantitative real-time PCR (**Table 2**). The β-actin gene was used as the housekeeping gene. All primers were synthesized and purified by Sangon Biotech Co. Ltd. (Shanghai, China). Reaction volume of 20 μL mixture contained 10 μL Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, United States), 1 μL cDNA template, 1 μL of the upstream and downstream primers for each targeting gene, and 7 μL sterilized deionized water. The amplification parameters for all the genes of the thermocycler (CFX Connect, Bio-Rad, Inc., Hercules, CA, United States) were a preheat period of 3 min at 95°C followed by 45 cycles of 95°C for 10 s and 55°C for 20 s, and a melting curve ramping from 65 to 95°C with an increasing temperature of 0.5°C. All sample analyses were carried out in triplicate and the average values were indexed. The target gene expressions were normalized to that of the selected reference gene, and the relative gene expressions were calculated using $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The mRNA levels were expressed as the fold change relative to the mean values of the control group, which was arbitrarily defined as 1.0.

[1]https://www.ncbi.nlm.nih.gov/genbank/

Statistical Analysis

Statistical analysis of data was conducted using Statistical Package for the Social Sciences 19.0 (IBM, Armonk, NY, United States). One-way ANOVA model was performed in the present research. Replicate was used as the experimental unit (As for the results of the biomarker analysis, it means that the results of two birds in each cage are summarized as an average value). Results were presented as means and pooled standard errors of the means (SEM). Differences among means of all groups were considered significant at $P < 0.05$. The P values between 0.05

TABLE 1 | Composition and nutrient levels of experimental diets (air-dry basis, %).

Items	Diets			
	0.00%	3.00%	6.00%	12.00%
Ingredients				
Corn	45.98	44.11	46.76	46.87
Soybean meal	25.58	24.64	24.43	23.22
Wheat flour	10.00	10.00	10.00	10.00
Rice husk	5.42	3.73	2.77	0.00
Ramie powder	0.00	3.00	6.00	12.00
Oil	2.57	3.30	2.65	2.94
Wheat bran	6.35	7.39	3.78	1.84
Limestone	1.37	1.12	0.78	0.21
$CaHPO_4{\cdot}2H_2O$	1.27	1.24	1.33	1.40
78.5% *L*- Lys	0.00	0.01	0.03	0.05
98.5% *DL*- Met	0.16	0.16	0.17	0.17
Salt	0.30	0.30	0.30	0.30
1% Premix[1]	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00
Nutrient levels[2]				
Metabolizable energy, MJ/kg	11.92	11.92	11.92	11.92
Crude protein	17.00	17.02	17.01	16.99
Crude fiber	5.55	5.54	5.56	5.57
Calcium	0.85	0.83	0.84	0.86
Total phosphorus	0.59	0.59	0.57	0.58
Available phosphorus	0.35	0.35	0.36	0.36
Lysine	0.91	0.90	0.89	0.91
Methionine	0.43	0.45	0.44	0.43

[1]The premix provided the following (per kilogram of complete diet) micronutrients: VA 12 000 IU, VD_3 2 500 IU, VE 20 mg, VK_3 3 mg, VB_1 3 mg, VB_2 8 mg, VB_6 7 mg, VB_{12} 0.03 mg, D-pantothenic acid 20 mg, nicotinic acid 50 mg, biotin 0.1 mg, folic acid 1.5 mg, Cu (as copper sulfate) 9 mg, Zn (as zinc sulfate) 110 mg, Fe (as ferrous sulfate) 100 mg, Mn (as manganese sulfate) 100 mg, Se (as sodium selenite) 0.16 mg, I (as potassium iodide) 0.6 mg.

[2]The contents of total energy, crude protein, crude fiber, calcium, total phosphorus, lysine, and methionine were analyzed.

and 0.10 were considered as a trend. Duncan's multiple range were further used when the main effect was significant. And the orthogonal polynomial contrasts were used to determine linear and quadratic responses of measured parameters of *Linwu* ducks to ramie powder levels.

RESULTS

Growth Performance

Growth performances of ducks in different treatment groups were shown in **Table 3**. In comparison with the control group, the groups fed diets supplemented with ramie level at 6 and 12% had a significant increase in the average final weight, and ADG ($P < 0.05$) and had a trend in increase the ADFI ($P = 0.063$). Moreover, the greatest average final weight, ADG, and ADFI were achieved for ducks fed with the 6% ramie supplemented diet. Positive linear relationships were found between ramie dose and the average final weight ($P = 0.009$), ADG ($P = 0.006$), and ADFI ($P = 0.013$). However, there were no significant differences ($P > 0.05$) in F/G ratio among groups.

Carcass Characteristics

Carcass characteristics were shown in **Table 4**. There were no significant differences ($P > 0.05$) in carcass characteristics among groups.

Meat Quality

Meat qualities of the *Linwu* ducks were shown in **Tables 5**, **6**. In breast muscles, no effect of dietary ramie supplementation was detected on the shear force, 24 h pH value, 45 min and 24 h meat color or cooking loss. However, there were trends in increase in the pH value at 45 min ($P = 0.081$) and decrease in the drip loss ($P = 0.096$) in the groups treated with ramie. In addition, quadratic effects ($P < 0.05$) on drip loss and pH value at 45 min were observed as the dietary ramie level increased from 0 to 12%, and the lowest drip loss value and the highest 45 min pH value were noted, respectively, in ducks fed with 6 and 3% ramie.

In leg muscles, compared with the control group and 12% ramie treated group, the 6% ramie treated group had a significant decrease in the cooking loss at 45 min ($P = 0.043$). Compared with the 12% ramie treated group, the 6% ramie treated group had a significant increase in the pH value at 45 min ($P = 0.048$). Quadratic effects ($P < 0.05$) for 45 min cooking loss and pH value were observed as the dietary ramie level increased from 0 to 12%. However, there were no significant differences ($P > 0.05$) on other characteristics of leg muscles quality among groups.

Serum Antioxidant Biomarkers

Levels of serum antioxidant biomarkers were shown in **Table 7**. In comparison with the control group, the groups supplemented with 6% ramie had a significant increase in the content of GSH and the activity of SOD ($P < 0.05$). In addition, there were increasing trends in the levels of GPX ($P = 0.082$) and T-AOC ($P = 0.092$) in the groups supplemented with ramie. Moreover, positive linear relationships were observed between ramie dose and serum levels of GPX and GSH ($P < 0.05$). Besides, significant quadratic relationships were observed between ramie dose and serum levels of SOD, and T-AOC ($P < 0.05$).

The mRNA Expression Levels of Muscular Antioxidant-Related Genes

The mRNA expression levels of muscular antioxidant-related genes were shown in **Table 8**. In breast muscles, compared with the control group, the group with ramie level at 6% had significant increase in the *GPX1* mRNA expression level ($P < 0.05$). A quadratic effect was observed between the dietary ramie level and for *GPX1* mRNA expression level ($P = 0.012$).

In leg muscles, compared with the control group and the 12% ramie treated group, the 6% ramie treated group showed a significant increase in the *SOD1* mRNA expression level ($P = 0.048$). There was an increasing trend in the *CAT* mRNA expression level in the ramie treated groups ($P = 0.098$). In addition, quadratic effects were observed between dietary ramie level and *SOD1* and *CAT* mRNA expression level ($P < 0.05$). However, dietary ramie supplementation did not influence the mRNA expression levels of *Nrf2/ECH* in either breast muscles or leg muscles of the ducks.

TABLE 2 | Primer sequences used for real-time quantitative PCR.

Primer name[1]	Sequences of the primer pair	GenBank accession NO.	Fragment length, bp
β-*Actin* sense	5′-AGTACCCCATTGAACACGGT-3′	EF667345	197
β-*Actin* antisense	5′-ATACATGGCTGGGGTGTTGA-3′		
GPX1 sense	5′-TTCGAGAAGTGCGAGGTGAA-3′	KU048803	156
GPX1 antisense	5′-GTTCCAGGAGATGTCGTTGC-3′		
CAT sense	5′-AATGTGCGTGACTGACAACC-3′	KU048802	196
CAT antisense	5′-ACGTTCATCCTCCTTCAGCA-3′		
SOD1 sense	5′-TGGACCAAAGGATGCAGAGA-3′	KU048808	200
SOD1 antisense	5′-CATTCCCAGTTAGCGTGCTC-3′		
Nrf2/ECH sense	5′-CGCCTTGAAGCTCATCTCAC-3′	KM109969	176
Nrf2/ECH antisense	5′-TTCTTGCCTCTCCTGCGTAT-3′		

[1]*GPX1, glutathione peroxidase-1; CAT, catalase; SOD1, superoxide dismutase-1; Nrf2/ECH, nuclear factor erythroid-2-related factor 2/erythroid-derived CNC homology factor.*

DISCUSSION

Feed shortage is one of the most critical factors to impede the development of animal husbandry in many regions, which is in particular severe in southern China (Yin et al., 2019). Ramie, a hardy perennial herbaceous plant with high production of DM, is widely grown in southern China (Lv, 2012). Furthermore, the nutritive value of ramie is reported to be similar to that of Lucerne (Kipriotis et al., 2015). We selected *Linwu* duck in the current research because it was a famous and excellent breed

TABLE 3 | Effect of varying ramie powder levels in diet on growth performance of *Linwu* ducks.

Item[1]	Ramie powder levels				*SEM*	*P*-value	Linear and quadratic effects of ramie powder	
							P-value	
	0.00%	3.00%	6.00%	12.00%			Linear	Quadratic
Average initial weight/(g)	685.897	684.615	687.180	683.333	1.218	0.736	0.655	0.618
Average final weight/(g)	1,569.662^{b}	1,599.508^{ab}	1,632.352^{a}	1,617.897^{a}	8.060	0.025	0.009	0.123
ADG/(g)	21.042^{b}	21.783^{ab}	22.504^{a}	22.252^{a}	0.188	0.021	0.006	0.134
ADFI/(g)	125.618	129.968	135.312	134.671	1.493	0.063	0.013	0.363
F/G	5.969	5.968	6.010	6.050	0.030	0.762	0.321	0.748

^{a,b}With in a row, values with different superscript letters mean significant difference ($P < 0.05$).
[1]ADG, average daily body weight gain; ADFI, average daily feed intake; F/G, feed/gain ratios.

TABLE 4 | Effect of different ramie powder levels in diet on carcass characteristics of 70 days *Linwu* ducks.

Item	Ramie powder levels				SEM	*P*-value	Linear and quadratic effects of ramie powder	
							P-value	
	0.00%	3.00%	6.00%	12.00%			Linear	Quadratic
Dressed percentage	84.729	86.153	85.530	84.588	0.769	0.894	0.887	0.475
Percentage of half-eviscerated yield	89.179	91.688	90.376	92.399	0.567	0.192	0.100	0.825
Percentage of eviscerated yield	78.979	81.224	80.108	82.357	0.640	0.288	0.123	0.999
Percentage of breast muscle	11.567	13.034	11.971	12.784	0.252	0.130	0.234	0.496
Percentage of leg muscle	9.979	9.713	9.769	9.832	0.234	0.984	0.866	0.745
Percentage of lean meat	21.546	22.746	21.741	22.616	0.369	0.596	0.522	0.832
Percentage of abdominal fat	1.475	1.281	1.393	1.281	0.072	0.760	0.494	0.789

TABLE 5 | Effect of different ramie powder levels in diet on meat quality of 70 days *Linwu* ducks' breast muscle.

Item[1]		Times	Ramie powder levels				*SEM*	*P*-value	Linear and quadratic effects of ramie powder	
									P-value	
			0.00%	3.00%	6.00%	12.00%			Linear	Quadratic
Shear force, kg·f		45 min	3.229	3.080	3.227	3.398	0.205	0.965	0.741	0.718
		24 h	3.169	3.121	3.282	2.965	0.155	0.922	0.762	0.687
Meat color	L*	45 min	34.663	34.073	35.253	33.772	0.566	0.821	0.781	0.711
		24 h	34.982	34.212	34.332	35.668	0.647	0.865	0.724	0.448
	a*	45 min	17.840	17.558	18.313	16.507	0.500	0.654	0.490	0.468
		24 h	20.643	19.805	20.362	18.570	0.538	0.563	0.262	0.669
	b*	45 min	4.127	4.833	4.525	4.982	0.206	0.495	0.239	0.767
		24 h	4.405	4.040	4.183	4.613	0.189	0.747	0.666	0.324
Cooking loss, %		45 min	38.800	37.002	37.208	37.925	0.542	0.668	0.634	0.274
		24 h	40.661	38.914	38.764	37.973	0.603	0.474	0.147	0.699
Drip loss, %		24 h	3.230	2.653	2.346	2.860	0.130	0.096	0.199	0.033
pH value		45 min	6.291	6.403	6.381	6.308	0.019	0.081	0.848	0.013
		24 h	6.142	6.190	6.169	6.202	0.023	0.824	0.466	0.868

[1]L, lightness; a*, redness; b*, yellowness.*

of duck in local southern China with outstanding meat quality and strong adaptability. Therefore, this research was important and practical in assisting the *Linwu* duck breeding industry with recommendation of utilization of a new feed ingredient.

In the present study, the effects of ramie powder as feed sources in the growth performance and carcass characteristics of *Linwu* ducks were tested. Among the diet composition of all groups, no differences in nutrition or ingredient levels in diets were designed, except for the content of corn, soybean meal, rice husk, and ramie powder. Ramie powder as a supplementation to traditional feed material was considered as a provider of crude protein and crude fiber in the diets. Previous study showed that, 9 and 12% inclusion of ramie in diet lowered the final body weight and ADG of the finishing pigs compared to the ones with control diets, but the ADFI did not changed (Li et al., 2019). It was partially different with our finding, that 6 and 12% of ramie supplement in diets significantly increased the final body weight and ADG of *Linwu* ducks, but no influence was found in ADFI by ramie inclusion. The possible explanation for the inconsistence could be the difference in dietary crude fiber level of the diet. It was reported that excessive intake of fiber could reduce the nutrient digestibility and increase the satiety of birds, leading to the reduction of weight gain (Nielsen et al., 2011). In the present study, the diets in all experimental groups possessed similar crude fiber contents, while in Li's study, the crude fiber level was raised as ramie inclusion level increased in diet.

On the other hand, no differences were noticed with the parameters of the carcass characteristics of *Linwu* ducks among groups, indicating that ramie supplementation in diets would not harm the productive efficacy of the ducks compared with the control diets. Duck meat was famous worldwide, especially in Asia, due to its attractive flavor, taste, and nutritious value

TABLE 6 | Effect of different ramie powder levels in diet on meat quality of 70 days *Linwu* ducks' leg muscle.

Item[1]	Times	Ramie powder levels,				*SEM*	*P*-value	Linear and quadratic effects of ramie powder	
								P-value	
		0.00%	3.00%	6.00%	12.00%			Linear	Quadratic
Shear force, kg·f	45 min	3.430	2.761	3.302	3.399	0.143	0.328	0.727	0.191
	24 h	3.820	3.326	3.391	3.238	0.166	0.638	0.283	0.623
Meat color L*	45 min	37.610	38.475	37.812	36.447	0.685	0.791	0.524	0.446
	24 h	36.638	36.405	37.305	33.893	0.605	0.209	0.174	0.187
a*	45 min	19.267	18.518	19.808	21.623	0.629	0.363	0.150	0.317
	24 h	19.938	18.190	19.885	20.405	0.470	0.380	0.468	0.239
b*	45 min	5.393	5.745	6.025	5.735	0.296	0.915	0.647	0.614
	24 h	4.365	4.408	4.988	5.530	0.317	0.548	0.174	0.704
Cooking loss, %	45 min	31.954^{a}	30.035^{ab}	27.178^{b}	32.647^{a}	0.771	0.043	0.899	0.013
	24 h	31.867	30.751	30.523	29.564	0.604	0.636	0.214	0.950
Drip loss, %	24 h	2.902	2.798	2.378	2.573	0.123	0.463	0.220	0.553
pH value	45 min	6.347^{ab}	6.370^{ab}	6.453^{a}	6.301^{b}	0.020	0.048	0.728	0.025
	24 h	6.162	6.272	6.245	6.214	0.018	0.159	0.404	0.051

^{a,b}With in a row, values with different superscript letters mean significant difference ($P < 0.05$).
^{1}L, lightness; a*, redness; b*, yellowness.*

TABLE 7 | Effect of different ramie powder levels in diet on serum antioxidant biomarks of 70 days *Linwu* ducks.

Item[1]	Ramie powder levels				*SEM*	*P*-value	Linear and quadratic effects of ramie powder	
							P-value	
	0.00%	3.00%	6.00%	12.00%			Linear	Quadratic
SOD, U/ml	61.005^{b}	66.378^{ab}	70.628^{a}	63.191^{b}	1.287	0.035	0.293	0.010
GPX, U/ml	248.918	260.983	276.539	268.266	4.003	0.082	0.035	0.178
CAT, U/ml	1.056	1.144	1.305	1.233	0.048	0.290	0.113	0.401
GSH, μmol/L	25.789^{b}	28.583^{ab}	34.169^{a}	33.020^{a}	1.249	0.049	0.012	0.383
T-AOC, mmol/L	0.735	0.820	0.805	0.762	0.014	0.092	0.552	0.018
8-OHdG, ng/ml	19.114	18.598	16.707	18.919	0.643	0.556	0.677	0.310
MDA, nmol/ml	6.210	6.157	5.764	6.114	0.114	0.534	0.520	0.398

^{a,b}With in a row, values with different superscript letters mean significant difference ($P < 0.05$).
^{1}SOD, superoxide dismutase; GPX, glutathione peroxidase; CAT, catalase; GSH, glutathione; T-AOC, total antioxidant capacity; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; MDA, malonaldehyde.

TABLE 8 | Effect of different ramie powder levels in diet on antioxidant-related genes expression in muscle of 70 days *Linwu* ducks.

Item[1]	Ramie powder levels				*SEM*	*P*-value	Linear and quadratic effects of ramie powder	
							P-value	
	0.00%	3.00%	6.00%	12.00%			Linear	Quadratic
Breast muscle								
GPX1	1.000^{b}	1.186^{ab}	1.284^{a}	1.086^{ab}	0.040	0.049	0.250	0.012
SOD1	1.000	1.092	1.186	0.975	0.066	0.709	0.975	0.301
CAT	1.000	1.101	1.106	1.035	0.035	0.699	0.747	0.269
Nrf2/ECH	1.000	1.057	1.088	1.069	0.048	0.939	0.621	0.721
Leg muscle								
GPX1	1.000	1.015	1.169	0.898	0.060	0.503	0.788	0.267
SOD1	1.000^{b}	1.111^{ab}	1.303^{a}	1.068^{b}	0.042	0.048	0.223	0.028
CAT	1.000	1.286	1.407	1.217	0.061	0.098	0.127	0.043
Nrf2/ECH	1.000	0.800	1.087	1.129	0.075	0.451	0.336	0.438

^{a,b}With in a row, values with different superscript letters mean significant difference ($P < 0.05$).
^{1}GPX1, glutathione peroxidase-1; SOD1, superoxide dismutase-1; CAT, catalase; Nrf2/ECH, nuclear factor erythroid-2-related factor 2/erythroid-derived CNC homology factor.

(Chen et al., 2010). Increasing demands for high-quality meat called for new feed formula and animal breeding for better meat quality (Mehta et al., 2015). The tenderness, flavor, juiciness and color were commonly accepted as the main elements associated with the meat quality (Qiao et al., 2017). In current research, the quality of breast and leg muscles of ducks in different groups were studied. It was found that 6% ramie supplementation in diets significantly decreased the cooking loss of leg muscle at 45 min post-slaughter, indicating that ramie powder would improve the water holding capacity and improved the juiciness of the duck meat. The result was partially corroborated by Li's study, that finishing pigs fed diets supplemented with less than 9% ramie demonstrated an improvement in the pork quality (Li et al., 2019). Moreover, the inclusion of ramie hay or raw ramie in diets showed a tendency for better meat quality in goats as well (Zhang et al., 2019).

Oxidation stress was mainly caused by overproduction of reactive oxygen species (ROS), which contributed to the biological damages that affect the growth and production in animals (Nisar et al., 2013). It also led to lipid peroxidation that deteriorated the meat tenderness by inhibiting calpain activity and suppressing proteolysis process (Harris et al., 2001). In organism and muscle tissue, the balance between the oxidants and antioxidants was modulated by a defense system, composed by enzymatic components such as SOD, CAT, and GPX, and non-enzymatic compounds such as vitamin C and GSH (Surai et al., 2019). Studies showed that dietary supplements containing natural antioxidant plant materials could promote the growth of poultry, improved the antioxidant defense system of heat stressed birds, and improved meat and egg quality (Abo Ghanima et al., 2019; Ashour et al., 2020; Dosoky et al., 2021). In poultry, the antioxidant defense system was mediated by Nrf2/ECH, which triggered the antioxidant response elements and promote the expression of antioxidative enzymes in various tissues (Nguyen et al., 2009). When oxidative damage happened, MDA and 8-OHdG were formed as predominant forms of ROS induced oxidative lesions, so that they were considered common biomarkers for oxidative stress (Valavanidis et al., 2009; Fu et al., 2013). In order to prevent the oxidative stress, dietary ingredients that promoted the activities of antioxidant defense system were widely used in animal feed (Akbarian et al., 2016). At present, there was growing interests in utilizing herbal medicines as supplementation in feed for poultry and livestock to maintain or improve their health and productivity. The benefits to host health were mainly attributed to the phytochemical metabolites, namely polyphenols. Polyphenols were natural antioxidants with effects as antioxidant, immune regulation, antibacterial, antiviral, detoxification, and prevention of liver toxicity (Abd El-Hack et al., 2019; Abdel-Moneim et al., 2020; Attia et al., 2020; Mesalam et al., 2021). Previous data showed that ramie leaf contained polyphenols and flavonoids which were considered excellent antioxidant (Lee et al., 2014). They could effectively activate the antioxidant enzymes in intestinal mucosa (Lee et al., 2020) and muscle tissue (Li et al., 2019). In the current study, 6 and 12% ramie supplementation in diets increased the serum activity of SOD and content of GSH compared to the control group. Moreover, increased expressions of SOD1 in leg muscle and GPX1 in breast muscle were noticed under the dietary supplementation of 6% ramie. Both results above were in lines with previous findings and confirmed the antioxidative efficacy of ramie.

In conclusion, this study suggested a dietary supplementation of 6% ramie powder could promote the growth performance of *Linwu* ducks with no adverse effect on meat quality, possibly due to improvements of the antioxidative capabilities in ducks. This study provided solid information for the utilization of ramie as feed source for poultry.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Care and Use Committee, Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences.

AUTHOR CONTRIBUTIONS

All authors participated in the discussion, edited the manuscript, and approved the submitted version.

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Effects of Different Levels of Garlic Straw Powder on Growth Performance, Meat Quality, Antioxidant and Intestinal Mucosal Morphology of Yellow-Feathered Broilers

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The full utilization of garlic straw can partially alleviate shortage of feedstuff and waste of resources. The purpose of this study was to investigate the effects of garlic straw as an unconventional feed on yellow-feathered broilers. 360 28-day-old yellow-feathered broilers were randomly divided into 4 groups with 6 replicates (cage) per group, 15 per cage. The 4 groups were as follows: control group (basal diet) and experimental group I (basal diet supplemented with 3% garlic straw powder), II (basal diet supplemented with 6% garlic straw powder) and III (basal diet supplemented with 9% garlic straw powder). There was no significant difference in the initial body weight of the broilers among groups ($p > 0.05$). The test period was 28 days in total. The experiment results showed that there were no significant difference in the average final weight, ADG, ADFI and F/G among groups ($p > 0.05$). On the one hand, for the breast muscle, the drip loss of experimental group I, II and III were reduced by 17.24% ($p < 0.05$), 20.11% ($p < 0.05$) and 20.50% ($p < 0.05$), respectively, compared with the control group; the redness a* of the experimental groups had a trend of improvement ($0.05 < p < 0.1$). On the other hand, compared with the control group, the redness a* of the experimental group II increased significantly by 23.18% for the leg muscles ($p < 0.05$). Furthermore, compared with the control group, GSH-Px of the experimental group III significantly increased by 21.38% ($p < 0.05$), and SOD of the experimental group I significantly increased by 21.85% ($p < 0.05$). Finally, there were no significant differences in the intestinal villus height, crypt depth, V/C and intestinal wall thickness among four groups ($p > 0.05$). In conclusion, dietary supplementation of different levels of garlic straw powder can improve meat quality and antioxidant capacity of yellow-feathered broilers without affecting growth performance and intestinal mucosal morphology.

Keywords: garlic straw powder, yellow-feathered broilers, growth performance, meat quality, antioxidant, intestinal mucosa morphology

INTRODUCTION

The sustainability of livestock production systems ensures global food security (Davis and White., 2020). However, as the number of humans and livestock soared, there was inevitably a situation of people and livestock competing for food. Non-conventional feed raw material resources are "a large number of scraps" or "garbage" neglected by the material production sector, including stems of agricultural crops, residues from slaughterhouses, excreta of livestock and poultry, and plant meal (Yin et al., 2019). These resources are widely available, low cost and high feeding value. In fact, it has become a reality to use this "garbage" to solve some problems in agriculture. A prime example is Singapore's use of technology, such as recycling nutrients from food waste, to address food shortages (Mok et al., 2020). This makes us think, can we also use non-conventional feed raw material resources to solve the issue of insufficient feed resources ?

Garlic straw is the main by-product of garlic. Studies showed that the crude protein content of garlic straw is similar to that of alfalfa hay and the crude fiber content is lower (Lee et al., 2017). Garlic straw is also rich in active functional components, including more than 30 kinds of allicin, organic selenium, superoxide dismutase, enzymes and glycosides (Karangiya et al., 2016; Zhang et al., 2019). Nicastro et al. (2015) highlight potential mechanisms of metabolites of allicin, including decreased bioactivation of carcinogens, antimicrobial activities, and redox modification. To be specific, allicin and other sulfide substances contained in garlic straw can inhibit the proliferation of both bacteria and fungi, including antibiotic-resistant strains like methicillin-resistant *Staphylococcus aureus* (Borlinghaus et al., 2014; Nakamoto et al., 2020). Long-term human intake of animal-derived antibiotics may cause pathogenic bacteria to develop drug resistance, thereby breaking the ecological balance of bacteria in the body (Chen et al., 2019). Feeding garlic by-product to livestock and poultry may maintain the balance of flora to a certain extent, and ultimately benefit humans (Wunderlich et al., 2014; Zhu et al., 2021).

Yellow-feather broiler accounts for more than half of Chinese broiler market, which satisfy consumer preferences regarding flavor in China and some other countries in South-East Asia (Gou et al., 2016). At present, garlic straw has achieved good feeding effect in rabbits (Liu L. et al., 2019), buffalo (Attri et al., 2020), sheep (Lee et al., 2017) and other animals. There is no relevant research on the feeding of garlic straw to yellow feather broilers. Therefore, this study took the high-quality broiler breed yellow-feather broiler as the research object, and explored the application effect of garlic straw as an unconventional feed resource in yellow-feather broiler.

MATERIALS AND METHODS

Garlic Straw Powder Preparation

The garlic straws used in this study were collected from Jinxiang County, Shandong Province, China. Garlic straw powder is made by cutting, drying and pulverizing, and stored in a closed dark room for preparation. After analysis and determination, the conventional nutrient content and apparent metabolizable energy of the garlic straw powder samples used in this study are as follows: dry matter content 88.83%, crude protein 10.81%, crude fiber 25.97%, crude fat 1.40%, crude ash 12.87%, calcium 3.02%, total phosphorus 0.24%, apparent metabolizable energy 1.65Mcal/kg.

Animals and Experimental Details

All the experiment procedures were conducted in accordance with the Chinese guidelines for animal welfare and approved by the Animal Care and Use Committee, Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences. In this study, 360 28-day-old yellow-feathered broilers were randomly divided into 4 groups with 6 replicates (cage) per group, 15 per cage. The 4 groups were as follows: control group (basal diet) and experimental group I (basal diet supplemented with 3% garlic straw powder), II (basal diet supplemented with 6% garlic straw powder) and III (basal diet supplemented with 9% garlic straw powder). The initial weight of chicks was approximately similar for all groups. The whole experiment period was 28 days.

The basal diet was formulated with reference to the nutritional requirements of broilers in NRC (1994) and NY/T33-2004 Chicken Feeding Standard. The raw material dosage and source of the whole feed were kept the same, and the specific composition and nutritional level were shown in **Table 1**.

This experiment was carried out in Yuanjiang Experimental Base of Hemp Research Institute, Chinese Academy of Agricultural Sciences. First, thoroughly clean and disinfect the coop 1 week before the experiment. Second, a 24-h intelligent light source and ventilator are installed in the broilers house to provide appropriate temperature and ventilation according to the temperature needs of broilers. Third, the broilers are fed different diets according to the experimental design. Broilers have free access to food and water. In addition, daily management and immunizations are carried out in strict accordance with the standard procedures of the broilers farm. Record the actual daily feed intake and observe the behavior and health of the chickens from time to time.

Sample Collection

On day 28 of the experiment, 6 broilers were randomly selected from each treatment for blood sampling. Blood samples were collected from the jugular vein of the broiler into a vacuum blood collection tube. The whole blood was coagulated in a tube at room temperature and centrifugated at 3,500 rpm for 15 min. The serum samples were separated and stored at −20°C until it was used for the determination of serum indicators After blood collection, the broilers were euthanized by carbon absorption. After cervical dislocation, muscle samples (left pectoral and leg muscles) and intestinal samples (duodenum, jejunum and ileum tissues) were quickly separated from the body in a sterile environment. Muscle samples were assayed rapidly. The middle section of each intestine was about 2 cm, cleaned gently with normal saline, and fixed with 4% paraformaldehyde solution.

TABLE 1 | Diet formulation and calculated nutrients (as fed basis).

Items	Control	Garlic straw powder supplementation concentration in diets		
		3%	6%	9%
Ingredients, %				
Corn	50.00	50.26	50.96	51.30
Soybean meal	30.52	30.00	29.57	29.04
Rice husk	4.60	3.03	1.56	0.00
garlic straw powder	0.00	3.00	6.00	9.00
Oil	3.43	3.13	2.69	2.35
Wheat bran	7.55	6.90	5.77	5.07
Limestone	1.71	1.47	1.22	0.97
CaHPO4·2H2O	0.74	0.74	0.74	0.76
L- Lys	0.00	0.02	0.03	0.05
98.5% DL- Met	0.15	0.15	0.16	0.16
NaCl	0.30	0.30	0.30	0.30
3% Premix1)	1.00	1.00	1.00	1.00
Total	100	100	100	100
Nutrient levels (%)				
ME (Mcal/kg)	2.85	2.85	2.85	2.85
Crude protein (%)	18.00	18.00	18.00	18.00
Crude fiber	5.71	5.71	5.71	5.71
Calcium (%)	1.00	1.00	1.00	1.00
Total phosphorus (%)	0.61	0.61	0.60	0.60
Available P	0.35	0.35	0.35	0.35
Lysine (%)	0.99	0.99	0.98	0.99
Methionine (%)	0.46	0.46	0.46	0.46
Methionine + cystine (%)	0.76	0.76	0.76	0.75

[a]*The premix provided the following (per kilogram of complete diet): vitamin A 12000 IU; vitamin D3 2500 IU; vitamin E 20 mg; vitamin K3 3 mg; vitamin B1 3 mg; vitamin B2 8 mg; vitamin B6 7 mg; vitamin B12 0.03 mg; Pantothenic acid 20 mg; Nicotinic acid 50 mg; Biotin 0.1 mg; Folic acid 1.5 mg; Cu 9 mg; Zn 110 mg; Fe 100 mg; Mn100 mg; Se 0.16 mg; I 0.6 mg. 2) Nutrient levels were calculated values.*

Growth Performance

Body weight of yellow feather broilers was individually measured at the beginning (day 28) and the end of the trial (day 56). Feed intake per cage was recorded daily. The average daily gain (ADG), average daily feed intake (ADFI), and food conversion ratio (FCR) were calculated according to the data from each cage.

Determination of Meat Quality

The meat colour of 45 min postmortem was, respectively, determined as the L*, a* and b*, the indicators of lightness, redness and yellowness, with a colorimeter (CR-400; Konica Minolta Co., Osaka, Japan). The water loss rate (WLR, using a filter paper press method) and pH values (45 min postmortem) were determined as described previously (Choi et al., 2016). The pH value was measured with a pH meter (Model 340, Mettler-Toledo GmbH, Schwerzenbach, Switzerland). The drip loss was scored based on a suspension method with the size-standardised samples (2.0 cm*1.5 cm*1.5 cm) from the leg muscle and breast muscle that were weighed, suspended in a plastic bag, held at 4°C for 24 h, and thereafter reweighed. Drip loss was expressed as a percentage. And, the samples which tested the cooking loss at 45 min postmortem were bagged in tin foil and immersed in a 75°C water bath for 30 min and cooled at room temperature for 30 min. After cooling to room temperature, the tin foil was opened and free juice was drained. The cooked samples were blotted with a paper towel and weighed. The cooking loss was determined by weighing the meat before and after cooking (Honikel., 1998). The shear force was further measured with the digital tenderness meter (C-LM3B, Northeast Agricultural University, Harbin, China) after measuring the cooking loss to evaluate tenderness. Test speeds were set at 2 mm/s. Data were collected and analysed on the basis of the shear force values to obtain the maximum force required to shear through each sample.

Antioxidant Indices

The serum and hepatic levels of glutathione peroxidase (GSH-Px), the activities of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), total antioxidant capacity (T-AOC) and malondialdehyde (MDA) were determined by the commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) with an automated fluorescence instrument (Thermo Fisher Scientific). The GSH-Px activity was assayed at 412 nm by quantifying the oxidation rate of reduced GSH to oxidised glutathione (Yin et al., 2013). The activity of SOD was determined at 450 nm by the nitrite coloration method (Liu et al., 2014). Briefly, the CAT activity was determined by incubating sample with a known concentration of H_2O_2, which was then measured at 405 nm by the ammonium molybdate method (Sun et al., 2015). The GSH concentration was measured according to the methods as described previously with minor modification (Zhan and Meng, 1989). The T-AOC was determined at 520 nm with the method of ferric reducing ability of plasma (Sun et al., 2015). The MDA concentration was determined at 532 nm by thiobarbituric acid reaction method (Yang et al., 2010).

TABLE 2 | Effects of different dietary levels of garlic straw powder on growth performance in yellow feather broiler (g).

Item	Control	Garlic straw powder supplementation concentration in diets			SEM	*p*-value		
		3%	6%	9%		ANOVA	Linear	Quadratic
Average final weight	1447.57	1464.03	1500.87	1481.23	11.706	0.436	0.204	0.451
ADG	34.58	34.95	36.71	35.37	0.501	0.486	0.373	0.407
ADFI	129.54	122.97	126.00	120.82	1.582	0.237	0.107	0.822
FCR	3.77	3.54	3.43	3.42	0.063	0.155	0.038	0.352

[a,b]Means in the same row with different superscript letters indicate differences (p < 0.05).

Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; FCR, food conversion ratio; SEM, standard error of the mean.

TABLE 3 | Effects of different dietary levels of garlic straw powder on breast muscle quality in yellow-feathered broilers (g).

Item		Control	Garlic straw powder supplementation concentration in diets			SEM	*p*-value		
			3%	6%	9%		ANOVA	Linear	Quadratic
Shear force		3.23	2.68	3.05	2.72	0.139	0.458	0.364	0.693
Meat colour	L*	50.92	52.87	50.76	53.80	0.647	0.273	0.261	0.667
	a*	8.00	8.26	9.01	8.66	0.150	0.073	0.035	0.274
	b*	7.71	7.44	7.72	8.08	0.189	0.724	0.440	0.430
Cooking loss/(%)		21.73	21.32	22.19	20.29	0.591	0.729	0.537	0.549
Drip loss/(%)		5.22[a]	4.32[b]	4.17[b]	4.15[b]	0.162	0.048	0.016	0.140
PH value		6.05	6.15	6.01	5.85	0.046	0.151	0.076	0.158

[a,b]Means in the same row with different superscript letters indicate differences (p < 0.05).

Abbreviations: L, brightness; B*, redness; A*, yellowness; SEM, standard error of the mean.*

Determination of Intestinal Mucosa Morphological Structure

The intestinal samples were cleaned and stained with hematoxylin and eosin (HE) and finally made into paraffin sections using microtome (RM-2235, Leica microsystems AG Co., Ltd., Black Forest, Germany). Microscopes were then used to observe tissue paraffin sections, multiple non-contiguous fields were randomly selected, and representative fields were selected to be imaged. Next, the Mingmei Microscope Digital Measurement and Analysis System V1.6.1 was used to observe and measure, to determine the thickness of the intestinal wall thickness, the villus height, and the crypt depth, and to calculate the ratio of villus height to crypt depth (V/C).

Statistical Analysis

The data were analysed using one way ANOVA model among four groups in Statistical Packages for Social Science 25.0 (IBM). Orthogonal polynomial contrasts were used to determine the Linear and quadratic responses of different levels of garlic straw powder to growth performance, meat quality, antioxidant capacity and intestinal mucosal morphology of broilers. The broilers selected from each group was experimental unit for the analyses of all the data of the present study. The results were expressed as arithmetic means and SEM. When the main test was significant, the differences among means were further analysed using Tukey–Kramer test for multiple comparison. Differences between means of all groups were considered significant at $p < 0.05$. The p values between 0.05 and 0.10 were considered as a trend.

RESULTS

Growth Performance

The effects of different levels of garlic straw powder on growth performance of yellow-feathered broilers is presented in **Table 2**. There were no significant differences in average final weight, ADG, ADFI, and FCR among four groups ($p > 0.05$). No mortality was observed throughout the trial period.

Meat Quality

Table 3 shows the effects of different levels of garlic straw powder on breast muscle of yellow-feathered broilers. Compared with the control group, the drip loss of breast muscle was decreased by 17.24% ($p < 0.05$), 20.11% ($p < 0.05$) and 20.50% ($p < 0.05$) at 3, 6 and 9% garlic straw powder supplemental levels, and showed a significant linear change ($p < 0.05$). The experimental groups supplemented with garlic straw powder tended to increase the redness of breast muscle a* ($0.05<p < 0.1$). In addition, there were no significant differences in shear force, L* and b* of meat color, cooking loss and pH value of breast muscle among four groups ($p > 0.05$).

Table 4 shows the effects of different levels of garlic straw powder on leg muscle of yellow-feathered broilers. Compared with the control group, the redness a* of drumsticks in the experimental group II significantly increased by 23.18% ($p < 0.05$). In addition, there were no significant differences in shear force, L* and b* of meat color, cooking loss, drip loss and pH ratio of leg muscle among four groups ($p > 0.05$).

TABLE 4 | Effects of different dietary levels of garlic straw powder on leg muscle quality in yellow-feathered broilers (g).

Item		Control	Garlic straw powder supplementation concentration in diets			SEM	*p*-value		
			3%	6%	9%		ANOVA	Linear	Quadratic
Shear force		1.59	1.60	1.35	1.38	0.080	0.608	0.254	0.959
Meat colour	L*	48.19	50.28	48.40	48.84	0.938	0.878	0.994	0.682
	a*	15.70^{b}	16.57^{b}	19.34^{a}	17.75^{ab}	0.476	0.029	0.025	0.150
	b*	9.06	9.78	10.16	10.30	0.468	0.810	0.361	0.769
Cooking loss/(%)		17.49	17.70	16.04	16.23	0.997	0.920	0.572	0.996
Drip loss/(%)		6.70	5.58	7.00	6.90	0.303	0.332	0.460	0.399
pH value		6.28	6.32	6.34	6.26	0.028	0.745	0.856	0.309

^{a,b}Means in the same row with different superscript letters indicate differences (p < 0.05).

Abbreviations: L,brightness; B*,redness; A*, yellowness; SEM, standard error of the mean.*

TABLE 5 | Effects of different dietary levels of garlic straw powder on serum antioxidant performance in yellow-feathered broilers.

Item	Control	Garlic straw powder supplementation concentration in diets			SEM	*p*-value		
		3%	6%	9%		ANOVA	Linear	Quadratic
GSH-Px/(U/mL)	2062.37^{b}	2303.23^{ab}	2201.08^{b}	2503.23^{a}	60.810	0.037	0.013	0.731
SOD/(U/mL)	484.50^{b}	590.35^{a}	473.48^{b}	465.30^{b}	18.917	0.031	0.175	0.061
CAT/(U/mL)	0.03	0.02	0.02	0.02	0.003	0.209	0.128	0.187
GSH/(umol/L)	5.40	7.56	7.10	6.64	0.436	0.371	0.418	0.161
T-AOC/(mmol/L)	1.00	1.23	0.96	1.31	0.110	0.674	0.543	0.804
MDA/(nmol/mL)	4.75	3.73	4.41	4.51	0.344	0.800	0.989	0.480

^{a,b}Means in the same row with different superscript letters indicate differences (p < 0.05).

Abbreviations: GSH-PX, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; GSH, glutathione; T-AOC, total antioxidant capacity; SEM, standard error of the mean.

TABLE 6 | Effects of different dietary levels of garlic straw powder on intestinal morphology in yellow-feathered broilers.

Item		Control	Garlic straw powder supplementation concentration in diets			SEM	*p*-value		
			3%	6%	9%		ANOVA	Linear	Quadratic
Duodenum	Villus height	600.12	605.04	629.53	540.41	13.606	0.130	0.185	0.078
	Crypt depth	115.95	117.87	113.16	103.67	4.426	0.788	0.424	0.603
	V/C	5.25	5.14	5.58	5.24	0.134	0.795	0.778	0.750
	Intestinal wall thickness	229.00	221.95	212.63	189.87	6.702	0.168	0.044	0.505
Jejunum	Villus height	455.08	493.99	460.24	470.86	8.214	0.379	0.981	0.436
	Crypt depth	95.84	102.49	100.56	111.82	2.586	0.178	0.058	0.610
	V/C	4.78	4.83	4.58	4.23	0.103	0.137	0.038	0.304
	Intestinal wall thickness	201.39	223.40	223.89	229.45	5.356	0.352	0.128	0.464
Ileum	Villus height	539.86	521.22	482.89	511.67	14.512	0.635	0.398	0.463
	Crypt depth	99.94	88.92	93.67	94.95	2.976	0.692	0.731	0.365
	V/C	5.42	5.91	5.14	5.40	0.132	0.227	0.461	0.649
	Intestinal wall thickness	195.86	201.06	200.77	187.93	3.646	0.612	0.503	0.274

^{a,b}Means in the same row with different superscript letters indicate differences (p < 0.05).

Abbreviations: V/C,the villus height/crypt depth; SEM, standard error of the mean.

Antioxidant Performance

Table 5 shows the effects of different levels of garlic straw powder on antioxidant performance of yellow-feathered broilers. Compared with the control group, the serum GSH-Px level in experimental group III significantly increased by 21.38% ($p < 0.05$). At the same time, compared with the control group, the serum SOD level in

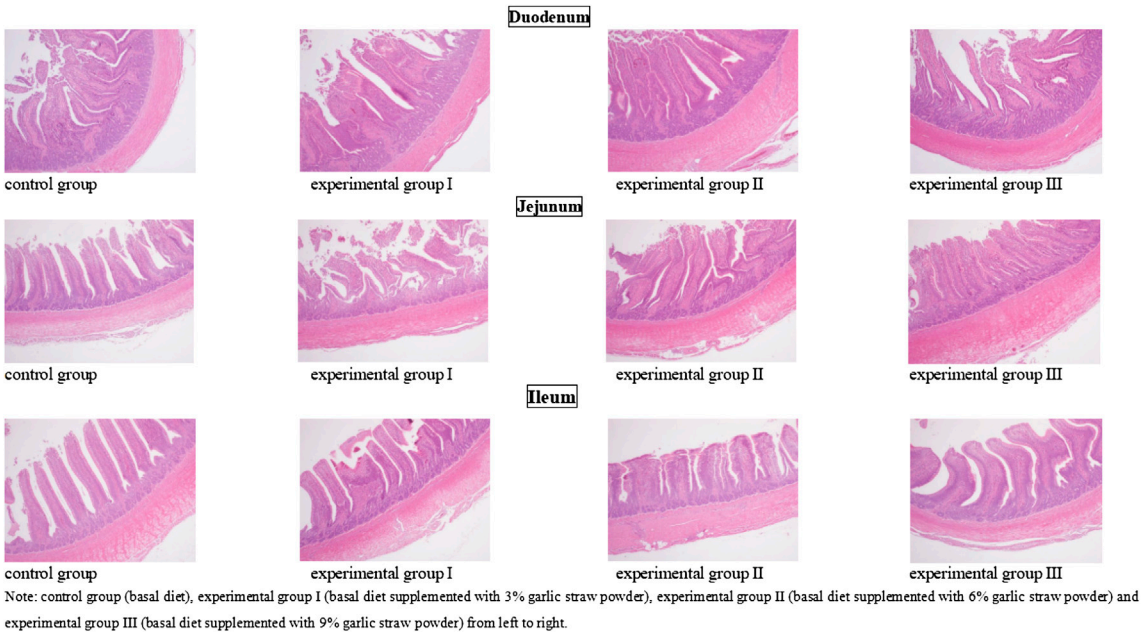

FIGURE 1 | Sections of duodenum jejunum and ileum of yellow-feathered broilers in each group (40×). Note: control group (basal diet), experimental group I (basal diet supplemented with 3% garlic straw powder), experimental group II (basal diet supplemented with 6% garlic straw powder) and experimental group III (basal diet supplemented with 9% garlic straw powder) from left to right.

experimental group I significantly increased by 21.85% ($p < 0.05$). In addition, there were no significant differences in CAT, GSH and T-AOC among four groups ($p > 0.05$).

Intestinal Mucosa Morphological Structure

The effects of different levels of garlic straw powder on intestinal structure of yellow-feathered broilers were shown in **Table 6** and **Figure 1**. There were no significant differences in intestinal wall thickness , villus height, crypt depth and V/C in duodenum, jejunum and ileum of among four groups ($p > 0.05$).

DISCUSSION

The addition of herbal extracts and herbs to poultry diets may have some beneficial effects on performance (Abd El-Hack et al., 2018). The multiple effective pharmacological component of garlic straw coincide highly with garlic (Majewski, 2014). It has been reported that the addition of 0.5 and 1.0% garlic in the diet can promote the feeding and improve the growth performance of broilers (Ismail et al., 2021). Studies showed that adding 0.25, 0.50 and 0.75 g/kg garlic powder in the diet promoted the increase of body weight and daily gain of broilers at 21 and 42 days (Ismail et al., 2021). It is speculated that garlic by-products are similar to garlic and can improve the performance of broiler. However, there were no significant differences of average final weight, ADG, ADFI, and FCR among four groups ($p > 0.05$), but no mortality was observed in the whole process in this study. A possible guess is that broilers have a certain tolerance threshold to garlic straw. Garlic and its products have been shown to have a pungent odor and anti-nutrition factors that can reduce the palatability of diets and lead to a decrease in feed intake (Abe et al., 2020; Gu et al., 2021). Some scholars suggest that garlic or garlic powder can be used with other plant to stimulate the digestive enzyme activities of broilers, and then improve the performance and economic benefits of broilers. For example, broilers showed better the weight gain and FCR when both garlic and black pepper were added to the diet (Kirubakaran et al., 2016).

Yellow-feathered broiler is one of the best broiler varieties in China, and its meat quality is valued by consumers (Liu W. et al., 2019). Improving meat quality of yellow-feathered broilers by diet is a common method in breeding industry (Jiang et al., 2018). Previous studies have shown that breast muscle and leg muscle of broilers treated with black garlic extract can significantly improve meat color (Barido et al., 2021). Under the conditions of this study, adding different levels of garlic straw powder tended to increase the a* value of broilers ($p < 0.05$). The color of meat is essentially the form and content of myoglobin (Barido et al., 2021). Choi et al. believed that adding 5% garlic powder can improve a* value of broilers, mainly because its antioxidant components can delay the formation and oxidation of myoglobin (Choi et al., 2010). In addition, this study showed that the drip loss of breast muscle was negatively correlated with the level of garlic straw meal supplementation ($p < 0.05$). Drip loss is closely related to muscle softness, which indirectly reflects human satisfaction with edible quality (Warner et al., 2022). This indicator also indirectly reflects the nutritional value and hygienic quality of broilers. Some studies have shown that increased oxidative damage can impair the tenderness of the meat,

thereby reducing the palatability and nutritional value of meat products (Zhang et al., 2013; Hauck et al., 2019). Garlic straw powder may inhibit the oxidation of protein and lipid in broilers by increasing the antioxidant content in broilers, and ultimately improve the tenderness and color of broilers.

Many factors, such as fast growth, high metabolism and intensive production, can produce a large number of free radicals in broilers (Lauridsen , 2019). These free radicals have been proved to induce disease in broilers, resulting in huge economic losses (Clark et al., 2019; Mishra and Jha. 2019). GSH-Px, SOD, and CAT together constitute the main antioxidant enzyme system in broilers, and the level of its content reflects the effect of scavenging free radicals (He et al., 2017). Plant or plant extracts can act on enzymes related to free radicals to indirectly organize the oxidation of free radicals, thereby enhancing the antioxidant capacity of animals (Gladine et al., 2007; Zhang et al., 2020). Previous studies have shown that the addition of garlic powder to the diet has a positive effect on the antioxidant properties of broilers (Ismail et al., 2021). The compound feeding of garlic root powder and Moringa leaf powder also increased the activity of glutathione peroxidase in broilers (Gbore et al., 2020). We speculate that perhaps garlic straw powder also has a similar potential to improve the antioxidant function of yellow feather broilers. In fact, compared with the control group, the serum GSH-Px level of broilers in the experimental group III significantly increased by 21.38% ($p < 0.05$). In addition, the serum SOD level in the experimental group I significantly increased by 21.85% compared with the control group ($p < 0.05$). Similar results were obtained by Locatelli who believed that organosulfur compounds such as allicin had strong anti-radical mechanisms and iron-reducing ability (Locatelli et al., 2017). L-theanine is a water-soluble, non-protein amino acid, mainly found in green tea leaves. L-theanine at 200 mg/kg improved the antioxidant status in broiler blood by increasing SOD, GSH-Px and relative CAT levels (Saeed et al., 2018). Combined with the results of meat quality in this study, it was further verified that adding garlic straw powder can improve the antioxidant capacity of yellow feather broilers.

Intestinal tract is the final site for nutrient digestion and absorption. Intestinal villus height, crypt depth, V/C and intestinal wall thickness indirectly indicate intestinal absorption function and immune function (Mowat and agace, 2014). Most scholars believe that the morphological and structural integrity of small intestine reflects the effective absorption rate of animal nutrients and resistance to related diseases (Solis de los Santos et al., 2005). Studies have shown that the addition of garlic and its by-product derivative (propane thiosulfonate) can improve the growth performance of broilers by protecting the integrity of the intestinal barrier and the composition of related microflora (Ruiz et al., 2015).The possible explanation is that some of the bioactive compounds in garlic can promote the absorption of chyme from the intestine (Hafidh et al., 2011). Previous studies have shown that multi-strain probiotic, citric acid, garlic powder or their combinations can improve the ileal structure of broilers (Elbaz et al., 2021). However, there were no significant differences in villus height, crypt depth, V/C and intestinal wall thickness in duodenum, jejunum and ileum among four groups ($p > 0.05$). We believe that this is related to dosage of garlic straw powder. Previous studies have shown that dietary 1000 mg/kg garlic powder can protect the integrity of intestinal structure of broilers at 21 days of age, while 1000 and 2000 mg/kg garlic powder can significantly protect the integrity of intestinal structure of broilers at 42 days of age (Yang et al., 2021). To sum up. these results indicate that dietary garlic straw powder has no adverse effects on intestinal development.

CONCLUSION

In conclusion, garlic straw has no adverse effects on growth performance and intestinal mucosal morphology of yellow-feathered broilers. 3–9% substitution rate of garlic straw powder can improve antioxidant capacity and meat quality.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by All the experiment procedures were conducted in accordance with the Chinese guidelines for animal welfare and approved by the Animal Care and Use Committee, Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences.

AUTHOR CONTRIBUTIONS

The research was designed and conducted by SL, LL, and PH. The animal experiment was conducted by XW, YW, and SZ. The detection and analysis works were conducted by TL, YL, TL, and ZF. SL, ZF, and QL prepared the manuscript draft. All authors participated in the discussion and editing of the manuscript.

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Effect of Ramie on the Production Performance of Laying Hens, and the Quality, Nutrient Composition, Antioxidation of the Eggs

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Ramie (*Boehmeria nivea*), which is rich in protein, fatty acid, vitamins and minerals, has become a potential alternative feed resource for poultry, and has attracted more and more attentions in nutrition research. The objective of this study is to evaluate the effect of dietary ramie at different concentrations on the production performance of the hens, and the quality, nutrient composition, and antioxidation of the eggs. A total of 432 34-week-old Lohmann commercial laying hens were divided into four groups, that were fed with corn-soybean meal-based control diet, control mixed with ramie at concentrations of 3, 6, or 9% separately for 8 weeks. Results showed that dietary ramie did not affect production performance. And egg yolk color gradually deepened as the inclusion levels of ramie increased. Ramie at tested concentration could significantly reduce the content of malondialdehyde (MDA) (p = 0.002) and 3% ramie supplementation significantly increased total antioxidative capacity (T-AOC) concentrations in egg yolk compared to the control group (p = 0.033). In addition, dietary supplementation with 6% ramie significantly reduced total cholesterol (T-CHO) content ($p < 0.05$) compared with controls. For egg nutrient composition, compared with the control group, the addition of 6% ramie significantly increased ($p < 0.05$) total omega-3 polyunsaturated fatty acids (n-3PUFA) and phenylalanine (Phe) in yolk. In conclusion, dietary inclusion of 6% ramie was most effective in improving the color, antioxidative capability, and reducing T-CHO contents of the egg yolks without any negative impacts on the production performance of the hens.

Keywords: ramie, production performance, egg quality, yolk antioxidation, egg nutrients composition

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INTRODUCTION

With the development of economy and living standards, there is an increasing consumption for livestock and poultry products (meat, eggs, milk, *etc.*), which promotes the vigorous development of animal husbandry and demand for feedstuffs. In the past a few years, the lack of feedstuffs has become an important factor restricting the development of the livestock industry due to the shortage of traditional feed resources and the rising prices (Li et al., 2019). To reduce the dependency on the

imported feeds and reduce the costs of production, seeking new feed sources with high quality and yield has become a hot spot in the field of animal nutrition (Reda et al., 2022).

Ramie (*Boehmeria nivea*) is a perennial herbaceous plant that belongs to the Urticaceae (Boehmeria) family; it is originated in China, commonly known as "Chinese grass" (Ni et al., 2018). As a traditional textile raw material, only 5% of ramie are utilized, resulting in a great waste of resources (Liu et al., 2013). In recent years, the development and utilization of high-quality unconventional feed resources have attracted much attention. Ramie is a source rich in nutrients, such as proteins, fatty acids (especially oleic acid, palmitic acids and linolerc acid), minerals, and vitamins. It is also well balanced in amino acids with the total amount up to 18.36% (Wang et al., 2019; Wang et al., 2021a). Ramie contains many biologically active compounds in its roots and leaves, such as flavonoids (Rutin, Rhoifolin, beta-ionone) and polyphenols compounds (chlorogenic acid, ferulic acid, caffeic acid), which have been shown to possess antibacterial and anti-inflammation activities *in vitro* (Xiao et al., 2014; Wang et al., 2019).

Previous studies have proved that other unconventional feedstuffs, such as almond hulls, alfalfa and nettle plants, are able to improve egg quality and reduced feed costs (Wang et al., 2021b; Grela et al., 2020; Zhang et al., 2020). It also have been proved that supplementary nettle (*Urtica dioica*), which belong to the same family as ramie, can significantly reduce the lipid metabolism and reduce the total cholesterol, triglyceride contents in blood plasma of mice (Avci et al., 2006). To date, most studies regarding to nettle as an alternative ingredient in laying hens' feeds have confirmed that it can increase yolk color, decrease total cholesterol contents, improve egg yolk fatty acid composition (Loetscher et al., 2013a; Zhang et al., 2020). Ramie is a plant of *Urticaceae*, and its high nutritional content makes it a potential new material feed. Therefore, the aim of this study was to assess the potential of ramie as a dietary ingredient for the laying hens, by determining the effects of dietary ramie supplementation on the production performance of hens, and the quality, nutrients composition, and oxidative status of the eggs.

MATERIALS AND METHODS

All experiment procedures in the present study were approved by the Animal Care Committee of the Institute of Bast Fiber Crops, Chinese Academic of Agricultural Sciences.

Forage Preparation

The leaves and tender tops of fresh ramie were purchased from Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences (Changsha, Hunan, China). The ramie (*Boehmeria nivea cv*. Qingsizhu No.1) was cut at about 60 cm height and immediately dried at 60°C for 4 days by placing in a heat drier room. Then, the dried stems and leaves were crushed to ramie powder using a grinder equipped with 1.5 mm sieve, and were kept in a well-closed and light-resistant place. The nutritional level of ramie powder was crude protein 16.84%, crude fiber 18.7%, calcium 3.3%, total phosphorus 0.30%, lysine 0.64% and methionine 0.04%.

Experimental Procedure

The experiment was performed at animal experiments base of Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences. A total of 432 34-week-old Lohmann Commercial laying hens were randomly divided into four groups, with six replicates of 18 birds each. The hens were raised in ladder cages with two birds each cage. Nine cages with the same diet trough were arranged sequentially as a replicate. After 2 weeks habituation, formal trial started and lasted for 6 weeks. The egg production, body weight, and feed intake of laying hens were measured during the adaptation period to confirm no statistical difference in the production performance among treatments. Laying hens were provided a control diet or 3, 6, 9% ramie powder replacement diet. All diets were formulated to meet recommendations of the National Research Council (NRC) for laying hens (1994) as shown in **Table 1**. All the diets contain the same ME, crude protein and amino acid *etc*. Free water and feed for all hens. The average temperature was 25 ± 2°C in the laying hens' house during the experimental period. The light time was according to the standard light procedure of commercial laying hens, 16 h of light per day until the end of the experiment.

Production Performance and Egg Quality

During the test, eggs were picked up at 16:30 pm daily. The feed intake was recorded weekly. laying conditions (egg production and egg weight) of each repetition were recorded in detail. Finally, the egg production, feed conversion ratio, egg weight, daily feed intake and daily egg weight were statistically calculated. Feed conversion ratio was calculated as grams of total feed intake per hen divided by grams of total egg mass per hen, and egg mass was calculated as egg weight multiplied by percentage of egg production. Weights of the albumen, yolk, and shell (12 eggs per replicate) were recorded separately on the last day of the experiment.

Egg quality were determined by parameters of shell strength, shell thickness, Haugh units, egg shaped index, egg yolk index and egg yolk color. Eggshell strength was evaluated using an eggshell force gauge (model II, Robotmation Co., Ltd., Tokyo, Japan). Shell thickness was measured using a vernier caliper and was taken as the mean value at three points: the two narrow ends and the middle part of the egg. Haugh unit and Yolk color were determined using an egg multi-tester (EMT-7300, Robotmation, Tokyo, Japan). Egg-shaped index meter (FHK, Mingao Instrument Equipment, Nanjing, China) was used to evaluate the egg shape index. In order to keep the egg yolk intact, the egg yolk diameter and height were accurately measured by vernier caliper. Each egg was measured three times, and the average value was taken. The ratio of egg yolk height to egg yolk diameter was egg yolk index.

Yolk Antioxidation

Twenty-four eggs (one eggs/replicate) were randomly selected to analyze the antioxidant index of the yolk on the last day. Egg yolk was separated by manual separator, and then placed at −20°C for

TABLE 1 | Diet formulation and calculated nutrients (air-dried basis, %).

Items	Control	Ramie power supplementation concentration in diets		
		3%	6%	9%
Ingredients, %				
Corn	51.61	51.34	51.23	51.03
Soybean meal	30.78	29.76	28.66	27.59
Rice husk	3.31	2.21	1.09	0.00
Ramie powder	0.00	3.00	6.00	9.00
Oil	2.85	2.50	2.10	1.73
Limestone	8.45	8.19	7.92	7.65
Premix	3.00	3.00	3.00	3.00
Total	100	100	100	100
Nutrient levels (%)				
ME(Mcal/kg)	2.75	2.75	2.75	2.75
Crude protein (%)	16.98	17.00	17.00	17.01
Crude fiber	4.44	4.44	4.44	4.45
Calcium (%)	3.50	3.50	3.50	3.50
Total phosphorus (%)	0.53	0.53	0.53	0.53
Available phosphorus (%)	0.32	0.32	0.32	0.32
Lysine (%)	0.95	0.95	0.94	0.93
Methionine (%)	0.36	0.35	0.35	0.34

[a]*The premix provided the following (per kilogram of complete diet): vitamin A 6000 IU, vitamin D3 2,500 IU, vitamin E 25 mg, vitamin K3 2.25 mg, vitamin B1 1.8 mg, vitamin B2 7 mg, vitamin B6 4 mg, vitamin B12 0.2 mg, D-pantothenic acid 12 mg, nicotinic acid 35 mg, biotin 0.14 mg, folic acid 0.8 mg, Cu (as copper sulphate) 11 mg, Zn (as zinc sulphate) 70 mg, Fe (as ferrous sulphate) 60 mg, Mn (as manganese sulphate) 115 mg, Se (as sodium selenite) 0.30 mg and I (as potassium iodide) 0.4 mg.*
[b]*Nutrient levels are calculated values.*

testing. Total antioxidative capacity (T-AOC) was determined by detecting the sample's ability to reduce Fe^{3+} to Fe^{2+} at 520 nm (Sun et al., 2015). Glutathione peroxidase (GSH-Px) activity were determined by measuring the oxidation rate of quantitative glutathione to oxidized glutathione at 412 nm (Fouad et al., 2016). Total superoxide dismutase (T-SOD) activity was determined by hydroxylamine method (Liu H. N. et al., 2014), catalase (CAT) activity was determined by ammonium molybdate method (Sun et al., 2015), and malondialdehyde (MDA) level was determined by thiobarbituric acid method (Yang et al., 2010). The above indicators were determined with the kits purchased from Nanjing Jiancheng Bioengineering Institute in strict accordance with the instructions.

Egg Nutrient Composition

During the sixth week of the study, 432 eggs were collected from each treatment (18 eggs/replicate) to analyze the nutrient composition of the egg. Among those, 144 eggs (36 eggs/treatment) were chosen for whole eggs chemical analysis, including moisture, crude protein (CP) (N × 6.25), ether extract (EE), and ash content (ASH), using methods developed by the AOAC (Association of Official Analytical Chemists). 144 eggs (36 eggs/treatment) were selected for the analysis of the amino acid content of whole eggs. The contents of 17 free amino acid (g/100 g) were determined by S433D amino acid automatic analyzer (Sykam, Germany) followed the method described by the regulations and requirements of determination of Amino Acids in foods (China, GB 5009.124-2016, China Food and Drug Administration, 2016). The content of Tryptophan in Freeze - dried Powder was individually determined by reversed-phase liquid chromatography (RP - HPLC). The remaining 144 eggs (36 eggs per treatment) were characterized for the biochemical parameters analysis in egg yolk. The total cholesterol content (mmol/gyolk) in egg yolk was measured by cholesterol oxidase method. The determination was carried out with the kit of Nanjing Jiancheng Institute of Biological Engineering in strict accordance with the instructions. The fatty acid composition of the yolk was analyzed using an Agilent 7890A gas chromatography (Agilent Technologies, Wilmington, United States) according to the regulations and requirements of Determination of National Food Safety Standard: Determination of Fatty Acids in Food (China, GB 5009.168-2016, China Food and Drug Administration, 2016).

Statistical Analysis

The data were tested by ANOVA for the control group and experiment groups with Statistical Packages for Social Science 18.0 (SPSS 18.0) software. Orthogonal polynomial contrasts were used to determine linear and quadratic responses of Lohmann commercial laying hens to different level ramie powder. For significant effects, means were compared by Duncan's multiple comparison test to determine specific differences between means. Statistical significance was assigned at $p < 0.05$. The p values between 0.05 and 0.10 were considered as having trends in difference.

RESULTS

Production Performance

The effect of dietary supplementation of ramie on the production performance of laying hens is presented in **Table 2**. No mortality was found during the 6 weeks experimental period. The dietary ramie supplementation had no effects on feed conversion ratio

TABLE 2 | Effects of dietary ramie supplementation on laying hen production performance.

Items	Control	Ramie supplementation concentration in diets			SEM	*p*-value		
		3%	6%	9%		ANOVA	Linear	Quadratic
Egg laying rate (%)	89.05	94.16	94.82	89.34	1.053	0.090	0.651	0.015
FCR	2.20	2.12	2.13	2.21	0.038	0.852	0.990	0.386
Egg weight (g)	56.51	56.59	56.91	56.91	0.139	0.640	0.225	0.913
Daily feed intake (g)	114.20	109.86	111.77	113.91	1.596	0.761	0.918	0.315
Egg mass per day (g)	51.97	51.94	52.41	51.48	0.585	0.979	0.828	0.803

[a,b]Means in the same row with different superscript letters indicate differences (p < 0.05); The p values between 0.05 and 0.10 were considered as having trends in difference. FCR, food conversion ratio; SEM, standard error of the mean.

TABLE 3 | Effects of dietary ramie supplementation on the egg quality of laying hens.

Items	Control	Ramie supplementation concentration in diets			SEM	*p*-value		
		3%	6%	9%		ANOVA	Linear	Quadratic
Egg weight (g per egg)	58.04	59.39	59.69	59.62	0.548	0.797	0.422	0.582
Eggshell strength (kgf)	4.58	4.62	4.61	4.56	0.049	0.976	0.936	0.665
Eggshell thickness (mm)	0.38	0.40	0.39	0.39	0.002	0.467	0.964	0.154
Haugh unit	75.83	76.18	76.84	77.57	0.587	0.767	0.303	0.882
Egg shape index	1.34	1.33	1.34	1.33	0.005	0.907	0.561	1.000
Yolk Index	0.36^{b}	0.36^{b}	0.37^{ab}	0.39^{a}	0.005	0.034	0.009	0.172
Yolk color	4.20^{b}	4.47^{b}	5.30^{a}	5.63^{a}	0.152	<0.001	<0.001	0.883
shell weight (g per egg)	5.75	5.68	5.72	5.80	0.084	0.970	0.810	0.683
yolk weight (g per egg)	15.84	16.17	16.54	16.33	0.196	0.681	0.332	0.522
Protein weight (g per egg)	36.71	36.97	38.41	37.49	0.463	0.638	0.493	0.438

[a,b]Means in the same row with different superscript letters indicate differences (p < 0.05). SEM, standard error of the mean.

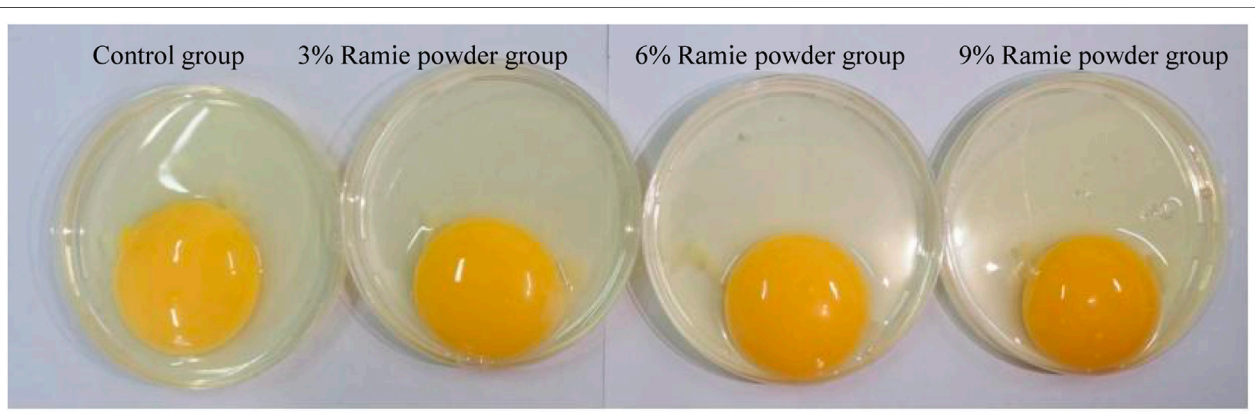

FIGURE 1 | Effect of diet on egg yolk color of laying hens (40 weeks of age). From left to right: control, 3, 6, 9% ramie supplementation diets in turn.

(FCR), egg weight, feed intake or the egg mass. There was an increasing trend in the average egg production in the ramie treated groups compared to the control throughout the whole experimental period (quadratic, $0.05 < p < 0.10$).

Egg Quality

As shown in **Table 3**, with the increase of dietary ramie content, egg yolk color gradually deepened, and ramie supplementation at 6%, 9% significantly increased the egg yolk color compared to the control (linear, $p < 0.001$; **Figure 1**). Ramie powder supplement of 9% increased the egg yolk index (linear, $p = 0.034$). None of the other egg quality traits (egg weight, egg strength, shell thickness, Haugh unit, shell weight, yolk weight, and protein weight) were affected by the dietary ramie supplementation ($p > 0.10$).

Yolk Antioxidation

Eggs from laying hens fed 3% ramie diet had significantly higher T-AOC in the yolk ($p < 0.05$) compared to the ones from the

TABLE 4 | Effects of dietary ramie supplementation on Yolk Antioxidation Indices.

Items	Control	Ramie supplementation concentration in diets			SEM	*p*-value		
		3%	6%	9%		ANOVA	Linear	Quadratic
T-AOC (U/ml)	12.11^{b}	15.98^{a}	13.85^{ab}	13.56^{ab}	0.490	0.033	0.565	0.024
T-SOD (U/g)	229.76	273.19	248.68	266.94	14.443	0.737	0.524	0.680
GSH-Px (U/g)	306.05	333.74	342.08	360.77	15.134	0.654	0.223	0.877
CAT (U/g)	30.43	31.86	36.76	35.95	1.273	0.227	0.065	0.655
MDA (nmol/g)	77.68^{a}	62.20^{b}	66.05^{b}	66.74^{b}	1.660	0.002	0.016	0.004

^{a,b}Means in the same row with different superscript letters indicate differences (p < 0.05).

T-AOC, total antioxidative capacity; T-SOD, total Superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; MDA, malondialdehyde; SEM, standard error of the mean.

TABLE 5 | Effects of dietary ramie supplementation on Nutritional Composition of Whole Egg and Total Cholesterol Content in Egg Yolk.

Items	Control	Ramie supplementation concentration in diets			SEM	*p*-value		
		3%	6%	9%		ANOVA	Linear	Quadratic
Moisture (%)	75.73	75.10	75.54	75.12	0.201	0.635	0.468	0.740
CP (%)	12.22	12.89	12.49	12.35	0.128	0.181	0.685	0.070
EE (%)	7.19	8.38	8.07	7.70	0.189	0.159	0.517	0.052
Ash (%)	0.78	0.73	0.74	0.77	0.009	0.290	0.965	0.091
T-CHO (mmol/gyolk)	5.95^{a}	5.22^{b}	5.01^{b}	5.36^{ab}	0.110	0.043	0.040	0.013

^{a,b}Means within a row with no common superscripts differ significantly (p < 0.05).

CP, crude protein; EE, ether extract; Ash, ash content; T-CHO, total cholesterol; SEM, standard error of the mean.

control group (**Table 4**). The antioxidative indices of the egg yolks was shown in **Table 4**. Ramie supplementation at 3% significantly increased the T-AOC level compared to the ones in the control groups ($p = 0.033$), and significant quadratic relation was nocited between them ($p = 0.024$). Moreover, the dietary supplementation with ramie significantly reduced the content of MDA in egg yolk ($p = 0.002$), and significant linear and quadratic relationships were found between the ramie concentration and MDA content ($p = 0.016$ and 0.004 respectively). However, not effect was noticed between the level of ramie on the contents of T-SOD, GSH-Px, or CAT ($p > 0.05$).

Nutritional Composition of the Eggs and Total Cholesterol Content of the Egg Yolk

The contents of total cholesterol in egg yolk was significantly decreased by 12.27%, 15.80% in 3 and 6% ramie treated groups compared to the control groups ($p = 0.043$), with significant linear and quadratic relationships ($p = 0.040$ and 0.013 respectively). But there were no differences in the content of moisture, CP, EE or Ash of eggs among the groups (**Table 5**).

Fatty Acids Composition of Egg Yolk

The fatty acids composition of the egg yolks was shown in **Table 6**. Compared to the control groups, 6% ramie supplement diets significantly increased the content of eicosadienoic acid ($p = 0.040$), ALA ($p = 0.033$), LA ($p = 0.004$), PUFA ($p = 0.002$), n-3 PUFA ($p = 0.003$), and EFA ($p = 0.004$) in egg yolks. Additionally, 6 and 9% ramie supplementation significantly increased the content of LA ($p = 0.004$), PUFA ($p = 0.002$), and EFA ($p = 0.004$) in the egg yolks compared to the ones in the control groups. Moreover, significant linear relationships were found between the levels of ramie supplementation and eicosadienoic acid ($p = 0.046$), LA ($p = 0.004$), PUFA ($p = 0.002$), and EFA ($p = 0.004$); and quadratic relationships were found between the levels of ramie supplementation and ALA ($p = 0.033$), LA ($p = 0.002$), PUFA ($p = 0.001$), n = 3 PUFA ($p = 0.001$), and EFA ($p = 0.002$).

Amino Acid Content of the Eggs

Eggs from hens fed ramie-supplemented diet exhibited a higher EAA/NEAA and EAA/TAA value compared to the ones in the control groups ($p = 0.037$ and 0.031, respectively). There was a tendency of increasing the content of EAA in whole eggs by ramie supplementation ($p = 0.055$). Additionally, as compared to the ones in the controls, 6% ramie supplementation significantly increased phenylalanine content ($p = 0.026$). Significant linear relationships were notice between the level of ramie supplementation and the contents of phenylalanine and EAA/TAA ($p = 0.019$ and 0.038, respectively).

DISCUSSION

Ramie leaves, which are commonly used for medicinal and edible purposes, are effective in reducing serum cholesterol and improving meat quality of farmed animals (Avci et al., 2006; Tang et al., 2021). However, there is only limited

TABLE 6 | Effects of dietary ramie supplementation on fatty acid composition in egg yolk (g/100 g FA).

Items	Control	Ramie supplementation concentration in diets			SEM	*p*-value		
		3%	6%	9%		ANOVA	Linear	Quadratic
C12:0	0.00	0.00	0.01	0.01	0.004	0.452	0.157	0.974
C14:0	0.33	0.34	0.33	0.35	0.009	0.867	0.652	0.733
C15:0	0.05	0.05	0.04	0.05	0.003	0.242	0.482	0.595
C16:0	25.38	24.77	24.40	25.09	0.331	0.781	0.612	0.404
C17:0	0.13	0.15	0.15	0.15	0.005	0.562	0.302	0.365
C18:0	8.68	8.29	9.02	8.79	0.171	0.609	0.579	0.782
C20:0	0.03	0.00	0.01	0.00	0.004	0.079	0.076	0.285
C22:0	0.05	0.03	0.03	0.02	0.007	0.291	0.075	0.919
C14:1	0.09	0.08	0.07	0.07	0.007	0.884	0.500	0.712
C16:1	3.54	3.26	2.72	3.12	0.287	0.812	0.484	0.616
C20:1	0.23	0.20	0.21	0.21	0.005	0.395	0.210	0.371
C18:1n-9t	0.14	0.12	0.14	0.13	0.004	0.414	0.640	0.976
C18:1n-9c (oleinic acid)	39.90	38.70	37.51	38.65	0.447	0.418	0.332	0.224
C20:2 (eicosadienoic acid)	0.16^{b}	0.17^{b}	0.22^{a}	0.18^{ab}	0.008	0.040	0.046	0.081
C22:6n-3 (DHA)	1.18	1.28	1.40	1.19	0.051	0.435	0.636	0.172
C18:3n-3 (ALA)	0.58^{b}	0.66^{b}	0.87^{a}	0.64^{b}	0.041	0.033	0.108	0.033
C18:3n-6 (GLA)	0.13	0.15	0.13	0.17	0.008	0.350	0.216	0.506
C18:2n-6c (LA)	13.57^{c}	19.24^{b}	22.17^{a}	18.44^{b}	1.196	0.004	0.004	0.002
C20:3n-6 (eicosatrienoic acid)	0.30	0.25	0.26	0.28	0.013	0.532	0.577	0.210
C20:4n-6 (AA)	2.75	3.02	2.80	3.00	0.060	0.322	0.371	0.657
SFA	34.64	33.63	33.99	34.45	0.278	0.612	0.754	0.238
MUFA	43.37	42.83	41.75	43.16	0.720	0.919	0.842	0.619
PUFA	18.75^{c}	24.78^{b}	28.10^{a}	24.03^{b}	1.286	0.002	0.002	0.001
UFA	64.55	66.43	66.61	66.44	0.382	0.148	0.076	0.135
n-6 PUFA	20.65	21.41	21.64	20.52	0.745	0.965	0.986	0.648
n-3 PUFA	1.58^{b}	1.94^{b}	2.57^{a}	1.82^{b}	0.117	0.003	0.078	0.002
n-6/n-3 PUFA	11.69	10.73	9.87	11.35	0.362	0.354	0.558	0.140
EFA	14.21^{c}	20.04^{b}	23.24^{a}	19.25^{b}	1.253	0.004	0.004	0.002

[a–c]Means in the same row with different superscript letters indicate differences (p < 0.05).

SEM, standard error of the mean; LA, linoleic acid; AA, arachidonic acid; ALA, α-linolenic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; EFA, essential fatty acid.

[1]SFA level were calculated as C15:0 + C16:0 + C17:0 + C18:0.

[2]MUFA levels were calculated as C16:1 + C17:1 + C18:1n-9 + C20:1n-9 + C22:1n-9 + C24:1n-9.

[3]PUFA levels were calculated as C18:2n-6 + C18:3n-6 + C18:3n-3 + C20:2 + C20:3n-6 + C20:3n-3 + C20:4n-6 + C20:5n-3 + C22:6n-3.

[4]n-6 was calculated as C18:2n-6 + C18:3n-6 + C20:3n-6 + C20:4n-6.

[5]n-3 was calculated as C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:6n-3.

[6]EFA was calculated as ALA + LA.

research on the effects of dietary ramie supplementation on laying hens. Thus, the aim of this study is to investigate whether the production performance, egg quality traits, yolk antioxidation indices and egg nutrients composition of laying hens are affected by the dietary supplementation of ramie. Ramie had no adverse effect on the overall production performance of hens in the present study. Many researches showed that the egg production increased in response to the nettle supplementation, such as *Urtica dioica*, *Urtica cannabina* and *Urtica fissa* (Loetscher et al., 2013a; Zhang et al., 2020; Liu et al., 2010). Ramie as a perennial dicotyledon of the Urticaceae family (Wei et al., 2014), might possess the same effect as nettle. Previous studies reported that egg production remained unchanged (Wang and Jie, 2012) or even tended to increase (Luo et al., 1989) with the supplement of ramie. In line with these studies, the supplementation of 3 and 6% ramie powder tended to increase the egg production in our study. Ramie is rich in cellulose, flavonoids compounds, polyphenol compounds, vitamin C, and minerals. It might partially because of the antioxidant properties of ramie contribute to the improved production performance. Recent studies showed that adding flavonoids to the diet can improve the performance of laying hens by improving the body's antioxidant capacity, reducing the occurrence of oxidative stress and promoting the absorption of nutrients in the intestine of laying hens (Brisibe et al., 2008; Galal et al., 2008; Seven, 2008; Liu L. L. et al., 2014; Zhu et al., 2021).

Yolk color is an important quality trait of eggs which is extremely critical to consumers. In our study, the yolk color was enhanced significantly by adding a certain amount (6–9%) of ramie. Similar results were also observed that yolk color gradually deepened as the addition levels of *urtica dioica* or *urtica cannabina* in diets (Like *Boehmeria nivea*, they are Urtica plants) (Loetscher et al., 2013a; zhang et al., 2020). *U. dioica* was found to be equally as effective as synthetic

pigmentation. It was shown that the inclusion of *U. dioica* in food increases the enrichment and bioaccessibility of lutein and *β*-carotene (provitamin A carotenoids) at the duodenum digestion stage (Kregiel et al., 2018). Lutein was considered an important natural pigment for ameliorating egg yolk and broiler skin color, which could be deposited in egg yolks (Hencken, 1992; Loetscher et al., 2013a; Loetscher et al., 2013b). Besides, nettle was reported to be rich in yellow-colored xanthophylls, with lutein (184 μg/g) being the predominant compound, followed by β-carotene (6.7 μg/g) (Marchetti et al., 2018). And xanthophylls were found to be absorbed in the digestive tract and deposited in subcutaneous fat and yolk leading to higher yolk color scores (Wen et al., 2019). Therefore, the higher yolk color observed in ramie (6–9%) treated groups in the present study might be due to the high levels of effective polar xanthophylls, such as lutein. Additionally, supplemental antioxidants to layer feed could also improve the yolk color (Yuan et al., 2016). Neohesperidin dihydrochalcone is a hydrogenated flavonoid derivative and has antioxidant, antimicrobial and anti-inflammatory properties in animals (Yeomans et al., 2007; Marti et al., 2008). It could improve the egg yolk color because it reduced the effects of lipid peroxides and free radicals on organisms (Zhu et al., 2021). It could also be possible that ramie (rich in flavonoid constituent) prevented the lutein from being oxidized and therefore increased the pigment deposition (Wang et al., 2019).

Egg yolk index can be used to measure the egg yolk nutrient concentration and egg freshness, and the higher egg yolk index means better egg processing grade and edible value. Previous studies showed that adding 2% nettle powder to the diet of laying hens could significantly increase the yolk index (Mansoub, 2011). Our study showed that egg yolk index was significantly increased in the 9% ramie group. The increase in egg yolk index might be related to the stability of yellow pigment in the lipid molecules located in egg yolk membrane, as ramie prevent the occurrence of the oxidative stress.

Nettle could also reduce lipid peroxidation (serum MDA content decreased) and liver enzyme activity in CCl4-treated rats, and improved the activity of antioxidant defense system (Kanter et al., 2003). Our study showed that ramie supplmentation significantly reduced MDA content in egg yolk. In addition, supplementation of 3% ramie powder significantly increased the T-AOC capacity of egg yolk. These indicated that by adding ramie powder, the antioxidant capacity of egg yolk was improved.

Total cholesterol content in eggs can be reduced by adding natural plants, cellulose and trace elements (Zhang et al., 2010). The addition of 15% *Urtica cannabina* in the diet of laying hens could significantly reduce the cholesterol content in yolk, without affecting the CP and EE content in eggs (Zhang et al., 2020). The addition of 6% *Urtica dioica* could significantly reduce the cholesterol content in quail egg yolk (Moula et al., 2019). Our study showed that ramie supplementation had no significant effect on the nutritional composition of whole eggs, but the total cholesterol concentration in yolk with 3 and 6% ramie treated groups was significantly decreased. This might be due to the phytosterol components in ramie which could reduce the absorption of cholesterol in intestinal tract, thereby lowering cholesterol levels in the blood, and subsequently, in animal products (Avci et al., 2006).

N-3PUFA, including ALA, EPA and DHA, has been recognized for its beneficial effects on the growth, health and immune function of humans and animals (Lee et al., 2009; Chen et al., 2014; Zhang et al., 2020). Numerous fatty acid desaturases played key roles in synthesizing PUFA. Several desaturases are absent in animals and humans, such as delta-12 and delta-15 desaturases (Lee et al., 2016). Thus, ALA must be obtained from the diet and be converted into EPA and DHA by delta-6 desaturase catalyzed dehydrogenation and the addition of two carbons by an elongase (Fraeye et al., 2012). Dietary supplementation with fresh nettle reduced the ratio of n-6/n-3 and increased the total n-3PUFA in yolk (Zhang et al., 2020). Similarly, it was reported that dietary supplementation with fresh nettle increased the contents of linoleic acid and linolenic acid, improved the proportion of PUFA and n-3PUFA and reduced the ratio of n-6/n-3 in the breast meat of broiler (Stojcic et al., 2016). Moreover, it was showed that the fat metabolism of pigs was modulated by 500 mg/kg nettle extract in diet, that the MUFA was decreased and PUFA was increased in muscle fat (Szewczyk et al., 2006). The composition of fatty acids stored in monogastric animals indicated the possibility that the lipid and fatty acid composition of poultry eggs could be altered by the diet (Kouassi et al., 2020). In the present study, 6% ramie significantly increased the content of icosaic acid and ALA in egg yolk, and significantly increased the content of n-3PUFA. In addition, the ramie supplementation was associated with a significant elevation in the LA, PUFA and EFA proportion compared to the control diet. This might be related to the rich sources of essential fatty acids in ramie leaves. ALA is the main fatty acid accounting for 40.7% of the fatty acids in mature leaves (Guil-Guerrero et al., 2003). Hence, eggs might receive the high content of n-3PUFA when the laying hens were fed by diets rich in n-3PUFA (Wen et al., 2019; Grõcevi´c et al., 2019). Another possible explanation for the higher total n-3PUFA content in the egg yolks of the groups treated with ramie could be because of the protective function of the antioxidative compounds such as lutein, tocopherol, flavonoids and phenolic compounds. Nettle is an abundant source of lutein, which has been considered to be an important natural pigment for improving egg yolk and broiler skin color (Loetscher et al., 2013b; Zhang et al., 2020). Lutein and flavonoids compounds are potent antioxidants due to their free radical quenching activities (Irgin et al., 2016; Kamoshita et al., 2016). In previous study, the addition of marigold powder (rich in lutein) to the laying hens' feed significantly increased egg lutein content, which helped to preserved a higher content of DHA in the yolks. Adding *Urtica cannabina* to the feed of laying hens (or due to the presence of antioxidant

TABLE 7 | Effects of dietary ramie supplementation on Amino Acid Content in Whole Egg (g/100 g DW).

Items	Ingredient	Control	Ramie supplementation concentration in diets			SEM	*p*-value		
			3%	6%	9%		ANOVA	Linear	Quadratic
nonessential amino acid	Asp	1.04	1.06	1.07	1.05	0.008	0.508	0.541	0.189
	Tyr	0.38	0.40	0.41	0.40	0.006	0.647	0.402	0.347
	Ser	0.52	0.54	0.55	0.52	0.006	0.179	0.652	0.040
	Glu	0.94	0.94	0.98	0.95	0.008	0.262	0.299	0.425
	Gly	0.42	0.41	0.43	0.42	0.005	0.629	0.758	0.930
	Ala	0.65	0.65	0.67	0.65	0.007	0.463	0.535	0.574
	Cys	0.44	0.47	0.46	0.45	0.006	0.397	0.513	0.193
	Arg	1.25	1.21	1.14	1.17	0.026	0.404	0.151	0.524
	Pro	0.53	0.56	0.59	0.49	0.018	0.302	0.632	0.094
	His	0.47	0.49	0.47	0.45	0.007	0.263	0.171	0.205
essential amino acid	Met	0.24	0.29	0.27	0.28	0.009	0.406	0.293	0.292
	Val	0.47	0.50	0.49	0.49	0.008	0.409	0.287	0.256
	Lys	0.78	0.82	0.80	0.80	0.009	0.449	0.605	0.249
	Ile	0.40	0.40	0.39	0.40	0.007	0.981	0.993	0.965
	Phe	0.34^{b}	0.36^{ab}	0.37^{a}	0.36^{ab}	0.005	0.026	0.019	0.050
	Leu	0.67	0.69	0.70	0.70	0.008	0.648	0.256	0.632
	Trp	0.20	0.23	0.23	0.24	0.009	0.569	0.193	0.722
	Thr	0.43	0.45	0.45	0.43	0.005	0.210	0.394	0.056
	EAA	3.42	3.72	3.68	3.60	0.045	0.055	0.152	0.023
	NEAA	6.64	6.72	6.75	6.55	0.048	0.507	0.719	0.166
	FAA	4.27	4.27	4.29	4.28	0.150	0.993	0.831	0.946
	TAA	10.05	10.44	10.43	10.15	0.082	0.223	0.608	0.047
	EAA/NEAA	0.51^{b}	0.56^{a}	0.54^{a}	0.55^{a}	0.006	0.037	0.061	0.061
	EAA/TAA	33.97^{b}	35.66^{a}	35.26^{a}	35.41^{a}	0.234	0.031	0.038	0.066
	FAA/TAA	41.56	41.78	41.52	41.75	0.716	0.968	0.864	0.954

[a,b]Means within a row with no common superscripts differ significantly (p < 0.05).

Asp, aspartic acid; Tyr, tryptophan; Ser, serine; Glu, glutamic acid; Gly, glycine; Ala, alanine; Cys, cysteine; Arg, arginine; Pro, proline; His, histidine; Met, methionine; Val, valine; Lys, lysine; Ile, I-isoleucine; Phe, phenylalanine; Leu, leucine; Trp, tryptophan; Thr, threonin; EAA, essential amino acid; NEAA, nonessential amino acid; FAA, umami amino acids; TAA, total amino acids.

compounds) can maintain high DHA content in egg yolk (Zhang et al., 2020). Based on these results, we assume that the richness of antioxidants in ramie had an impact on the conservation of PUFA in the lipids of the egg yolks by protecting them from oxidation and damage, thereby maintaining a higher level of PUFA in the ramie group. Collectively, these findings indicated that, as an important n-3 PUFA source, ramie contributed to the excessive production of n-3 PUFA in the eggs.

As one of the main animal products, eggs are welcome in the market as excellent protein source (Perić et al., 2011). The nutritional value of protein mainly depended on the variety and content of the amino acids. Sufficient content of essential amino acid and balance amino acid composition played an extremely important role in the nutritional value and edible flavor of food (Wu et al., 2013). Many studies showed that the nutrition and flavor of eggs were affected by factors such as dietary nutrients and feeding methods (Perić et al., 2011; Bashir et al., 2015; Dong et al., 2018; Bagheri et al., 2019). The tender stems and leaves of ramie contained high level of crude protein. After Boer goats feeding with silage of ramie tender stems and leaves, the content of umami amino acids and proline in the mutton were increased, which was beneficial in the umami taste, the protein bioavailability, and the quality of mutton (Gao et al., 2016). The addition of silage ramie as a source of roughage to Simmental beef cattle diets not only maintained the quality and nutritional value, but also improved the composition of flavor amino acids (umami amino acids, bitter amino acids, and sour amino acids) in beef (Yang et al., 2017). The results of the present experiment show that supplementation of ramie had no effect on the content of umami amino acids in eggs. It was different from previous researches, and the possible explanations might be the different processing methods of ramie before feeding (without silage), and the difference in experiment animals, who possessed different digestion and absorption capabilities. It was reported that 9% canola meal supplementation in diet increased the total amino acid content in eggs of Roman laying hens (Wang et al., 2013). As in the present study, supplementation of 6% ramie significantly increased phenylalanine content in the whole eggs, and different levels of ramie significantly increased EAA/TAA and EAA/NEAA in whole eggs. The trend of increased content of essential amino acids in the whole eggs in ramie treated groups might be because the amino acid content in ramie leaves is quite rich (EAA/TAA value was greater than 40%, and EAA/NEAA value was greater than 69%) (Sun et al., 2013). After the essential amino acids in ramie leaves being digested and absorbed by laying hens, they were deposited in eggs. It was also possible that ramie contains a variety of active substances, which improves intestinal health and nutrient absorption, thereby increased the content of essential amino

acids in eggs (Graf, 1992; Nardini et al., 1995; Van Acker et al., 2000; Miyake et al., 2003; AOAC International, 2005).

CONCLUSION

In summary, with a dietary supplement of ramie, no negative effects on egg production and egg quality in laying hens were found, but increase of the antioxidant capacity of the egg yolk and the nutritional content of the whole egg were achieved. The yellow color of egg yolk deepened as the increase of ramie content, and 9% ramie supplements contributed the darkest egg yolk. Compared with other groups of laying hens, supplementation of 6% ramie in the diet significantly reduced the total cholesterol content in the egg yolk; and increased the content of n-3 polyunsaturated fatty acids and essential amino acids in the whole eggs. However, how the biologically active ingredients in ramie affect the content of unsaturated fatty acids in eggs needs further research. These findings support the potential application of ramie as a dietary supplementation for laying hens (**Table 7**).

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

All the experiment procedures were approved by the Animal Care Committee of the Institute of Bast Fiber Crops, Chinese Academic of Agricultural Sciences, Changsha, China.

AUTHOR CONTRIBUTIONS

The research was designed and conducted by QL, S-YZ, H-JQ, and Z-YF. The animal experiment was conducted by XW, S-MP, YL, and G-YD. The detection and analysis works were conducted by SL, H-HZ, Y-MW, C-JL, and Y-ZW. XW, T-ML, and Y-HL prepared the manuscript draft. All authors participated in the discussion and editing of the manuscript.

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Effects of Feeding Fermented *Medicago sativa* (Plus Soybean and DDGS) on Growth Performance, Blood Profiles, Gut Health, and Carcass Characteristics of Lande (Meat) Geese

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The objective was to investigate the effects of alfalfa (*Medicago sativa Linn*)-mixed silage fermentation material (AMSFM) on various aspects of growth, function, and carcass characteristics of Lande (meat) geese. Based on a previous study, we used the following AMSFM: 80% Alfalfa +10% soybean meal +10% DDGS ensiled for 45 days. Lande geese, n = 264, 77 days of age, were randomly allocated into four groups with six replicates in each group. Control geese were fed a basal diet, whereas experimental groups were fed a basal diet supplemented with 6, 12, or 24% AMSFM. The experiment lasted 21 days. The AMSFM promoted some aspects of growth, with increase ($p < 0.05$) in leg muscle rate, lean meat rate, muscle protein content, and total energy content of leg muscle plus concurrent decreases ($p < 0.05$) in crude fat content and abdominal fat rate in chest muscle. In addition, AMSFM increased ($p < 0.05$) glutathione content in chest and leg muscles and serum superoxide dismutase activity, and it reduced ($p < 0.05$) muscle malondialdehyde content and serum concentrations of triglycerides, total cholesterol, urea, and aspartate aminotransferase, consistent with good liver and kidney function. Moreover, AMSFM improved ($p < 0.05$) ileum morphology. In conclusion, the optimal supplemented rate of AMSFM in the meat geese diet (12%) improved immunity and antioxidant status and enhanced growth performance and carcass characteristics of meat geese.

Keywords: alfalfa, mixed silage, serum biochemical indices, goose, antioxidative capacity, intestinal development

1 INTRODUCTION

Poultry production is increasing, but traditional protein sources, including soybean and fish meal, are becoming less available and more expensive, prompting the need for alternatives. Alfalfa (*Medicago sativa L.*) is termed the "king of forage" due to its high yield and that it contains a favorable nutrient content and various active substances (Chen et al., 2020; Ni et al., 2020). Ensiling alfalfa simplifies its preservation and reduces feed costs (Chen et al., 2013) and minimizes competition with humans for food sources (Wang and Yu, 2020). In addition, alfalfa-mixed silage is easy to produce and is a very good feed source (Plaizier, 2004; Wang et al., 2019).

TABLE 1 | Nutrient composition of alfalfa-mixed silage fermentation material (AMSFM).

Item	Content in AMSFM
DM (% FW)	35.41
CP (%DM)	28.44
NDF (%DM)	28.01
ADF (%DM)	16.84
CF (%DM)	17.71
ASH (%DM)	6.06
EE (%DM)	5.28
GE (MJ/kg)	17.17
Ca (%)	0.61
P (%)	0.17

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; CF, crude fiber; Ash, Ash content; EE, ether extract; GE, gross energy; Ca, calcium; P, phosphorus.

Meat geese are efficient herbivores, capable of consuming more crude fiber and less grain and generating high economic value for meat, liver, and feathers (Gao et al., 2016). However, there are few reports on feeding alfalfa-mixed silage to meat geese. The objective was to investigate the effects of alfalfa (*Medicago sativa Linn*)-mixed silage fermentation on various aspects of growth, function, and carcass characteristics of Lande (meat) geese.

2 MATERIALS AND METHODS

2.1 Experimental Materials

Based on previous studies, the alfalfa-mixed silage fermentation material (AMSFM) used was 80% alfalfa +10% soybean meal +10% DDGS. Alfalfa was collected from the alfalfa planting base of Hunan Deren Animal Husbandry Technology Co., Ltd. It was cut on sunny days at the budding stage, processed to a straw length of <1 cm, mixed with other ingredients, and seal-silaged for 45 days. The nutritional analysis of AMSFM is in **Table 1**.

2.2 Experimental Design

This study was conducted at the Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences. Lande geese (n = 264), 77 days old, were randomly allocated into four groups. Each group had six replicates (11 geese per replicate). Control geese (CONT) were fed a basal diet, whereas experimental groups were fed a basal diet supplemented with 6, 12, or 24% AMSFM (TRT6, TRT12, and TRT24, respectively), with *ad lib* access to feed and water. The animal test was conducted at the Shiji Lake Animal Test Base (112.38 ^{0}E, 28.82 ^{0}N) in Yuanjiang, Institute of Hemp, Chinese Academy of Agricultural Sciences. Durations of the preexperimental and trial periods were 7 and 21 days, respectively, with geese maintained under conditions with good ventilation and natural light. Immunization and disinfection were done according to standard procedures. Diets (**Table 2**) were prepared with reference to National Research Council (1994) requirements for meat geese.

2.3 Sample Collection

At the end of the experiment (105 days of age), the total weight of geese in each replicate was determined and recorded. On the 21st day of the experiment, geese were fasted for 6 h, and one goose was randomly selected from each replicate (a total of 6 geese in each treatment group) and weighed. Blood (5 ml) was collected by venipuncture of a wing vein, using a 10-ml syringe, put into centrifuge tubes, and after 30 min, centrifuged at ? x g for 10 min. Thereafter, serum was separated and stored at −20°C. Then, selected geese were killed by cutting the carotid artery and dissected. Chest and leg muscles were excised to determine meat nutrient quality and antioxidant indexes. The duodenum, jejunum, and ileum were identified and excised and a 1.5-cm portion of the middle of each intestinal segment was recovered, washed with 0.9% normal saline, and placed in a 50 ml test tubes containing formaldehyde.

TABLE 2 | Composition and nutrient levels of basal diets for meat geese (air-dry basis, %). Control geese (CONT) were fed a basal diet, whereas TRT6, TRT12, and TRT24 were fed a basal diet supplemented with 6, 12, or 24% alfalfa-mixed silage fermented material, respectively.

Item	CONT	TRT6	TRT12	TRT24
Ingredient			—	
Corn	45.70	45.95	44.84	40.00
Soybean meal	18.34	20.17	21.57	24.05
Defatted rice bran	15.00	10.00	6.90	0.80
Wheat bran	15.00	10.00	6.90	0.80
Rice husk powder	0.00	0.90	1.45	2.45
AFSM	0.00	6.00	12.00	24.00
Soybean oil	2.70	2.60	2.91	4.35
limestone	1.00	0.90	0.90	0.90
$CaHPO_4{\cdot}2H_2O$	0.95	1.13	1.13	1.22
98.5% methionine	0.00	0.00	0.00	0.03
78.5% lysine	0.01	0.00	0.00	0.00
NaCl	0.30	0.30	0.30	0.30
1% premix[1)]	1.00	1.00	1.00	1.00
Total	100	100	100	100
Nutrient levels[2)]			—	
ME (Mcal/kg)	2.70	2.70	2.70	2.70
CP(%DM)	15.62	15.63	15.62	15.62
CF(%DM)	4.69	4.70	4.68	4.69
Ca (%)	0.66	0.66	0.65	0.66
Available P (%)	0.36	0.37	0.36	0.36
Lys	0.81	0.81	0.82	0.83
Met	0.26	0.26	0.26	0.27
Met + Cys	0.55	0.54	0.53	0.54

The premix provided the following (per kilogram of complete diet) microelements: VA, 12000 IU; VD1 2500 IU; VE, 20 mg; VK3 3 mg; VB1 3 mg; VB2 8 mg; VB5 7 mg; VB12 0.03 mg; D-pantothenic acid 20 mg; nicotinic acid 50 mg; biotin 0.1 mg; folic acid 1.5 mg; Cu (as copper sulfate) 9 mg; Zn (as zinc sulfate) 110 mg; Fe (as ferrous sulfate) 100 mg; Mn (as managanese sulfate) 100 mg; Se (as sodium selenite) 0.16 mg; and I (as potassium iodide) 0.6 mg.

Nutrient levels are calculated values.

AFSM, alfalfa-mixed silage fermentation material.

2.4 Measurements and Methods

2.4.1 Growth Performance

The body weight of experimental geese was determined on Days 1 and 21 of the formal experiment. Throughout the experimental period, delivered and residual feed were weighed and recorded daily. Growth, feed intake, and feed efficiency were determined as follows:

$$\text{Average daily feed intake}(\text{g}) = \frac{\text{total feed intake}}{\text{test days}},$$

$$\text{Average daily gain}(\text{g}) = \frac{\text{final weight} - \text{initial weight}}{\text{test days}},$$

$$\text{Feed weight ratio} = \frac{\text{average daily feed intake}}{\text{average daily gain}}.$$

2.4.2 Carcass Characteristics

Another goose was selected from each replicate to determine post-slaughter status. The goose was weighed, slaughtered, and feathers removed. Then, we determined the following weights: slaughter, semi-clean bore, full clean bore, abdominal fat, the chest muscle, the leg muscle, the spleen, the bursa, the thymus, the liver, the muscular stomach, the glandular stomach, and viscera. We used the following formulas:

$$\text{Slaughter rate}(\%) = \frac{\text{weight after slaughter}}{\text{weight before slaughter}} \times \mathbf{100\%},$$

$$\text{Half clean bore rate}\ (\%) = \frac{\text{half clean bore weight}}{\text{weight before slaughter}} \times \mathbf{100\%},$$

$$\text{Full clean bore rate}\ (\%) = \frac{\text{weight of both pectoral muscles}}{\text{total net bore weight} * 1000} \times \mathbf{100\%},$$

$$\text{Pectoral muscle rate}\ (\%) = \frac{\text{full clean bore weight}}{\text{weight before slaughter}} \times \mathbf{100\%},$$

$$\text{Leg muscle rate}\ (\%) = \frac{\text{weight of both leg muscles}}{\text{full clean bore weight}\ \times 1000} \times \mathbf{100\%},$$

$$\text{Lean meat rate}\ (\%) = \frac{\text{chest muscle weight}\ + \text{leg muscle weight}}{\text{full clean bore weight}\ \times 1000} \times \mathbf{100\%},$$

$$\text{Organ index}(\text{g/kg}) = \frac{\text{fresh weight of internal organs}}{\text{weight before slaughter}} \times \mathbf{100\%},$$

$$\text{Abdominal fat rate}\ (\%) = \frac{\text{abdominal fat}\ +\ \text{muscle and gastric peripheral fat}}{\text{total net bore weight}\ \times 1000} \times \mathbf{100\%}.$$

2.4.3 Muscle Characteristics

Portions (~15 g) of pectoral and leg muscles were excised from each sample goose, processed to a constant weight with a freeze-drying machine, and dry matter contents measured. Crude protein (CP) was determined by the Kjeldahl method, crude fat was extracted by the Soxhlet method, and crude ash was determined by a high-temperature (550°C) burning method. Total energy contents were determined by a 5E calorimeter.

2.4.4 Antioxidant Indexes

Portions (0.15–0.2 g) of chest and leg muscle tissue samples were excised and placed into 2 ml homogenization tubes, with 9 times the volume of precooled normal saline and sterilized homogenization beads added. The samples were then centrifuged at 3000 x g for 15 min at 4°C and tissue supernatants collected. Total antioxidant capacities (T-AOC), malondialdehyde (MDA) and glutathione (GSH) contents, catalase (CAT) and superoxide dismutase (SOD) activities of chest and leg muscles as well as serum T-AOC, MDA content, GSH content, CAT activity, SOD activity, and glutathione peroxidase (GSH-Px) activity were measured.

2.4.5 Serum Indexes

The Mairui bs-420 Automatic Biochemical Instrument was used to determine serum physiological and biochemical indexes, including serum concentrations of glucose (Glu), triglyceride (TG), total cholesterol (CHO), urea (urea), uric acid (UA), albumin (ALB), total protein (TP), globulin (GLB) and creatinine as well as the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

2.4.6 Intestinal Morphology

After fixation, intestinal tissue samples were dehydrated through an alcohol series, embedded in paraffin, sectioned (5 μm), stained with H&E, and observed under an optical microscope. Five intact villi and crypts were selected from each slice in independent visual fields, and the thickness of each segment, from the top of the villi to the opening of the crypt was measured and recorded as villi height (VH). Furthermore, the distance from the recess opening to the recess base was recorded as the recess depth (CD). The ratio of VH to CD was recorded as V/C.

2.5 Data Analyses

Data were analyzed with ANOVA, with LSD used to locate differences. All analyses were done with SPS 19.00, $p < 0.05$ was considered significant, and results were reported as mean ± SD.

3 RESULTS

3.1 Growth Performance

There were no differences ($p > 0.05$) among groups for final weight, ADG, ADFI, or F/G (**Table 3**).

3.2 Carcass Characteristics

The leg muscle ratio was lower ($p = 0.010$) in CONT compared to the other three groups (**Table 4**). The abdominal fat ratio in TRT24 was lower $p = 0.012$) than that in TRT6 or CONT and the lean meat ratio in TRT24 exceeded ($p < 0.05$) that of the CONT.

3.3 Muscle Characteristics

The content of DM in chest muscle was lower ($p = 0.049$) in TRT24 than in CONT (**Table 5**). The content of CP in chest muscle was higher ($p = 0.039$) in TRT12 than in CONT. The contents of EE in the chest muscle of CONT were higher ($p = 0.003$) than in the other three groups. In leg muscle, DM content was higher ($p = 0.041$) in TRT12 than in CONT or TRT24, CP content was greater ($p = 0.012$) in TRT12 or TRT24 than in CONT, and GE content was higher ($p = 0.023$) in TRT12 compared to CONT or TRT24.

3.4 Serum Indexes

Serum concentrations of triglycerides and urea were highest in CONT ($p = 0.011$ and $p = 0.003$, respectively), whereas

TABLE 3 | Effects of alfalfa-mixed silage fermentation material (AMSFM) on growth performance of meat geese. Control geese (CONT) were fed a basal diet, whereas TRT6, TRT12, and TRT24 were fed a basal diet supplemented with 6, 12, or 24% AMSFM, respectively.

Team	CONT	TRT6	TRT6	TRT24	*p value*
Initial weight (kg)	3.81 ± 0.02	3.82 ± 0.01	3.82 ± 0.01	3.81 ± 0.02	0.313
Final weight (kg)	4.06 ± 0.13	4.21 ± 0.11	4.09 ± 0.32	4.08 ± 0.21	0.717
ADG (g/d)	14.43 ± 2.90	18.38 ± 4.14	17.30 ± 5.16	17.52 ± 5.27	0.624
ADFI (g/d)	182.29 ± 14.40	182.26 ± 20.51	178.86 ± 9.93	174.63 ± 21.01	0.845
F/G	13.7 ± 3.25	11.39 ± 2.58	10.93 ± 2.27	10.42 ± 1.64	0.352

Values in the table are mean ± SD (n = 11).
ADG, average daily gain; ADFI, average daily feed intake; F/G, feed weight ratio, ADFI/ADG.

TABLE 4 | Effects of alfalfa-mixed silage fermentation material (AMSFM) on carcass characteristics of meat geese. Control geese (CONT) were fed a basal diet, whereas TRT6, TRT12, and TRT24 were fed a basal diet supplemented with 6, 12, or 24% AMSFM, respectively.

	CONT	TRT6	TRT12	TRT24	*p value*
Carcass ratio (%)	88.72 ± 3.02	89.79 ± 4.65	89.22 ± 11.01	89.22 ± 1.58	0.993
Semi-eviscerated ratio (%)	79.69 ± 7.33	82.53 ± 4.68	81.15 ± 0.88	80.48 ± 3.39	0.758
Eviscerated ratio (%)	67.41 ± 2.85	70.90 ± 4.39	67.55 ± 3.10	67.33 ± 3.32	0.269
Chest muscle ratio (%)	13.12 ± 1.40	13.89 ± 1.68	13.93 ± 1.02	14.91 ± 2.10	0.374
Leg muscle ratio (%)	10.61 ± 1.03^{b}	12.26 ± 0.84^{a}	12.31 ± 1.44^{a}	13.19 ± 0.64^{a}	0.010
Abdominal fat ratio (%)	3.83 ± 0.62^{a}	3.75 ± 0.58^{a}	3.30 ± 0.58^{ab}	2.65 ± 0.50^{b}	0.012
Lean meat ratio (%)	23.73 ± 2.12^{b}	26.10 ± 1.23^{ab}	26.19 ± 1.52^{ab}	28.31 ± 2.51^{a}	0.018

Values in the table are mean ± SD (n = 11).
^{a,b}Pairs of means with the same superscript letter within the same row are not significantly different at least at the p > 0.05 level.

TABLE 5 | Effects of alfalfa-mixed silage fermentation material (AMSFM) on muscle characteristics of meat geese. Control geese (CONT) were fed a basal diet, whereas TRT6, TRT12, and TRT24 were fed a basal diet supplemented with 6, 12, or 24% AMSFM, respectively.

Item		CONT	TRT6	TRT12	TRT24	*p value*
Chest muscle	**DM (%FW)**	25.06 ± 0.98^{a}	24.28 ± 1.04^{ab}	24.48 ± 0.55^{ab}	23.67 ± 0.42^{b}	0.049
	CP (%DM)	80.64 ± 2.80^{b}	85.64 ± 1.51^{ab}	92.33 ± 8.72^{a}	86.85 ± 4.81^{ab}	0.039
	EE (%DM)	13.22 ± 1.83^{a}	10.29 ± 1.74^{b}	10.54 ± 1.60^{b}	8.68 ± 1.11^{b}	0.003
	ASH (%DM)	1.63 ± 0.48	2.04 ± 0.24	1.92 ± 0.08	2.07 ± 0.15	0.693
	GE (MJ/kg)	21.70 ± 0.79	21.81 ± 0.70	21.96 ± 0.32	21.96 ± 0.76	0.889
Leg muscle	**DM (%FW)**	24.11 ± 1.57^{b}	25.97 ± 2.29^{ab}	27.14 ± 1.96^{a}	24.70 ± 1.29^{b}	0.041
	CP (%DM)	74.26 ± 2.23^{b}	76.98 ± 2.39^{ab}	79.83 ± 3.47^{a}	80.76 ± 3.41^{a}	0.012
	EE (%DM)	18.19 ± 6.12	17.83 ± 4.62	17.77 ± 3.56	15.88 ± 5.98	0.890
	ASH (%DM)	1.16 ± 0.64	1.39 ± 0.47	1.13 ± 0.23	0.72 ± 0.10	0.465
	GE (MJ/kg)	23.26 ± 0.51^{b}	24.20 ± 1.01^{ab}	25.37 ± 1.13^{a}	23.99 ± 1.22^{b}	0.023

Values in the table are mean ± SD (n = 11).
^{a,b}Pairs of means with the same superscript letter within the same row are not significantly different at least at the p >0.05 level.
DM, dry matter; CP, crude protein; EE, ether extract; Ash, Ash content; GE, gross energy.

serum AST activity was highest (p = 0.005) in CONT (**Table 6**).

3.5 Muscle Antioxidant Status

In the chest muscle, the MDA concentration was higher (p = 0.014) in CONT versus TRT6 or TRT12, GSH was lowest (p < 0.010) in CONT, and CAT was highest (p = 0.023) in TRT6 and TRT12 (**Table 7**). In the leg muscle, MDA was lowest (p = 0.040) in TRT6 and TRT12 and GSH was higher (p = 0.022) in TRT12 and TRT24 than in CONT.

3.6 Serum Antioxidant Status

Regarding serum antioxidant capacity, the SOD activity was greater (p = 0.047) in TRT6 and TRT12 than in CONT (**Table 8**).

3.7 Small Intestine Histomorphological Indexes

Effects of AMFSM on small intestine histology of meat geese are shown in **Table 9** and **Figures 1–3**. In the ileum, TRT12 had the least (p = 0.022) CD and the highest (p = 0.038) V/C ratio.

TABLE 6 | Effects of alfalfa-mixed silage fermentation material (AMSFM) on serum biochemistry of meat geese. Control geese (CONT) were fed a basal diet, whereas TRT6, TRT12, and TRT24 were fed a basal diet supplemented with 6, 12, or 24% AMSFM, respectively.

Item	CONT	TRT6	TRT12	TRT24	*p value*
GLU (mmol/L)	9.09 ± 1.10	9.21 ± 1.06	8.95 ± 1.36	9.72 ± 0.72	0.766
TG (mmol/L)	1.33 ± 0.32^{a}	0.87 ± 0.26^{b}	0.78 ± 0.02^{b}	0.72 ± 0.13^{b}	0.011
CHO (mmol/L)	5.79 ± 1.28	5.09 ± 0.44	5.12 ± 0.58	5.52 ± 1.38	0.720
UREA (mmol/L)	2.11 ± 0.15^{a}	1.62 ± 0.20^{b}	1.57 ± 0.15^{b}	1.64 ± 0.20^{b}	0.003
UA (μmol/L)	0.15 ± 0.03	0.15 ± 0.03	0.12 ± 0.04	0.14 ± 0.04	0.683
ALB (g/L)	12.53 ± 1.44	13.14 ± 1.69	12.58 ± 1.87	12.88 ± 1.10	0.939
TP (g/L)	50.10 ± 3.12	54.27 ± 9.29	46.35 ± 7.79	50.12 ± 7.95	0.537
GLB (g/L)	37.57 ± 1.89	41.13 ± 7.78	33.77 ± 6.70	37.24 ± 7.09	0.466
ALT (U/L)	10.28 ± 1.56	9.93 ± 1.21	9.68 ± 0.71	10.80 ± 2.38	0.771
AST (U/L)	38.27 ± 1.94^{a}	31.78 ± 3.76^{b}	30.33 ± 1.81^{b}	29.40 ± 1.14^{b}	0.005
Creatinine (μmol/L)	5.08 ± 0.98	4.76 ± 0.72	4.78 ± 0.78	5.35 ± 0.62	0.751

Values in the table are mean ± SD (n = 11).
^{a,b}Pairs of means with the same superscript letter within the same row are not significantly different at least at the p >0.05 level.
GLU, glucose; TG, triglyceride; CHO, total cholesterol; UREA, UREA; UA, uric acid; ALB, albumin; TP, total protein; GLB, globulin; ALT, Glutamic-pyruvic Transaminase; AST, glutamic oxalacetic transaminase; Creatinine, Creatinine.

TABLE 7 | Effects of alfalfa-mixed silage fermentation material (AMSFM) on antioxidant indexes of chest and leg muscles from meat geese. Control geese (CONT) were fed a basal diet, whereas TRT6, TRT12, and TRT24 were fed a basal diet supplemented with 6, 12, or 24% AMSFM, respectively.

Team	CONT	TRT6	TRT12	TRT24	*p value*
			Chest muscle		
MDA (nmol/mL)	0.72 ± 0.14^{a}	0.50 ± 0.14^{bc}	0.44 ± 0.12^{c}	0.66 ± 0.13^{ab}	0.014
T-AOC (U/mL)	0.09 ± 0.02	0.10 ± 0.02	0.10 ± 0.03	0.09 ± 0.01	0.775
GSH (umol/L)	1.91 ± 0.44^{b}	2.96 ± 0.33^{a}	3.01 ± 0.44^{a}	3.35 ± 0.94^{a}	<0.010
CAT (U/mL)	0.80 ± 0.17^{b}	1.21 ± 0.26^{a}	1.15 ± 0.17^{a}	1.01 ± 0.22^{ab}	0.023
SOD (U/mL)	21.54 ± 5.06	25.50 ± 7.77	22.87 ± 4.52	22.54 ± 5.87	0.695
			Leg muscle		
MDA (nmol/mL)	0.39 ± 0.08^{a}	0.26 ± 0.06^{b}	0.22 ± 0.08^{b}	0.29 ± 0.07^{ab}	0.040
T-AOC (U/mL)	0.16 ± 0.04	0.17 ± 0.04	0.19 ± 0.05	0.19 ± 0.05	0.609
GSH (umol/L)	2.46 ± 1.04^{c}	3.25 ± 0.57^{bc}	3.99 ± 1.24^{ab}	4.69 ± 0.96^{a}	0.022
CAT (U/mL)	0.97 ± 0.19	1.06 ± 0.18	0.98 ± 0.13	1.07 ± 0.26	0.738
SOD (U/mL)	23.07 ± 5.13	25.63 ± 5.19	26.23 ± 6.13	24.41 ± 4.89	0.810

Values in the table are mean ± SD (n = 11).
$^{a–c}$Pairs of means with the same superscript letter within the same row are not significantly different at least at the p >0.05 level.
T-AOC, total antioxidant capacity; MDA, malondialdehyde; GPX, glutathione content; CAT, catalase; SOD, superoxide dismutase.

TABLE 8 | Effects of alfalfa-mixed silage fermentation material (AMSFM) on serum antioxidant indexes in meat geese. Control geese (CONT) were fed a basal diet, whereas TRT6, TRT12, and TRT24 were fed a basal diet supplemented with 6, 12, or 24% AMSFM, respectively.

Team	CONT	TRT6	TRT12	TRT24	*p value*
MDA (nmol/mL)	9.57 ± 2.23	7.86 ± 1.14	6.65 ± 2.30	9.12 ± 2.69	0.272
T-AOC (U/mL)	0.99 ± 0.33	1.21 ± 0.32	1.03 ± 0.26	1.06 ± 0.33	0.667
GSH (umol/L)	9.12 ± 3.61	10.01 ± 1.46	10.71 ± 3.57	9.21 ± 2.11	0.759
CAT (U/mL)	0.07 ± 0.02	0.09 ± 0.02	0.07 ± 0.01	0.07 ± 0.02	0.217
SOD (U/mL)	630.53 ± 120.87^{b}	847.04 ± 191.04^{a}	804.78 ± 87.20^{a}	756.34 ± 69.28^{ab}	0.047
GSH-P$_X$ (nmol/mg prot)	934.53 ± 202.41	1045.36 ± 129.37	1211.87 ± 244.26	1143.44 ± 184.81	0.168

Values in the table are mean ± SD (n = 11).
^{a,b}Pairs of means with the same superscript letter within the same row are not significantly different at least at the p >0.05 level.
T-AOC, total antioxidant capacity; MDA, malondialdehyde; GPX, glutathione content; GSH-Px, glutathione peroxidase; CAT, catalase; SOD, superoxide dismutase.

4 DISCUSSION

Compared to the CONT, geese consuming AMSFM had ~2% more rapid ADG and ~2% higher final weight, although there were no significant differences among groups for either endpoint. Similarly, in a previous study (Xu et al., 2021), there were no significant differences in the ADG and ADFI of meat geese when adding biofermented feed at the later growth stage. Perhaps

TABLE 9 | Effects of alfalfa-mixed silage fermentation material (AMSFM) on intestinal histomorphological indexes in meat geese. Control geese (CONT) were fed a basal diet, whereas TRT6, TRT12, and TRT24 were fed a basal diet supplemented with 6, 12, or 24% AMSFM, respectively.

Location		CONT	TRT6	TRT12	TRT24	*p value*
Jejunum	**VH (μm)**	1083.79 ± 172.04	1331.91 ± 143.99	1266.09 ± 202.10	1110.42 ± 211.26	0.293
	CD (μm)	227.07 ± 9.05	253.67 ± 33.86	245.18 ± 21.90	236.13 ± 21.87	0.441
	V/C	4.78 ± 0.77	4.68 ± 1.23	4.60 ± 1.02	4.69 ± 0.67	0.994
Ileum	**VH (μm)**	977.96 ± 45.47^{b}	1197.35 ± 107.65^{a}	1210.25 ± 37.82^{a}	1088.33 ± 103.28^{ab}	0.012
	CD (μm)	237.19 ± 7.35^{a}	245.14 ± 17.81^{a}	216.22 ± 9.90^{b}	229.38 ± 2.98^{ab}	0.022
	V/C	4.57 ± 0.56^{b}	4.76 ± 0.62^{b}	5.60 ± 0.11^{a}	4.54 ± 0.56^{b}	0.038
Duodenum	**VH (μm)**	954.21 ± 190.83	1040.42 ± 101.76	1081.33 ± 278.37	1005.42 ± 89.56	0.850
	CD (μm)	248.13 ± 35.31	197.99 ± 28.94	220.13 ± 14.81	240.38 ± 39.12	0.152
	V/C	4.18 ± 0.45	4.80 ± 0.85	4.40 ± 1.13	4.22 ± 0.44	0.702

Values in the table are mean ± SD (n = 11).

^{a,b}Pairs of means with the same superscript letter within the same row are not significantly different at least at the p >0.05 level.

VH, villus height; CD, crypt depth; V/C, villus height/crypt depth.

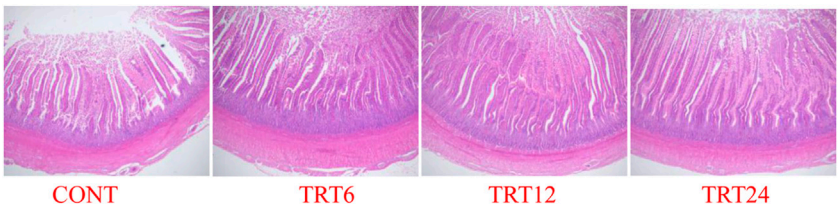

FIGURE 1 | Light micrograph of jejunum morphology in meat geese (40 × multiple). Control geese (CONT) were fed a basal diet, whereas TRT6, TRT12, and TRT24 were fed a basal diet supplemented with 6, 12, or 24% alfalfa-mixed silage fermented material, respectively.

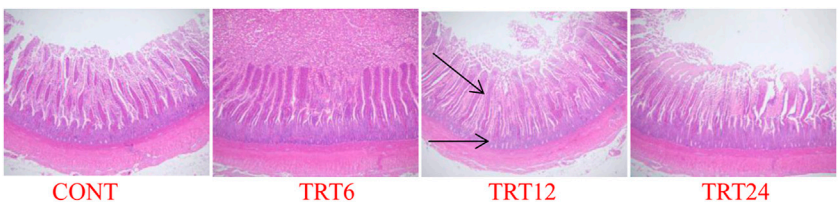

FIGURE 2 | Light micrograph of ileum morphology in meat geese (40 × multiple). Control geese (CONT) were fed a basal diet, whereas TRT6, TRT12, and TRT24 were fed a basal diet supplemented with 6, 12, or 24% alfalfa-mixed silage fermented material, respectively.

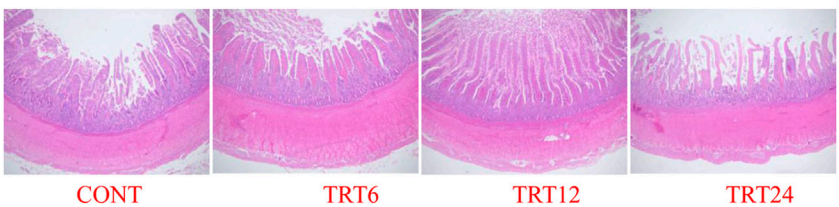

FIGURE 3 | Light micrograph of duodenum morphology in meat geese (40 × multiple). Control geese (CONT) were fed a basal diet, whereas TRT6, TRT12, and TRT24 were fed a basal diet supplemented with 6, 12, or 24% alfalfa-mixed silage fermented material, respectively.

weight development of meat geese decreases at a later growth stage, and the basal diet met the needs of growth and development so that the fermented feed had no significant impact on growth performance. In previous studies, adding alfalfa to the diet promoted animal growth (Hu et al., 2021), with fermented alfalfa better than alfalfa meal (Yin and Zhou, 2015). Fermentation can improve nutritional quality and palatability, and enhance digestion, absorption, and growth

performance. Adding a fermented feed to a goose diet enhanced the composition of goose intestinal flora, which improved nutritional status and intestinal health, and enhanced the growth performance of meat geese (Yan et al., 2019). Furthermore, feeding grass powder had no significant effect on the slaughter performance of geese, but reduced fat deposition, (Jiang et al., 2012), similar to current results.

In this experiment, the leg muscle ratio, abdominal fat ratio, and lean meat ratio of the three AMSFM groups were significantly lower than those of the control group. Perhaps AMSFM improved feed nutritional value and utilization. AMSFM is rich in amino acids and effective components such as alfalfa polysaccharide, saponins, and carotene, which could improve the metabolic rate of protein, crude fat, and other substances, promote digestion and absorption of nutrients, reduce fat deposition, and improve lean meat yield (Francisco et al., 2020).

The CONT geese had the lowest CP content in both chest and leg muscles; this was attributed to alfalfa, DDGS, and soybean meal in AMSFM being rich sources of protein and amino acids. Furthermore, the range of water content of chest and leg muscles was 72.86%–76.33%, similar to a previous report (71.32%–77.20%) from five breeds of geese (Tang et al., 2010). The water content of muscle is directly related to the taste, juiciness, and chewiness of meat, with implications for the economic value of meat (Biesek et al., 2020). The EE of the chest muscle was highest in the CONT, consistent with a reduction in fat content in geese supplemented with AMSFM. Perhaps the alfalfa saponins, fatty acids, and other substances in AMSFM reduced the crude fat content of chest muscle in meat geese.

Serum TG concentrations were significantly higher in CONT geese than those in the other three groups. In a previous study, alfalfa meal significantly reduced TG in animals (Li et al., 2019). Furthermore, lower serum urea concentrations in geese supplemented with AMSFM were consistent with greater incorporation of nitrogen into muscle. In addition, significantly lower serum AST concentration in geese supplemented with AMSFM indicated good liver function, consistent with previous studies (Li et al., 2015; Yang et al., 2022).

After fermentation, portions of saponins, flavonoids, vitamins, and other probiotics in AMSFM were converted into amino acids that could be effectively absorbed by livestock and poultry, maintaining the dynamic balance of amino acids in the animal body, and improving antioxidant capacity (Gungor et al., 2021; Lu et al., 2021). Organic acids produced during fermentation removed oxygen free radicals and enhanced antioxidative ability (Zhan et al., 2017). Antioxidant peptides are formed by fermentation of bioactive substances, which could prevent peroxidation, and protect the normal structures and functions of various tissues and organs as well (Choi et al., 2010). CAT, SOD, and GSH-Px decompose hydrogen peroxide and remove free radicals, serving as the main endogenous antioxidative enzymes to protect the body from oxidative damage (Fang et al., 2002).

After slaughter, numerous reactive oxygen species are produced, affecting meat color and lipid peroxidation in the muscle. As antioxidant substances in the muscle are limited, meat quality eventually decreases (Echegaray et al., 2021). In previous studies, feeding alfalfa saponins tended to reduce MDA concentrations in layers and increased activities of GSH-Px and SOD (Fan et al., 2018). In a comparison of adding alfalfa meal or fermented alfalfa to the diet of geese, fermented alfalfa had better effects in improving antioxidant performance, attributed to the greater content of vitamins, alfalfa polysaccharides, isoflavones, and small molecule peptides (Pleger et al., 2020). Overall, these effects were similar to those in the present study.

The small intestine is not only critical for poultry to digest and absorb nutrients but also the largest immune organ in the body. It constitutes the first barrier to preventing invasion of pathogenic bacteria. Intestinal health is one of the most important indicators to reflect poultry health (Chen et al., 2013). The functions of villi and crypt in the small intestine are to promote digestion and absorption of nutrients. Intestinal crypt cells differentiate into villi to replace shed or damaged villous cells (Woyengo et al., 2010). The higher the villus height and the ratio of the villus to the crypt in the morphology of the small intestine, the larger the surface area and the greater the nutrient absorption capacity (Jazi et al., 2017). Missotten et al. (2013) reported that feeding broilers fermented feed improved the villus height and the ratio of the villus to the crypt, and decreased crypt depth in their small intestine. Li et al. (2020) added fermented soybean meal to the diet of broilers and improved the duodenum and jejunum. In this study, AMSFM significantly improved ileal morphology, consistent with the previous studies. With the increase of the AMSFM proportion, the villus height and the villus ratio of the small intestine increased first and then decreased, the crypt depth gradually decreased, and the thickness of the intestinal wall also decreased, which helped the transportation and absorption effect of nutrients. Therefore, AMSFM had positive effects on the intestinal structure of meat geese, and promoted nutrient absorption and utilization, with 6–12% AMSFM having the best effects. In addition, there were some microbial metabolites, e.g., butyric acid after fermentation of alfalfa polysaccharide (Flint et al., 2008), and organic acids, amino acids, and small peptides produced by the metabolism of other substances, acting on the intestine and inducing the proliferation and differentiation of intestinal cells, which also improved the biological environment and maintained the balance and health of intestinal flora (Zahran et al., 2014; Jazi et al., 2018).

5 CONCLUSIONS

Adding AMSFM to the diet of meat geese improved some aspects of growth performance, lean meat ratio, and muscle protein, and reduced the fat content in muscle. Moreover, dietary AMSFM improved the antioxidant capacity and immune organ index, improved the morphological structure of the ileum, and promoted good liver and kidney function. In this study, supplementing the diet of meat geese with 12% AMSFM provided the best overall response.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

HL: methodology, data analysis, and writing; YL: writing and editing and data analysis; LW: investigation and statistical analysis; QL: project design, constructive discussion, and validation; and ZZ: project design, validation, and supervision.

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

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Effects of Lecithin Supplementation in Feed of Different fat Levels on Serum Indexes and Liver Health of Laying Hens

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The aim of this experiment was to investigate the effect of soy lecithin on serum-related indicators and liver health in laying hens under the influence of high-fat diets. 180 peak laying hens at 40 weeks of age were randomly assigned to one of the four diets using a 2 × 2 factorial and fed for 5 weeks. The results showed that compared to the low-fat group, the high-fat group had lower egg production ($p < 0.05$) and higher average daily feed intake and feed-to-egg ratio ($p < 0.05$). At the 21st day, the serum levels of triglyceride (TC) and superoxide dismutase (SOD) were higher ($p < 0.05$), high-density lipoproteins cholesterol (HDL-C) levels were lower ($p < 0.01$), catalase (CAT) activity was lower ($p < 0.05$), TC and malondialdehyde (MDA) levels in liver were higher ($p < 0.01$) and SOD activity in liver was lower ($p < 0.05$) in layers supplemented with soy lecithin. CAT activity in serum was increased ($p < 0.01$) and total antioxidant capacity (T-AOC) activity in the liver was decreased ($p < 0.05$) after increasing the dietary fat concentration. The addition of soy lecithin and the increase in dietary fat concentration had a highly significant interaction on serum CAT activity and liver TC content in layers ($p < 0.01$). At the 35th day, the serum alanine aminotransferase (ALT) activity was higher ($p < 0.01$), serum glutathione peroxidase (GSH-Px) and CAT activity were higher ($p < 0.05$), and serum triglyceride (TG) content and total T-AOC capacity activity were lower ($p < 0.05$) in layers supplemented with soy lecithin. Increasing dietary fat concentration decreased alanine aminotransferase (ALT), aspartate aminotransferase (AST) and GSH-Px activity in serum ($p < 0.05$). However, it increased TG and MDA content in liver ($p < 0.05$), and highly decreased SOD content in liver ($p < 0.01$) in layers. The addition of soy lecithin and increasing dietary fat concentration had a highly significant reciprocal effect on serum ALT viability and CAT viability ($p < 0.01$) and liver TG and MDA content and SOD viability ($p < 0.05$) in layers. In conclusion, feeding high-fat diets will adversely affect the laying performance of laying hens, while long-term addition of lecithin can improve the blood lipids and liver lipids of laying hens, enhance the antioxidant capacity of the liver, and maintain liver health.

Keywords: soy lecithin, biochemical indexes, antioxidant index, laying performance, liver health

INTRODUCTION

With the rapid development of the animal husbandry industry, the breeding environment became more and more complex, which potentially causes various diseases. As the most important detoxification organ in the body, the liver plays an important role. It has been observed that in the long-term breeding process of laying hens, the abdomens of some hens were enlarged and soft, and their egg production declined. In hens that died unexpectedly, postpartum revealed hemorrhages and liver damage, all of which were caused by fatty liver disease (Liu, 2017). The liver health of livestock and poultry is positively related to their growth. Liver damage often occurs during the breeding process. Therefore, it requires more attention to repair the liver damage.

Soy lecithin is mainly a mixture of various phospholipids, including phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, etc., (Scholfield, 1981). The active ingredients contained in soy lecithin play an important role in maintaining the integrity, fluidity, and functions of cell membranes (Pagheh, et al., 2018). It was reported that under carbon tetrachloride modeling, phospholipids could slow down the increase in liver weight, promote the regeneration of hepatocytes, and gradually restore the fascicular and lobular structures of the liver (Drozdov, 2014). There were also reports suggesting that adding lecithin to the diet could improve the pathological changes in the liver of laying hens, as well as the abnormal expression of apoA I and apoB100 genes in the liver (Yalu et al., 2017). It has also been found that supplementation of 0.5–2.0% soybean lecithin (SL) improved the production performance of laying hens and total phospholipid contents in whole eggs and egg yolks were also increased at 1, 2, and 4 and 2% SL supplementation than the control group, respectively (Sun et al., 2010). Further, Lu et al. (2018) also found that supplementation of 0.1 and 0.2% lecithin in high-fat diets improved the laying hens' egg production rate and reduced liver index, blood fat content, and occurrence of fatty liver syndrome. However, limited research is available on the effect of lecithin on the liver health of laying hens. Therefore, this study examined the effects of dietary fat concentration and lecithin on serum markers and liver health in laying hens.

MATERIALS AND METHODS

Test Material

The lecithin (acetone insoluble content ≥ 90%) used in this study was provided by Centree Bio-tech (Wuhan) Co., Ltd. China.

Animals and Experimental Details

All the experimental procedures were approved by the Animal Care Committee of the Institute of Bast Fiber Crops, Chinese Academic of Agricultural Sciences, Changsha, China. A total of 180 Lohmann Commercial laying hens (40-week-old) were randomly divided into four groups, with five replicates of 9 birds each. Experimental laying hens were pre-fed for 30 days and rearranged after confirmation of no clinical abnormality.

TABLE 1 | Composition and nutrient levels of experimental diets (air-dried basis) %.

Items	Diets	
	Basal diet	**High-fat diet**
Ingredients		
Corn	58.08	57.52
Soybean meal	28.37	20.16
Rice husk	0.00	0.51
Oil	1.08	6.10
Wheat bran	1.00	4.20
Limestone	8.47	8.51
$CaHPO_4{\cdot}2H_2O$	0.00	0.00
98.0% *L*- Lys	0.00	0.00
98.5% *DL*- Met	0.00	0.00
NaCl	0.00	0.00
3% Premix1)	3.00	3.00
Total	100.00	100.00
Nutrient levels2)		
ME/(Mcal/kg)	2.75	3.00
CP	16.50	13.50
CF	3.05	3.05
Ca	3.50	3.50
Total *p*	0.54	0.53
Available *p*	0.32	0.32
NaCl	0.31	0.31

1) The premix provided the following (per kilogram of complete diet) micronutrients: VA, 6 000 IU, VD3 2 500 IU, VE, 25 mg, VK3 2.25 mg, VB1 1.8 mg, VB2 7 mg, VB6 4 mg, VB12 0.2 mg, D-pantothenic acid 12 mg, nicotinic acid 35 mg, biotin 0.14 mg, folic acid 0.8 mg, Cu (as copper sulfate) 11 mg, Zn (as zinc sulfate) 70 mg, Fe (as ferrous sulfate) 60 mg, Mn (as manganese sulfate) 115 mg, Se (as sodium selenite) 0.30 mg, I (as potassium iodide) 0.4 mg.

2) Nutrient levels are calculated values.

A 2 × 2 factorial was randomly assigned to 4 groups with the basal diet was a corn-soybean meal diet, the dietary fat rate of 1 and 6% and dietary supplemented lecithin concentration of 0 and 1 kg/t as the main treatment factors. The normal dietary protein level was 16.5% and the high-fat diet protein level was 13.5%. Other nutrient levels met the recommendations of the National Research Council (NRC) for laying hens (1994) and "NY/T 33–2004 Chicken Breeding Standards" as shown in **Table 1**. The hens were raised in ladder cages with one bird in each cage. After 4 weeks of the adaptation period, the main experiment started and lasted for 7 weeks. The egg production, body weight, and feed intake of each laying hen were measured on the first day of the experiment. No statistical differences in the production performance were found among treatments. Free water and feed were provided for all hens. The average temperature was 25 ± 2°C in the laying hens' house during the experimental period. The light time was according to the standard light procedure of commercial laying hens, which was 16 h of light per day, until the end of the experiment.

Egg Production Performance

The laying hens of each treatment were fed with the corresponding diet according to the experimental design. The feed intake was recorded. The egg production of chickens was recorded and each egg was weighed. The deaths were also recorded every day.

TABLE 2 | Effect of adding lecithin on egg laying performance of laying hens on 35 days.

Items		1% Fat		6% Fat			Lecithin		Fat		*p*- value		
	Lecithin	0 kg/t	1 kg/t	0 kg/t	1 kg/t	SEM	0 kg/t	1 kg/t	1%	6%	Fat	Lecithin	Interaction
Egg production rate (%)		96.63	96.41	91.60	88.92	1.22	94.12	92.67	96.52	90.26	0.045	0.607	0.663
Average egg weight (g)		60.96	59.22	59.68	60.88	0.40	60.32	60.05	60.09	60.28	0.846	0.790	0.167
Average daily feed intake (g)		116.08	116.83	125.49	125.57	1.60	120.79	121.20	116.46	125.53	0.010	0.887	0.910
The egg mass/feed consumption ratio		1.90	1.97	2.11	2.06	0.02	2.01	2.02	1.94	2.08	0.001	0.704	0.108

In the same row, values with no letter or the same letter superscripts mean no significant difference (p > 0.05), with different small letter superscripts mean significant difference (p < 0.05), and with different capital letter superscripts mean extremely significant difference (p < 0.01). The same as below.

Determination of Serum Biochemical Indexes and Antioxidant Indexes

On the morning of the 21st and the 35th day, 5 test chickens were randomly selected from each treatment (1 bird per replicate), and about 8 ml of blood were collected from the wing vein after weighting with 10 ml centrifuge tubes and placed in a tilted position to allow the blood to coagulate naturally. After 0.5 h of hemagglutination, the blood samples were centrifuged at 3000 r/min for 15 min to separate the serum. The upper serums were collected and stored at −40°C. Serum biochemical indices, including triglyceride (TG), cholesterol (TC), aspartate aminotransferase (AST), alanine aminotransferase (ALT), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured by using commercial kits following the instructions assay kits (BS-200, Shenzhen Mairui Medical International Co., Ltd., China). Antioxidant indexes, including total antioxidative capacity (T-AOC), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and malonaldehyde (MDA) were examined using assay kits (H249, Nanjing Jiancheng Bioengineering Institute, China) with an automated fluorescence instrument (MultiskanSkyHigh, Thermo Fisher Scientific, Waltham, MA, United States) following the manufacturers' instructions.

Detection of Liver Indexes

The liver indexes were calculated after peeling and weighing the livers of the slaughtered chickens. Three samples of suitable size were collected from each liver sample. They were cut in the middle of the large liver lobe, wrapped in tin foil, and stored at −80°C in refrigerator. Afterwards, 0.3 g of the liver sample was taken and homogenized with cold physiological saline using an Ultra-Turrax homogenizer from Tekmar (Cincinnati, United States), followed by centrifugation at 4000 × g for 15 min at 4°C for indexes analysis. Antioxidant indexes in the liver, including T-AOC, CAT, SOD, GSH-Px, and MDA were examined using assay kits (H249, Nanjing Jiancheng Bioengineering Institute, China) with an automated fluorescence instrument (MultiskanSkyHigh, Thermo Fisher Scientific, Waltham, MA, United States) following the manufacturers' instructions.

Data Processing and Statistical Analysis

After the experimental data was preliminarily processed with Excel 2007 software, two-way ANOVA was performed with SPSS 19.0 statistical software. $p < 0.05$ and 0.01 suggested significant differences and extremely significant differences respectively. The test results were expressed as the mean of each group. The dataset standard error (SEM) results were also presented.

RESULTS

Laying Performance

It could be seen from **Table 2** that on the 35th day, the egg production rate, average egg weight, average daily feed intake, and the egg mass/feed consumption ratio between the layers treated with or without lecithin were not affected ($p > 0.05$). Compared with the low-fat group, the laying rate of the hens in the high-fat group was decreased ($p < 0.05$). The average daily feed intake and the egg mass/feed consumption ratio were increased ($p < 0.05$). The average egg weight had no change ($p > 0.05$). The interactions of lecithin and fat on egg production rate, average egg weight, average daily feed intake, and the egg mass/feed consumption ratio were not affected ($p > 0.05$).

Serum Biochemical Indicators

It could be seen from **Table 3** that on the 21st day, the content of TC in the serum of the group treated with lecithin was increased ($p < 0.05$) compared to the treatment group without lecithin, whereas the content of HDL-C was decreased ($p < 0.01$). The content of HDL-C showed an upward trend ($p = 0.081$), while the activities of ALT, AST, and TG in serum had no differences ($p > 0.05$). Compared with the low-fat group, the serum ALT and AST activities, TG, TC, HDL-C, and LDL-C contents of laying hens in the high-fat group had no difference ($p > 0.05$). The interactions of lecithin and fat on ALT, AST activities, and TG, TC, HDL-C, and LDL-C content in laying hens' serum were not affected ($p > 0.05$).

On the 35th day, compared to those without the lecithin supplementation treatment group, the serum ALT activity was highly increased ($p < 0.01$). The TC content was decreased ($p < 0.05$) in the lecithin supplemented treatment group. But the serum AST activity, contents of TG, HDL-C, and LDL-C had no difference ($p > 0.05$). Compared with the low-fat group, the serum AST and ALT activities of the laying hens in the high-fat group were decreased ($p < 0.01$), whereas the serum AST activity, TC, TG, HDL-C, and LDL-C contents had no difference ($p > 0.05$). There was an interaction between lecithin and ALT activity in the serum of laying hens ($p < 0.05$). But the interactions on the

TABLE 3 | Effects of adding lecithin on serum biochemical indexes of laying hens.

Items			1% Fat		6% Fat			Lecithin		Fat		*p*- value		
		Lecithin	0 kg/t	1 kg/t	0 kg/t	1 kg/t	SEM	0 kg/t	1 kg/t	1%	6%	Fat	Lecithin	Interaction
	ALT (U/L)		1.89	2.10	2.15	2.31	0.09	2.02	2.2	2	2.23	0.228	0.335	0.897
	AST (U/L)		18.24	19.81	16.89	18.14	0.53	17.56	18.98	19.03	17.51	0.160	0.186	0.879
21d	TC (mmol/L)		23.99	28.15	22.22	27.79	1.45	23.1	27.97	26.07	25	0.718	0.112	0.811
	TG (mmol/L)		3.25	3.84	2.67	3.55	0.18	2.96	3.69	3.54	3.11	0.206	0.038	0.655
	HDL-C (mmol/L)		0.41	0.30	0.36	0.31	0.01	0.39	0.31	0.36	0.34	0.443	0.002	0.143
	LDL-C (mmol/L)		1.35	1.69	1.47	1.8	0.09	1.41	1.74	1.52	1.63	0.535	0.081	0.971
	ALT (U/L)		2.22^{A}	2.20^{A}	1.37^{B}	2.25^{A}	0.11	1.79	2.22	2.21	1.81	0.010	0.007	0.005
	AST (U/L)		18.17	20.95	16.41	16.79	0.62	17.29	18.87	19.56	16.60	0.010	0.138	0.252
35d	TC (mmol/L)		31.74	26.97	35.43	29.33	1.61	33.59	28.15	29.35	32.38	0.348	0.102	0.835
	TG (mmol/L)		4.18	3.30	4.01	3.54	0.17	4.10	3.42	3.74	3.77	0.923	0.048	0.538
	HDL-C (mmol/L)		0.32	0.33	0.25	0.36	0.02	0.28	0.34	0.32	0.30	0.741	0.222	0.318
	LDL-C (mmol/L)		1.56	1.48	1.57	1.38	0.06	1.56	1.43	1.52	1.48	0.728	0.263	0.655

ALT, alanine aminotransferase; AST, aspartate aminotransferase; TC, triglyceride; TG, Total cholesterol; HDL-C, high density liptein cholesterol and LDL-C, low-density lipoprotein cholesterol.

TABLE 4 | Effects of adding lecithin on serum Antioxidant Index of Laying Hens.

Items			1% Fat		6% Fat			Lecithin		Fat		*p*- value		
		Lecithin	0 kg/t	1 kg/t	0 kg/t	1 kg/t	SEM	0 kg/t	1 kg/t	1%	6%	Fat	Lecithin	Interaction
21d	T-SOD (U/mgprot)		124.42	130.75	124.02	149.19	4.12	124.22	139.97	127.59	136.61	0.239	0.049	0.220
	MDA (nmol/mgprot)		7.49	8.94	7.71	8.50	0.55	7.60	8.72	8.21	8.11	0.927	0.348	0.777
	GSH-PX (U/mgprot)		462.44	739.44	572.99	562.70	39.9	517.71	651.07	600.94	567.84	0.649	0.080	0.061
	CAT (U/mgprot)		2.40B	1.31C	2.93AB	3.08A	0.18	2.67	2.20	1.86	3.01	< 0.001	0.035	0.008
	T-AOC (mmol/gprot)		1.05	1.26	1.31	1.17	0.07	1.18	1.22	1.16	1.24	0.550	0.779	0.232
35d	T-SOD (U/mgprot)		135.73	131.52	131.06	138.36	4.04	133.39	134.94	133.63	134.71	0.902	0.861	0.517
	MDA (nmol/mgprot)		10.97	7.47	11.10	10.90	0.60	11.04	9.18	9.22	11.00	0.109	0.096	0.134
	GSH-PX (U/mgprot)		794.06	1004.68	711.07	780.61	37.10	752.56	892.65	899.37	745.84	0.020	0.031	0.252
	CAT (U/mgprot)		1.18B	3.22A	2.46A	2.16A	0.24	1.82	2.69	2.20	2.31	0.765	0.032	0.006
	T-AOC (mmol/gprot)		1.55	1.21	1.49	1.40	0.06	1.52	1.30	1.38	1.45	0.528	0.044	0.237

T-SOD, total superoxide dismutase; MDA, malondialdehyde; GSH-PX, glutathione peroxidase; CAT, catalase and T-AOC, total antioxidant capacity.

contents of AST, TG, TC, HDL-C, and LDL-C in serum were not affected ($p > 0.05$).

Serum Antioxidant Indicators

It could be seen from **Table 4** that on the 21st day, compared with the treatment group without lecithin supplementation, the CAT activity in the serum was decreased ($p < 0.01$), whereas the SOD, GSH-Px, T-AOC in the serum were significantly reduced ($p < 0.01$) in the lecithin supplemented treatment group. But the MDA content had no difference ($p > 0.05$). Compared with the low-fat group, the activities of SOD and CAT in the serum were increased ($p < 0.05$), and the activity of GSH-Px was decreased ($p = 0.080$) in the laying hens in the high-fat group. There was an interaction between lecithin and fat on the activity of CAT in the serum of laying hens ($p < 0.01$). But the interactions on the activities of SOD, GSH-Px, and T-AOC and the content of MDA in serum were not affected ($p > 0.05$).

On the 35th day, compared to without lecithin supplemented treatment group, the serum GSH-Px activity in the lecithin supplemented treatment group was increased ($p < 0.01$), whereas the SOD, CAT, T-AOC activity, and MDA content in the serum did not show differences ($p > 0.05$). Compared with the low-fat group, the serum GSH-Px and CAT activities of the laying hens were decreased ($p < 0.05$), the T-AOC activity was increased ($p < 0.05$), and the serum MDA content had a tendency to increase ($p = 0.096$) in the high-fat group. There was no change in SOD activity in serum ($p > 0.05$). Besides, there was an interaction between lecithin and fat in the activity of CAT in the serum of laying hens ($p < 0.01$), but the interactions in the activities of SOD, T-AOC, GSH-Px, and MDA content in serum were not obvious ($p > 0.05$).

Liver Index and Liver Triglyceride Content

It could be seen from **Table 5** that on the 21st and the 35th day, the liver index of the laying hens of the four groups was not affected ($p > 0.05$). The interactions between fat content and lecithin on the liver index of laying hens did not reach a level ($p > 0.05$).

It could be seen from **Table 5** that on the 21st day, compared with the treatment group without lecithin supplementation, the

TABLE 5 | Effects of adding lecithin on layer Liver Index and TG content of liver in laying hens.

Items			1% Fat		6% Fat			Lecithin		Fat		*p*- value		
		Lecithin	0 kg/t	1 kg/t	0 kg/t	1 kg/t	SEM	0 kg/t	1 kg/t	1%	6%	Fat	Lecithin	Interaction
21d	Liver index (%)		2.250	2.380	2.270	2.040	0.05	2.260	2.210	2.320	2.150	0.121	0.634	0.092
	TG (mmol/gprot)		0.5^{B}	0.88^{A}	0.65^{B}	0.63^{B}	0.04	0.58	0.76	0.69	0.64	0.414	0.008	0.003
35d	Liver index (%)		2.530	2.460	2.190	2.390	0.08	2.360	2.430	2.500	2.290	0.203	0.684	0.407
	TG (mmol/gprot)		0.93^{B}	0.79^{B}	0.87^{B}	1.18^{A}	0.05	0.90	0.98	0.86	1.02	0.040	0.260	0.010

TG content in the liver of the lecithin supplemented treatment group was increased ($p < 0.01$). Compared with the low-fat group, the high-fat group had no difference on the liver TG content of laying hens ($p > 0.05$). However, lecithin and fat had an interaction on the liver TG content of laying hens ($p < 0.01$). On the 35th day, the hepatic TG content of the lecithin supplemented treatment group and without lecithin supplemented group had no difference ($p > 0.05$). Compared with the low-fat group, the TG content in the liver of the laying hens in the high-fat group was increased ($p < 0.01$). Besides, there was an interaction between lecithin and fat on the TG content in the liver of the laying hens ($p < 0.05$).

Liver Antioxidant Indicators

It could be seen from **Table 6** that on the 21st day, compared with the treatment group without lecithin supplementation, the SOD activity was decreased, and the MDA content was increased ($p < 0.05$) in the liver of the lecithin-added treatment group. There was no change in the activity of T-AOC ($p > 0.05$). Compared with the low-fat group, the liver T-AOC activity decreased ($p < 0.05$), and the MDA content increased ($p < 0.01$) of the laying hens in the high-fat group. There were no interactions between lecithin and fat on the measured antioxidant indexes in the liver of laying hens ($p > 0.05$).

On the 35th day, compared to the without lecithin supplementation group, the hepatic T-AOC activity in the lecithin supplemented treatment group had a tendency to increase ($p = 0.084$), while the SOD, GSH-Px, CAT activity and MDA content in the liver were not affected ($p > 0.05$). Compared with the low-fat group, the SOD activity was significantly decreased, and the MDA content was increased ($p < 0.01$) in the liver of the laying hens in the high-fat group, while the activities of GSH-Px, CAT, and T-AOC in the liver had no differences ($p > 0.05$). There were interactions between lecithin and fat on the hepatic SOD activity and MDA content in laying hens ($p < 0.05$).

DISCUSSION

The purpose of fat addition in the diet for laying hens was mainly to reduce the pulverization of the feed to improve feed utilization. But high levels of dietary fat increased egg masses and laying hen body weights (Grobas et al., 2001). In the present study, it has been observed that with the increase in dietary fat concentration, the egg production decreased, whereas egg mass/feed consumption ratio of laying hens increased significantly. This was consistent with the previous report by Weiss and Fisher (1957) showing that a high level of animal fat resulted in decreased egg production and increased laying hen body weight. Zhang et al. (2008) also found that increased dietary fat concentration reduced the laying rate of laying hens. The decrease in egg production might be due to fat deposition in the abdomen because of a high energy fat diet, which increased the weight and created an excessive burden on the production performance of the layer (Li et al., 2009). In the current study, it was found that the lecithin supplementation in diet had no significant effect on the laying performance of laying hens, and the interaction between dietary lecithin and fat on the laying performance was not significant. A previous study by Attia et al. (2009) also revealed no significant changes in laying performance with lecithin supplementation. Further, our study also revealed no significant effects of dietary lecithin on the liver index of laying hens on the 21st and 35th days. A study by Mandalawi et al. (2015) also revealed that no significant effect on liver size in laying hens with lecithin supplementation in diets between 23 and 55 weeks of age.

In this study, it was found that on the 35th day, the serum ALT activity was higher in the high-fat group, and the high-fat + lecithin group than in the diet supplemented with lecithin. The high levels of ALT activity in the high-fat group might be happened due to feeding a high-fat diet to laying hens for a long time which can result in an increased burden on the liver and can cause liver damage, especially in the late stage, because of the long growth cycle and high egg production pressure in the layer farming industry. Fatty liver hemorrhage syndrome is a common disease of laying hens, with an incidence rate of 5–30% (Guo et al., 2021). When the liver is damaged, the permeability of the liver cell membrane increases, and a variety of enzymes in the liver cells are released into the blood, such as ALT and AST. Therefore, the activities of these enzymes in the blood would increase (Cray et al., 2008). In poultry, the liver is involved in fat metabolism (Zaefarian et al., 2019). So, Increasing the dietary fat can increase the metabolic burden on the liver resulting in liver damage (Charradi et al., 2013). A previous study also showed that a high-fat diet could induce fatty liver, and the activities of ALT and AST in serum were significantly increased compared with the normal diet group (Zhang et al., 2008). Lecithin is the major component of cell membranes and can play a key role in cell repair and liver health. The results of the current study showed that on the 21st day, dietary lecithin increased the content of TC, and LDL-C, whereas decreased the content of HDL-C in serum. As a surfactant, soybean lecithin could emulsify fat and might affect the absorption of fatty acids in the small intestine (Jenkins

TABLE 6 | Effects of adding lecithin on antioxidant index of liver of laying hens.

Items		1% Fat		6% Fat		SEM	Lecithin		Fat		*p*- value		
	Lecithin	0 kg/t	1 kg/t	0 kg/t	1 kg/t		0 kg/t	1 kg/t	1%	6%	Fat	Lecithin	Interaction
21d	SOD (U/mgprot)	1089.98	961.08	1026.33	985.63	14.98	1058.16	973.36	1025.53	1005.98	0.381	0.001	0.059
	MDA (nmol/mgprot)	0.30	0.42	0.37	0.44	0.02	0.34	0.43	0.36	0.41	0.099	0.004	0.464
	GSH-PX (U/mgprot)	15.62	15.34	15.82	18.26	0.58	15.72	16.8	15.48	17.04	0.182	0.348	0.241
	CAT (U/mgprot)	5.77	7.46	8.24	9.36	0.65	7.01	8.41	6.62	8.8	0.103	0.282	0.823
	T-AOC (mmol/gprot)	0.23	0.21	0.20	0.20	0.01	0.22	0.20	0.22	0.20	0.045	0.141	0.419
35d	SOD (U/mgprot)	895.97^{b}	995.91^{a}	846.23^{b}	819.98^{b}	19.08	871.10	907.95	945.94	833.11	< 0.001	0.152	0.020
	MDA (nmol/mgprot)	0.57^{b}	0.48^{c}	0.68^{a}	0.72^{a}	0.03	0.62	0.60	0.52	0.70	< 0.001	0.407	0.030
	GSH-PX (U/mgprot)	17.27	20.91	18.52	20.55	0.83	17.89	20.73	19.09	19.53	0.793	0.105	0.631
	CAT (U/mgprot)	8.21	7.39	8.76	6.51	0.49	8.49	6.95	7.80	7.63	0.862	0.133	0.470
	T-AOC (mmol/gprot)	0.18	0.20	0.19	0.22	.010	0.18	0.21	0.19	0.20	0.314	0.084	0.825

et al., 1989). It was also reported that soybean lecithin could promote the secretion of endogenous bile acids and improve the utilization of fat (Liu et al., 2020), thereby increasing the fat content in the blood. But on the 35th day, the TC content in serum was significantly reduced by dietary lecithin. Soybean lecithin contains phosphatidylcholine, which is an important component of lipoprotein and plays an essential role in the process of lipid metabolism. Exogenous soybean lecithin could accelerate the decomposition of liver fat in laying hens resulting in a decrease in TC (Shen et al., 2021). Siyal et al. (2017) and Li et al. (2015) found that adding medium and high doses of lecithin to the feed could reduce the content of TC in the serum of broilers. The main active ingredient in lecithin was phosphatidylcholine. Lecithin metabolism releases phosphatidylcholine into the blood, which acts as a substrate for the conversion of cholesterol into cholesterol ester in the body and is associated with the stability of apolipoprotein. Moreover, lecithin has good hydrophilic, lipophilic, and emulsifying properties, so that it can convert cholesterol from large particles to small particles which can be easily absorbed by the tissues via the blood vessel wall, which ultimately decreases the blood lipids levels (Li et al., 2015). It was found in the current study that when laying hens were fed with the high-fat diet for 35 days, the TG content in the liver increased significantly, indicating that the high-fat diet increased the deposition of fat in the liver and increase the incidence of fatty liver. The addition of lecithin decreased the hepatic TG content compared with the high-fat diet group, which showed that lecithin could reduce the deposition of TG in the liver.

Laying hens are usually reared for a longer time duration to get maximum egg production. This can results in an accelerated oxidative rate in laying hens and oxidative damage is severe in the peak laying period. Continuous high production and vigorous metabolism increase the content of active oxygen free radicals in the body. This results in decreased activities of the antioxidant enzymes in laying hens, causing the excessive accumulation of the oxygen free radicals in the body. Excessive deposition of fat in the liver can trigger lipid peroxidation, which damages the liver (Zhao et al., 1995). A study in mice by Tm et al. (2020) showed that long-term feeding of a high-fat diet resulted in an increase in MDA content in the liver and a decrease in the activities of T-AOC, T-SOD, and GSH-Px in the liver. In this study, it was found that the content of MDA, a lipid peroxidation product, in the liver of high-fat-fed laying hens increased. More importantly, the antioxidant capacity of the liver also gradually decreased, which was manifested as a decrease in the activity of the antioxidant enzyme SOD. It was reported that phosphatidylcholine had good antioxidant activity and could be used as an antioxidant. Experiments showed that adding soybean lecithin to super palm oil could reduce the rate of oil oxidation (Pan et al., 2016). The current study revealed that long-term use of lecithin reduced the content of MDA (product of lipid peroxidation in the liver) resulting in an increase in the content of SOD, T-AOC, and GSH-Px in the liver, ultimately improving the antioxidant capacity of the liver of laying hens. Siyal et al. (2017) also mentioned that the addition of 0.05 and 0.1% soybean lecithin

increased the activity of T-AOC, T-SOD, and GSH-Px in serum, and decreased the content of MDA in serum, which was consistent with the results in the current study. This might be because lecithin supplementation could repair cell membranes damaged by oxidative stress, increased the unsaturation of cell membrane fatty acids, and improved the metabolic, self-healing, and regeneration capabilities of cells.

CONCLUSION

In conclusion, feeding high-fat diets adversely affects laying hens, while lecithin supplementation promotes fat absorption. When used for a long time, it can reduce the blood lipid and liver fat of laying hens. Meanwhile, it can improve the antioxidant capacity of the liver of laying hens. Therefore, adding lecithin has a positive effect on liver health.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study and all the experiment procedures were reviewed and approved by the Animal Care Committee of the Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences, Changsha, China.

AUTHOR CONTRIBUTIONS

G-LH, QL, H-JY, LH, and PC designed and conducted the study. XW, SL, TL, and C-JL conducted the animal. XW, SL, TL, C-JL, and PH conducted the detection and analysis works. G-LH, JX, YL, and QL prepared the manuscript draft. All authors participated in the discussion and editing of the manuscript.

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Effects of Dietary Bopu Powder Supplementation on Serum Antioxidant Capacity, Egg Quality, and Intestinal Microbiota of Laying Hens

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The purpose of this study was to investigate the effects of dietary Bopu powder supplementation on the serum antioxidant capacity, serum biochemical indices, egg quality, and intestinal microbiota. Six hundred and forty-eight 33-week-old Lohmann Brown commercial laying hens were randomly allocated into six groups and fed a basal diet supplemented with 0, 25, 50, 100, 200, and 400 mg/kg Bopu powder for 8 weeks, denoted BP0, BP25, BP50, BP100, BP200, and BP400, respectively. The results showed that dietary Bopu powder supplementation reduced serum cholesterol concentrations (linear, $p < 0.01$) while increasing serum globulin and albumin concentrations (linear, $p < 0.05$). Furthermore, the BP50 and BP100 groups had greater serum catalase and glutathione peroxidase activity ($p < 0.05$). The egg Haugh Units were considerably higher in BP25 and BP50 ($p < 0.05$), and eggshell thickness was higher in BP25, BP200, and BP400 ($p < 0.05$) when compared to BP0. Dietary treatment with Bopu powder at doses ranging from 25–100 mg/kg improved glutathione peroxidase and catalase activities while decreasing malondialdehyde concentrations in the yolk ($p < 0.05$). The addition of Bopu powder increased the diversity of microbiota and the relative abundance of Bacteroidota in the gut. For instance, dietary Bopu powder supplementation of 25–50 mg/kg significantly raised the relative abundance of *Enterococcus, Bacteroides*, and *Fusobacterium* in the foregut. Supplementing the diet with 50–100 mg/kg of Bopu powder improved the relative abundance of *Lactobacillus* in the hindgut. In conclusion, dietary Bopu powder supplementation enhanced the abundance of beneficial bacteria in the foregut of laying hens and improved egg quality and antioxidant capacity. Furthermore, in the laying hen diet, the optimal dosage of Bopu powder additive was 25–50 mg/kg.

Keywords: Bopu powder, antioxidant capacity, egg quality, gut microbiota, laying hens

INTRODUCTION

Eggs are one of the most cost-effective sources of high biological value and well-balanced protein. The nutritional value and customer preferences are influenced by the eggshell and internal quality, which is crucial to the egg industry's economic viability (Roberts, 2004). Antibiotic growth promoters (AGPs) are commonly used in food-animal feeds in modern animal husbandry to boost production performance and protect animals from diseases (Castanon, 2007). Although utilizing AGPs in laying hen diet increased laying production and egg quality, it also increased antibiotic residues in eggs and the potential for antibiotic-resistant strains of bacteria to cause health problems in humans. Thus, it is necessary to investigate alternative ways for improving laying performance, egg quality, and preventing laying hen diseases, to ensure healthy and sustainable laying hen production.

Natural plant (Chinese herbal medicine) extracts are known for their multi-functionality, no known bacteria resistance, and low toxicity. Recent studies indicate that natural plant extracts have positive effects on the laying performance and egg quality of laying hens (Abdelli et al., 2021; Righi et al., 2021). Natural antioxidants in the diet, such as tea polyphenols, may help to lay hens increase their performance, albumen quality, magnum shape, antioxidant status, and egg antioxidant capacity (Wang et al., 2018; Zhou et al., 2021). Wang et al. (2019) discovered that dietary tea polyphenol supplementation could mitigate the negative effects of high molybdenum exposure on performance, egg quality, and antioxidant status in laying hens. Also, dietary tea polyphenol supplementation differentially enriched microbial compositions in the cecum enhanced the enrichment of *Bacilli*, *Lactobacillates*, *Lactobacillus*, and *Lactobacillus gasseri*, and indicated that dietary tea polyphenols maintain eubiosis of the cecum microbiota in molybdenum-challenged layers (Wang et al., 2019).

Intestinal microbiota plays a vital role in maintaining gut health and influences the overall performance of laying hens. Gut microbiota is actively involved in the development of the immune system, can confer protection from pathogen infection *via* competitive exclusion and production of antimicrobial compounds, supplies micronutrients, amino acids, and short-chain fatty acids, and influences the development of intestinal epithelium (Khan et al., 2020). Throughout the laying hen production cycle, the gut microbiota composition is altered with age in distinct ways (Joat et al., 2021; Sun et al., 2021). Understanding the baseline and evolution of gut microbiota in laying hens throughout the course of their lives was crucial to obtaining optimal performance and gut health. According to new research, supplementing natural plant extracts enhances laying hen performance and egg quality while also modifying the gut microbiome (Kim et al., 2018; Abad et al., 2020; Dilawar et al., 2021).

Macleaya cordata (Chinese name "Bo-luo-hui"), also known as plume poppy, is a perennial traditional medicinal herb of the Papaveraceae family that is widely distributed in southern China. Its main active compounds are benzophenanthridine alkaloids (sanguinarine and chelerythrine) and protopine alkaloids (protopine and allocryptopine). Our previous studies showed that sanguinarine extracted from *Macleaya cordata* modulated the gut microbiome and intestinal morphology to enhance growth performance in broilers (Liu et al., 2020). Compounds comprising sanguinarine and chelerythrine derived from *Macleaya cordata* were recognized as feed additives in the European Union in 2005, and are widely utilized in poultry and livestock production to replace antibiotic growth promoters. In 2019, compounds containing protopine and allocryptopine isolated from *Macleaya cordata* were registered as veterinary medications in China as Bopu powder (Veterinary Drug No. 180415374), which can be used to treat chicken diarrhea caused by *E. coli*.

Propine and allocryptopine have a wide range of therapeutic biological actions, including anti-inflammatory, antibacterial, antiviral, liver repair, and neuroprotective properties (Vacek et al., 2010; Huang et al., 2021). However, there is limited research in the scientific literature on the effects of protopine and allotytopine on the health and egg quality of laying hens. As a result, the purpose of this study was to assess the effects of dietary supplementation with various amounts of Bopu powder on laying performance, egg quality, serum antioxidant capacity, and gut microbiota in laying hens.

TABLE 1 | Composition and nutrient levels of basal diets (air-dry basis, %).

Items	Diets
Ingredients, %	
Corn	55.45
Soybean meal	29.8
Soybean Oil	1.6
Wheat bran	1.2
Limestone	8.45
CaHPO4·2H2O	1.2
NaCl	0.3
Premix[a]	2
Total	100.00
Nutrient content[b]	
Metabolic energy, MJ/kg	11.5
Crude protein, %	17.00
Crude fiber, %	3.14
Ca, %	3.50
Total phosphorus, %	0.55
Available phosphorus, %	0.33
Lys, %	0.95
Met, %	0.36
Met + Cys, %	0.65

[a]*The premix provided the following (per kilogram of complete diet) micronutrients: VA, 6,000 IU, VD_3 2,500 IU, VE, 25 mg, VK_3 2.25 mg, VB_1 1.8 mg, VB_2 7 mg, VB_6 4 mg, VB_{12} 0.2 mg, D-pantothenic acid 12 mg, nicotinic acid 35 mg, biotin 0.14 mg, folic acid 0.8 mg, Cu (as copper sulfate) 11 mg, Zn (as zinc sulfate) 70 mg, Fe (as ferrous sulfate) 60 mg, Mn (as manganese sulfate) 115 mg, Se (as sodium selenite) 0.30 mg, and I (as potassium iodide) 0.4 mg.*

[b]*Nutrient levels are calculated values.*

MATERIALS AND METHODS

Birds, Diets, and Management

Six hundred and forty-eight 33-week-old Lohmann laying hens with an initial egg production of 89.97% ± 6.05% were randomly

and equally distributed among the six dietary treatments. Six experimental diets were formulated based on corn and soybean meal supplemented with 0, 25, 50, 100, 200, and 400 mg/kg Bopu powder, expressed as BP0, BP25, BP50, BP100, BP200, and BP400, respectively. At the expense of corn, Bopu powder was added to the basal diet (**Table 1**). The Bopu powder consisted of 1% protopine, 0.5% allotypotopine, and 98.5% starch, was produced by the Micolta Bioresource Company Ltd. (Changsha, 410331, PR China). Each treatment had 6 replicates with 18 hens each. Replicates were equally distributed into upper, middle, and lower cage levels to minimize the replicate level effect. Three hens were housed in a 45-by-45-by-45-cm cage, with six surrounding galvanized steel cages (two cages on each floor) serving as a replicate. Ambient temperature and humidity in the laying hen barn were maintained at 23 ± 2°C and 50 to approximately 65%, respectively. The photoperiod was set to 16L:8D throughout the study. All hens were given free access to feed and water. All of the birds were fed a basal diet for 2 weeks prior to the feeding trial (33 weeks of age). The experiment lasted 8 weeks (from 35 to 42 weeks of age). During the study, the animals were housed and handled according to the Lohmann Brown Laying Hens Management Guide's guidelines.

Laying Performance and Sampling

The egg production and egg mass were recorded daily. The feed intake of each replicate was recorded weekly. The feed conversion ratio was calculated as the ratio of total feed consumed to total egg mass-produced. Egg production was expressed as an average daily production. At the end of the trial, a total of 36 hens (6 replicates/treatment, 1 hen/replicate) were randomly collected from each treatment and humanely slaughtered after a 12-h fast (water offered *ad libitum*) to collect foregut contents and hindgut contents. The collected intestinal contents were frozen immediately in liquid nitrogen, and then stored at −80°C for subsequent analyses. Before slaughter, blood was collected from the wing vein and centrifuged at 3,000 X g for 10 min to separate the serum, and then frozen at −20°C for further analysis.

Egg Quality and Antioxidant Capacity of Yolk

Eggs (six replicates/treatment, eight eggs/replicate) were collected on the final day of the experiment to measure egg quality. Egg yolk color, albumen height, and Haugh unit were evaluated using an egg multitester (EMT-7300, Robotmation Co. Ltd., Tokyo, Japan). Eggshell breaking strength was evaluated using an eggshell force gauge model II (Robotmation Co. Ltd., Tokyo, Japan). Eggshell thickness was measured at the large end, equatorial region, and small end using an eggshell thickness gauge (Robotmation Co., Ltd., Tokyo, Japan). Eggshell ratio was calculated as eggshell weight/egg weight x100. The egg shape index was calculated as the length of the egg divided by its width. Total antioxidant capacity (T-TAOC), total superoxide dismutase (T-SOD) activity, glutathione peroxidase (GSH-Px) activity, catalase (CAT) activity, and malondialdehyde (MDA) content in yolk were determined by assay kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions.

Serum Biochemical Parameters and Antioxidant Enzyme Activity

Serum total protein levels, albumin, globulin, total cholesterol (CHO), triglyceride (TG), urea, glucose (GLU), urea acid (UA), Ca, phosphorus (P), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), GSH-Px, superoxide dismutase (SOD), T-AOC, and MDA were assayed with commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's guidelines.

Intestinal Microflora DNA Extraction, Library Preparation, and Sequencing

Total microbial genomic DNA was extracted from intestinal contents using the E. Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, United States) according to the manufacturer's instructions. The quality and concentration of DNA were determined using 1.0% agarose gel electrophoresis and a NanoDrop® ND-2000 spectrophotometer (Thermo Scientific Inc., United States), which was then stored at −80°C until further use. The V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified with primer pairs 338F (5′-ACT CCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, United States). The PCR reaction mixture includes 4 μl 5 × Fast Pfu buffer, 2 μl 2.5 mM dNTPs, 0.8 μl each primer (5 μM), 0.4 μl Fast Pfu polymerase, 10 ng of template DNA, and ddH_2O to a final volume of 20 μl. The cycling conditions for PCR amplification were as follows: initial denaturation at 95°C for 3 min, followed by 27 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°Cfor 45 s, and single extension at 72°C for 10 min, and end at 4°C. All samples were amplified in triplicate. The PCR product was extracted from a 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, United States) according to the manufacturer's instructions and quantified using the Quantus™ Fluorometer (Promega, Madison, WA, United States). Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego, United States) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw sequencing reads were deposited into the NCBI Sequence Read Archive (SRA), PRJNA837752. After demultiplexing, the resulting sequences were quality filtered with fastp (0.19.6) and merged with FLASH (v1.2.11) as described previously by Chen et al. (2018) and Magoc and Salzberg (2011). Then the high-quality sequences were denoised using the DADA2 plugin in the Qiime2 (version 2020.2) as described previously by Callahan et al. (2016) and Bolyen et al. (2019) pipeline with recommended parameters,

TABLE 2 | Effect of dietary Bopu powder supplementation on the performance of laying hen.

Item		Bopu powder, mg/kg						SEM	*p* value		
		0	25	50	100	200	400		ANOVA	Linear	Quadratic
Egg production, %	Week 1 to 4	89.25	89.88	90.04	90.59	90.48	91.11	0.627	0.977	0.373	0.676
	Week 5 to 8	89.66	89.00	89.93	88.69	89.90	88.41	0.758	0.989	0.759	0.936
	Week 1 to 8	89.46	89.44	89.99	89.64	90.19	89.76	0.643	0.999	0.802	0.959
Average egg mass, g/hen/day	Week 1 to 4	51.88	53.20	52.82	52.80	53.36	53.45	0.400	0.906	0.313	0.587
	Week 5 to 8	54.54	54.94	55.66	54.76	55.26	54.23	0.461	0.967	0.857	0.479
	Week 1 to 8	53.20	54.10	54.22	53.78	54.28	53.85	0.387	0.979	0.688	0.783
Average daily feed intake, g/hen/day	Week 1 to 4	115.14	115.00	115.10	114.76	115.01	114.87	0.057	0.714	0.173	0.371
	Week 5 to 8	116.02	116.04	116.00	116.02	116.08	116.03	0.015	0.943	0.478	0.777
	Week 1 to 8	115.58	115.52	115.56	115.38	115.57	115.45	0.031	0.714	0.308	0.563
FCR, g/g	Week 1 to 4	2.23	2.17	2.18	2.19	2.16	2.16	0.017	0.840	0.266	0.497
	Week 5 to 8	2.13	2.12	2.09	2.13	2.11	2.15	0.018	0.971	0.795	0.751
	Week 1 to 8	2.18	2.14	2.14	2.16	2.13	2.15	0.016	0.966	0.662	0.740

FCR, feed conversion ratio.

which obtains single-nucleotide resolution based on error profiles within samples. DADA2 denoised sequences are usually called amplicon sequence variants (ASVs).

Statistical Analysis

Firstly, all the data were recorded and sorted in Excel. Then, data analysis of performance, egg quality, serum indices, and antioxidant capacity was performed using the IBM SPSS Statistics 22 statistical package (SPSS Inc., Chicago, IL, United States). The normality of the data was initially tested using the Shapiro–Wilk test. The data were then analyzed using one-way ANOVA and Orthogonal Polynomial Contrasts to determine linear and quadratic responses to different levels of Bopu powder. When the ANOVA showed statistical significance, Duncan's multiple range test was conducted. Differences were considered statistically significant at $p < 0.05$. The p values between 0.05 and 0.10 were considered a trend. Data were expressed as the mean and pooled SEM.

Bioinformatic analysis of the gut microbiota was carried out using the Majorbio Cloud platform (https://cloud.majorbio.com). Based on the ASVs information, rarefaction curves and alpha diversity indices including observed ASVs, Chao1 richness, ace index, Shannon index, and Simpson index were calculated with Mothur v1.30.1 (Schloss et al., 2009). Similarity among the microbial communities in different samples was determined by principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity using the Vegan v2.5-3 package. The linear discriminant analysis (LDA) effect size (LEfSe) (http://huttenhower.sph.harvard.edu/LEfSe) was performed to identify the significantly abundant taxa (phylum to genus) of bacteria among the different groups (LDA score >2, $p < 0.05$) as described previously by Segata et al. (2011).

RESULTS

Production Performance

The effect of dietary Bopu powder supplementation on the performance of laying hens was presented in **Table 2**. No mortality was found during the 8-week experimental period. The dietary Bopu powder supplementation had no significant effects on egg production, average egg weight, average daily feed intake, or feed conversion ratio.

Serum Biochemical Parameter Indices

The effect of dietary Bopu powder supplementation on serum biochemical parameters indices of laying hens was presented in **Table 3**. Serum CHO concentrations decreased (linear, $p < 0.01$) with increasing Bopu powder supplementation, whereas serum GLB and ALB concentrations increased (linear, $p < 0.05$) in laying hens. Dietary supplementation with Bopu powder had no influence on serum GLU, TG, UA, ALT, or AST levels.

Antioxidant Capacity of Serum

Table 4 shows that serum GSH-Px activity increased significantly in the Bopu powder supplemented groups (50–100 mg/kg) compared to the BP0 group ($p < 0.05$). Serum CAT activity in laying hens was increased (linear, $p < 0.05$) when Bopu powder supplementation increased.

Egg Quality and Antioxidant Capacity of the Yolk

As shown in **Table 5**, the egg Haugh units and eggshell thickness were significantly affected by dietary Bopu powder supplementation ($p < 0.05$). Compared with the BP0 group, BP25 and BP50 groups significantly increased egg Haugh units ($p < 0.05$), and BP25, BP200, and BP400 groups significantly enhanced eggshell thickness ($p < 0.05$). The effects of dietary Bopu powder supplementation on antioxidant enzyme activities in the yolk were presented in **Table 6**. The Bopu powder supplementation groups (25–100 mg/kg) had significantly higher yolk GSH-Px activity than the BP0 group ($p < 0.05$). Furthermore, the BP25 and BP50 groups exhibited significantly higher CAT activity in the yolk ($p < 0.05$) than the BP0 group; the MDA concentration was greatly lowered in the BP25 group ($p < 0.05$) but significantly increased in the BP200 group ($p < 0.05$).

TABLE 3 | Effect of dietary Bopu powder supplementation on serum biochemical parameters indices of laying hens.

Item	Bopu powder, mg/kg						SEM	*p* value		
	0	25	50	100	200	400		ANOVA	Linear	Quadratic
GLU, mmol/L	10.00	10.21	10.90	10.80	11.02	11.07	0.156	0.214	0.052	0.053
TG, mmol/L	24.31	23.89	25.82	24.76	22.05	22.17	0.637	0.530	0.117	0.298
CHO, mmol/L	5.42^{b}	5.58^{b}	5.41^{b}	5.36^{b}	5.01^{a}	4.90^{a}	0.064	<0.001	<0.001	<0.001
UA, umol/L	0.223	0.210	0.220	0.213	0.277	0.270	0.014	0.689	0.128	0.300
GLB, g/L	67.27^{b}	77.99^{a}	71.68^{ab}	67.4^{b}	70.44^{ab}	75.13^{ab}	2.673	0.045	0.017	0.337
ALB, g/L	14.91^{b}	14.13^{b}	14.20^{b}	14.04^{b}	15.48^{a}	15.48^{a}	0.195	0.038	0.021	0.077
TP, g/L	82.18^{b}	92.12^{a}	85.88^{ab}	81.44^{b}	85.92^{ab}	90.61^{a}	3.430	0.023	0.653	0.464
ALT, U/L	5.276	4.698	5.899	5.99	5.565	4.64	0.233	0.431	0.416	0.238
AST, U/L	210.43	234.47	223.27	243.33	187.67	197.83	7.22	0.180	0.112	0.293

^{a,b}Means in the same row with different superscript letters indicate differences (p < 0.05).

GLU, glucose; TG, triglyceride; CHO, cholesterol; UA, uric acid; GLB, globulin; ALB, albumin; TP, total protein; ALT, glutamic-pyruvic transaminase; AST, glutamic-oxaloacetic transaminase.

TABLE 4 | Effect of dietary Bopu powder supplementation on serum antioxidant capacity of laying hens.

Item	Bopu powder, mg/kg						SEM	*p* value		
	0	25	50	100	200	400		ANOVA	Linear	Quadratic
T-AOC, mmol/L	1.10	1.21	1.20	1.40	1.20	1.33	0.040	0.362	0.238	0.407
T-SOD, U/ml	722.32	682.78	703.92	712.93	736.85	649.18	12.94	0.475	0.238	0.172
GSH-Px, U/ml	175.86^{a}	219.36 ^{ab}	240.00^{b}	257.49^{b}	227.52 ^{ab}	256.12^{b}	88.17	0.042	0.062	0.086
GSH, umol/L	40.11	44.23	41.80	40.71	44.43	45.51	1.034	0.634	0.170	0.403
CAT, U/ml	3.8^{b}	8.0^{a}	7.9^{a}	7.6^{a}	8.8^{a}	8.1^{a}	0.110	0.001	0.032	0.585
MAD, nmol/ml	8.66	8.33	7.50	8.26	8.15	6.79	0.804	0.993	0.557	0.837

^{a,b}Means in the same row with different superscript letters indicate differences (p < 0.05).

T-AOC, total antioxidative capacity; T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase; GSH, glutathione; CAT, catalase; MAD, malondialdehyde.

TABLE 5 | Effect of dietary Bopu powder supplementation on egg quality of laying hens (8 weeks).

Item	Bopu powder, mg/kg						SEM	*p* value		
	0	25	50	100	200	400		ANOVA	Linear	Quadratic
Egg shape index	1.35	1.35	1.34	1.36	1.32	1.34	0.005	0.499	0.181	0.285
Eggshell strength, kgf	4.60	4.73	4.52	4.30	4.56	4.54	0.064	0.574	0.769	0.645
Egg weight, g	60.78	61.60	62.22	59.96	60.81	61.58	0.257	0.085	0.432	0.812
Haugh units	58.15^{b}	66.42^{a}	65.08^{a}	64.59^{ab}	58.76^{b}	58.40^{b}	1.039	0.046	0.065	0.172
Yolk color score	4.10	3.90	4.00	3.83	4.00	3.90	0.146	0.981	0.777	0.933
Yolk width, mm	39.65	40.03	38.93	40.36	39.33	40.48	0.263	0.534	0.367	0.560
Yolk height, mm	16.55	16.39	16.32	16.30	16.16	16.42	0.113	0.960	0.841	0.616
Yolk weight, %	27.19	28.20	28.53	27.78	27.47	27.39	0.214	0.431	0.365	0.630
Shell thickness, mm	0.335^{b}	0.352^{a}	0.346^{ab}	0.332^{b}	0.351^{a}	0.351^{a}	0.002	0.001	0.080	0.191
Shell weigh, %	9.36	9.47	9.44	9.79	9.80	9.54	0.080	0.513	0.416	0.150

^{a,b}Means in the same row with different superscript letters indicate differences (p < 0.05).

TABLE 6 | Effect of dietary Bopu powder supplementation on the antioxidant capacity of yolk.

Item	Bopu powder, mg/kg						SEM	*p* value		
	0	25	50	100	200	400		ANOVA	Linear	Quadratic
T-AOC, U/ml	15.57	16.38	13.09	12.24	13.55	13.55	1.014	0.866	0.010	0.064
T-SOD, U/ml	425.00	397.58	451.00	372.08	427.58	350.17	23.248	0.135	0.235	0.552
GSH-Px, U/ml	207.19^{b}	490.13^{a}	690.56^{a}	390.77^{a}	215.45^{b}	345.92^{ab}	50.955	0.034	0.130	0.268
CAT, U/ml	65.15^{b}	73.55^{a}	72.85^{a}	67.60^{ab}	69.65^{ab}	61.74^{b}	3.358	0.001	0.685	0.207
MDA, nmol/ml	204.25^{b}	144.80^{a}	170.30^{ab}	177.14^{ab}	294.16^{c}	212.00^{b}	11.810	0.001	0.004	0.033

^{a,b,c}Means in the same row with different superscript letters indicate differences (p < 0.05).

T-AOC, total antioxidative capacity; T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase; GSH, glutathione; CAT, catalase; MAD, malondial.

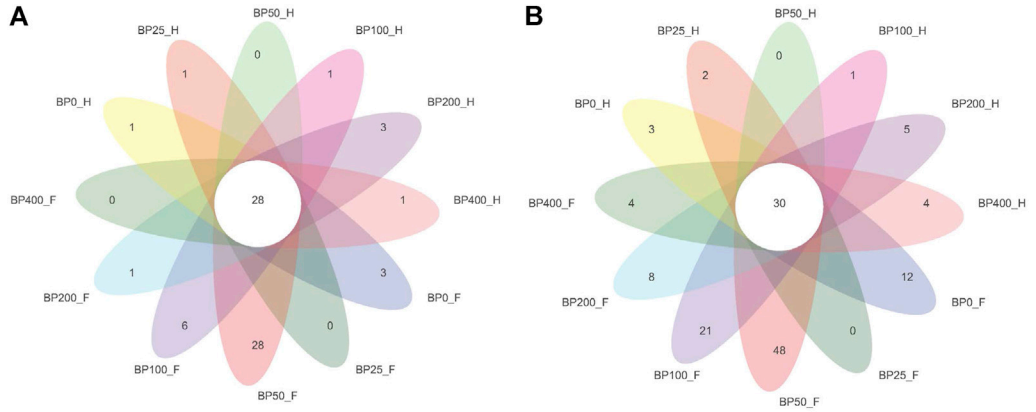

FIGURE 1 | (A) Venn diagram of intestinal microbiota at family level. **(B)** Venn diagram of intestinal microbiota at genus level.

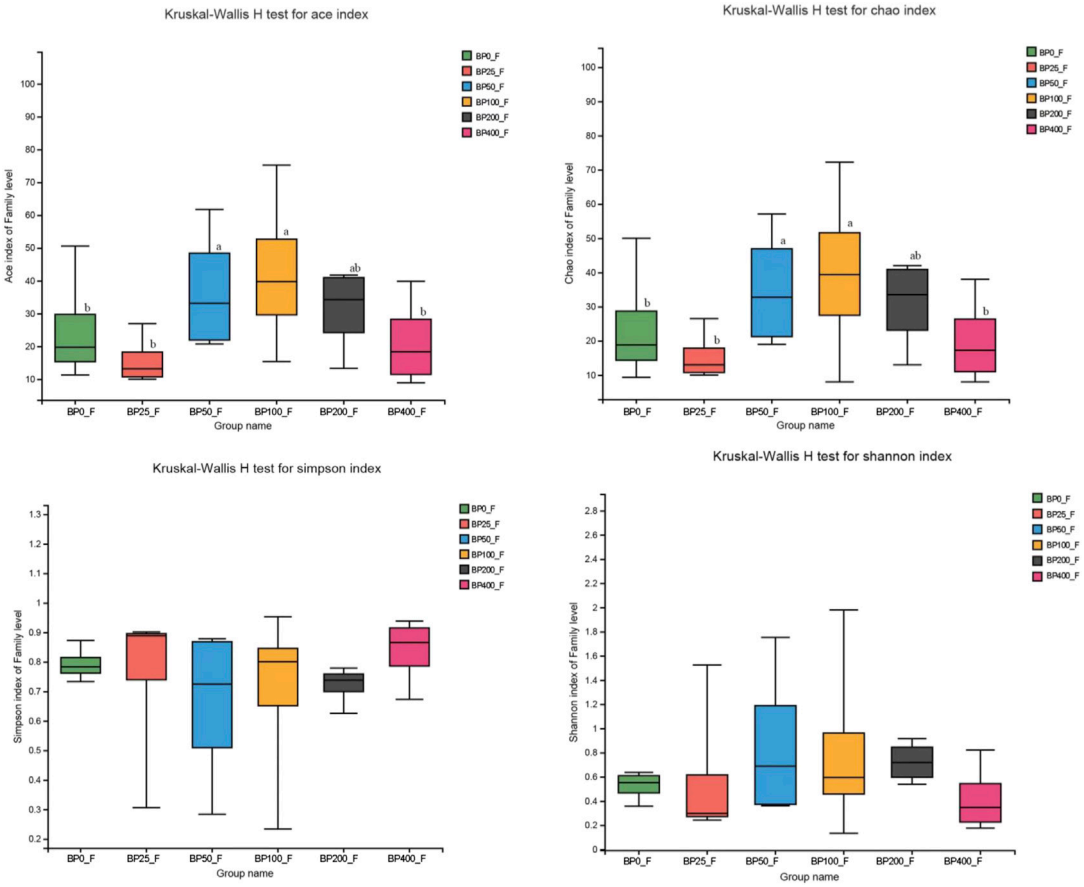

FIGURE 2 | Alpha diversity analysis in foregut.

Intestinal Microbiota

A total of 3486992 sequences in the dataset representing 3,657 ASVs were obtained after quality filtering and chimera checking, among which 25 phyla, 178 families, and 376 genera of intestinal microbiota were annotated. As shown in **Figure 1A**, in the foregut, there were 31, 28, 56, 34, 29, and 28 families and 42, 30, 78, 51, 38, and 34 genera were unique in the BP0, BP25, BP50, BP100, BP200, and BP400 groups, respectively. In the hindgut, there were 29, 29, 28, 29, 31, and 29 families, and 33, 32, 30, 31, 35, and 34 genera were unique in the BP0, BP25, BP50, BP100,

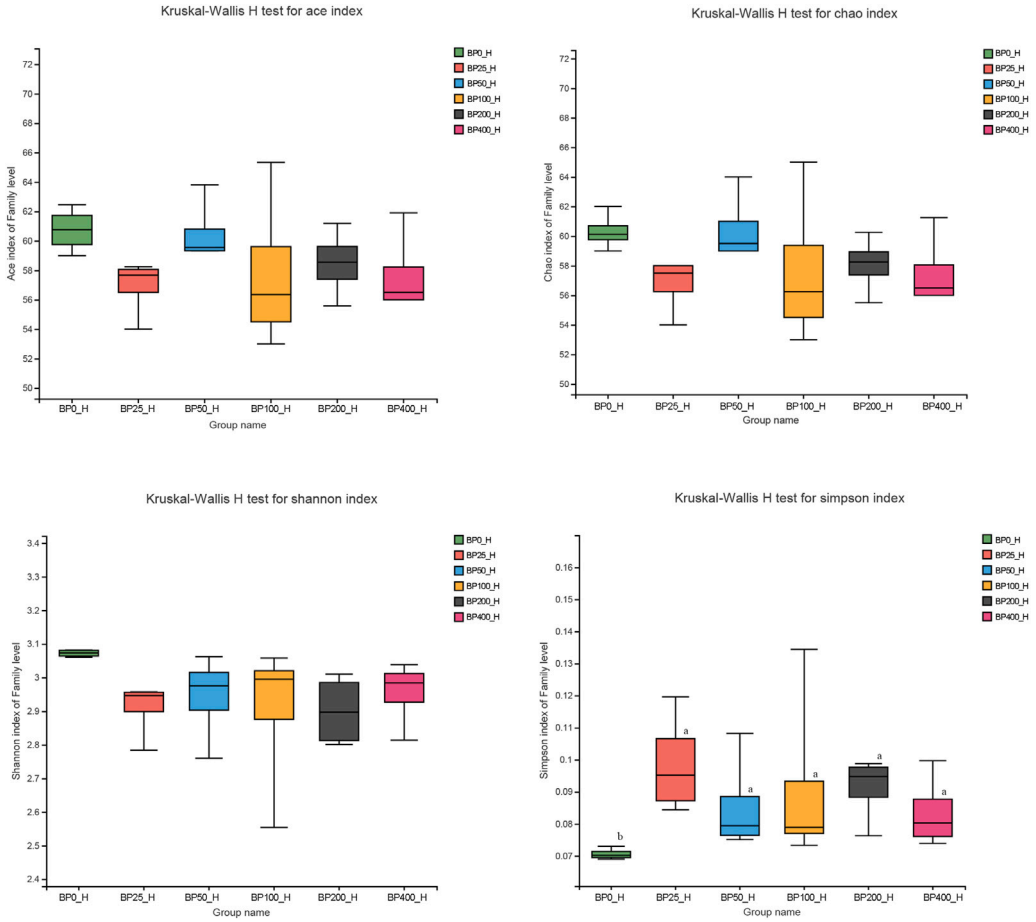

FIGURE 3 | Alpha diversity analysis in hindgut.

BP200, and BP400 groups, respectively (**Figure 1B**). The Alpha Diversity Analysis showed that the BP50 and BP100 groups exhibited higher diversity of microbiota in the foregut but there was no significant difference among the experimental groups in the hindgut (**Figures 2**, **3**). Hierarchical clustering analysis was performed according to the beta diversity distance matrix, and the UPGMA algorithm was used to construct a tree structure to analyze the degree of difference in the distribution of microbial communities in the anterior and posterior intestines. The results showed that there were significant differences between the foregut microbial communities and hindgut microbial communities (**Figure 4**). PcoA analysis of the microbial community structure of the foregut and hindgut at the family level demonstrated that Bopu powder affected the microbial community structure in the hindgut but had almost no effect on the microbial community structure of the foregut (**Figure 5**). Among all experimental groups, the distribution of microflora in the foregut and hindgut was significantly different. At the phylum level, the dominant flora in the foregut is *Firmicutes*, while the dominant flora in the hindgut is *Firmicutes* and *Bacteroidota*, and dietary supplementation with 50–400 mg/kg Bopu powder increased the relative abundance of *Bacteroidota* in the foregut (**Figure 6A**). At the family level, dietary 25–100 mg/kg Bopu powder supplementation significantly increased the relative abundance of Enterococcaceae. In the hindgut, the highest relative abundance of Bacteroidaceae and Fusobacteriaceae was found in the BP25 group, while the highest relative abundance of Lachnospiraceae was found in the BP100 group (**Figure 6B**). At the genus level, dietary 25–100 mg/kg Bopu powder supplementation significantly increased the relative abundance of *Enterococcus* in the foregut. In the hindgut, dietary supplementation of 25–50 mg/kg Bopu powder increased the relative abundance of *Bacteroides* and *Fusobacterium*, and dietary supplementation of 50–100 mg/kg Bopu powder increased the relative abundance of *Lactobacillus* (**Figure 6C**). In addition, the results of LEfSe (Linear discriminant analysis Effect Size) showed that there were significant differences in species of hindgut microbe among the experimental groups. In the hindgut, the relative abundance of Tannerellaceae and

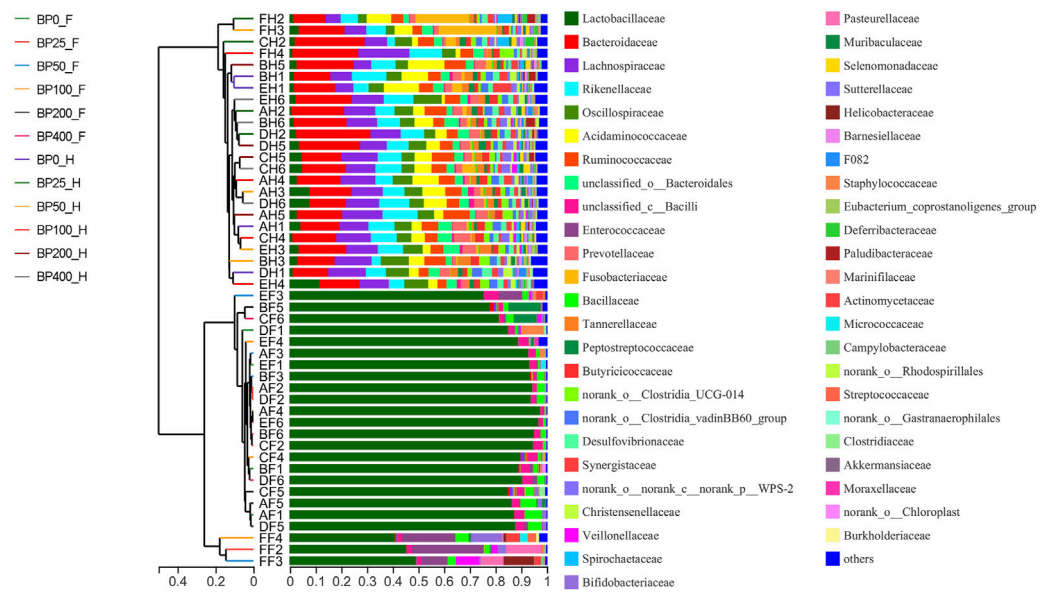

FIGURE 4 | Beta Hierarchical clustering analysis of intestinal microbiota on family level.

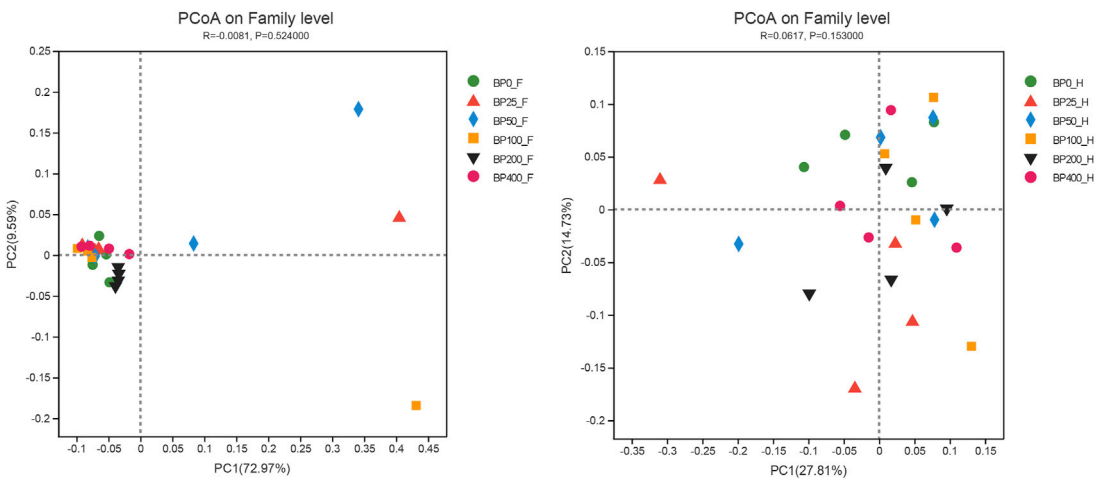

FIGURE 5 | PcoA analysis of intestinal microbiota on family level.

Muribaculaceae were significantly enriched in BP50, while BP0 significantly enriched the abundance of Desulfovibrionaceae (**Figure 7**).

DISCUSSION

When using new alkaloid resources for animal production, safety is critical. More than 290 components of *Macleaya cordata* have been discovered and/or isolated, with anti-inflammatory, anticarcinogenic, antibacterial, and insecticidal activities (Lin et al., 2018). In animal production, Isoquinoline alkaloids (sanguinarine and chelerythrine) were frequently employed as natural growth promoters with anti-inflammatory and antibacterial properties (Khadem et al., 2014; Ni et al., 2016; Liu et al., 2020). Furthermore, the main constituents of Bopu powder are protopine alkaloids (protopine and allocryptopine), which have anti-inflammatory properties and can be used to treat poultry *E. coli* diarrhea. The oral administration of LD_{50} of protopine in ICR mice was reported to be 313.10 mg/kg, and large dosages of protopine induced brain and liver damage, showing that protopine was moderately hazardous (Hu et al.,

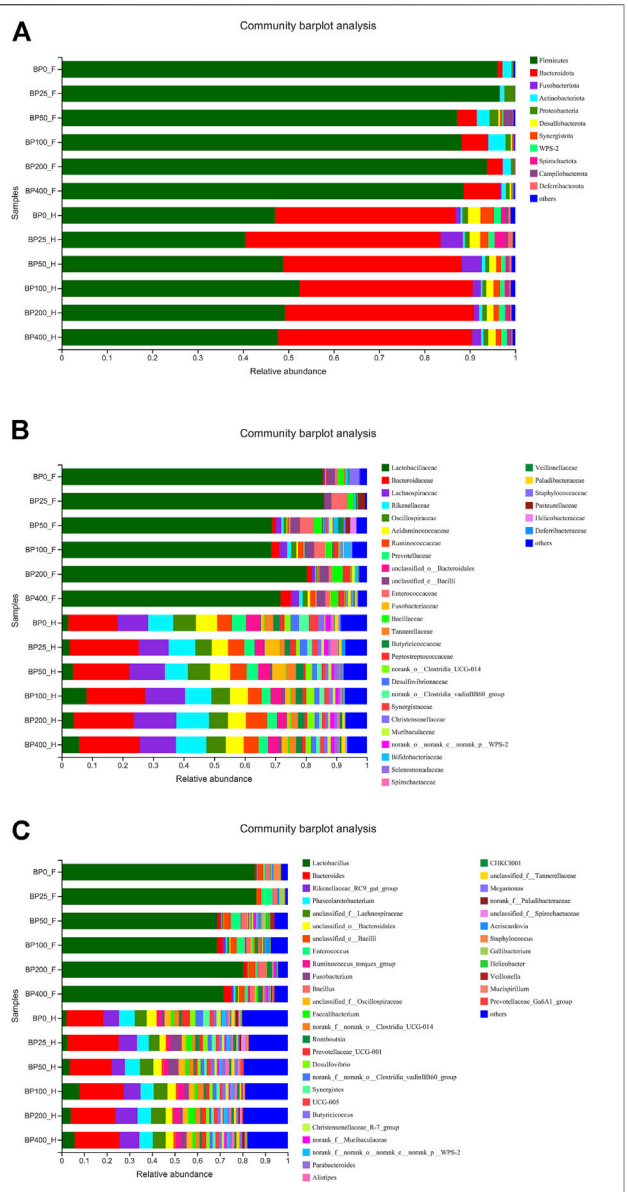

FIGURE 6 | (A) The community barplot analysis of intestinal microbiota at phylum level. **(B)** The community barplot analysis of intestinal microbiota at family level. **(C)** The community barplot analysis of intestinal microbiota at genus level.

2021). In the present study, we found no mortality or morbidity of laying hens, and there was no significant difference in production performances among the experimental groups, indicating that dietary 25–400 mg/kg Bopu powder supplementation had no adverse effects on laying hens. Similarly, a prior study indicated that supplementing broilers with benzophenanthridine and protopine in their drinking water enhanced their productive efficiency index (Previato do Amaral et al., 2016). Furthermore, daily consumption of 1.5 mg of benzophenanthridine alkaloids and protopine alkaloids reduced the unfavorable impacts of extreme heat on feedlot ewes' growth performance (Estrada-Angulo et al., 2016). This study is the first, to our knowledge, to show that dietary Bopu powder supplementation of 25–400 mg/kg has no deleterious effect on laying hens in a 56-day feeding trial.

The liver function index includes AST and ALT, which were frequently employed as indications of liver health (Diaz et al., 1999; Limdi and Hyde, 2003; Lala and Minter, 2021). Dietary Bopu powder supplementation of 25–400 mg/kg showed no influence on serum AST and ALT activity in this investigation, showing that Bopu powder supplementation had no detrimental effects on the liver of laying hens. Furthermore, serum CHO was linked to liver lipid metabolism, which was significantly elevated in fatty liver-laying hens (Harms and Simpson, 1979; Dong and Tong, 2019; Lin et al., 2021). According to the findings of this study, dietary Bopu powder supplementation significantly reduced serum CHO concentrations, suggesting that Bopu powder supplementation may improve hepatic lipid metabolism and protect the liver against fatty liver disease. Previous research found that protopine extracted from *Fumariaindica Pugsley* has the same hepatoprotective effect as the conventional medication, silymarine (Rathi et al., 2008). Similarly, nutritional supplementation with the antioxidant of plant spices such as garlic and tamarind reduced serum cholesterol in laying hens (Chowdhury et al., 2002; Chowdhury et al., 2005). Furthermore, the liver produces globulin fraction, which contains hundreds of serum proteins, carrier proteins, enzymes, complements, and immunoglobulins, except immunoglobulins, which are produced by plasma cells. In this study, dietary Bopu powder supplementation significantly elevated serum GLB and ALB concentrations, suggesting that Bopu powder could improve liver health and immunological function. It could be because protopine, which contains anti-inflammatory and antioxidant properties, can regulate hepatic lipid metabolism and increase immune cell activation. A study *in vitro* demonstrated that protopine reduced the inflammatory response in lipopolysaccharide-stimulated murine macrophages (Bae et al., 2012). Furthermore, in a carrageenan-induced mouse model, protopine attenuated inflammatory symptoms through modulation of MAPKs/NF-κB signaling cascades (Alam et al., 2019). In this study, dietary Bopu powder supplementation significantly enhanced the serum GSH-Px and catalase activities, demonstrating that Bopu powder increased the antioxidant capacity of laying hens. In line with a previous study, pretreatment of protopine increased serum superoxide dismutase activity in the middle cerebral artery occlusion in rats (Xiao et al., 2007). Similarly, Xiao et al. (2008) demonstrated that protopine relieved H_2O_2-induced oxidative stress and apoptosis in PC12 cells. Therefore, supplementation of Bopu powder might ameliorate hepatic lipid metabolism and protect the laying hens from fatty liver disease via antioxidant and anti-inflammatory mechanisms.

Egg quality, including shell and interior quality, is critical to the global egg industry. The hen's egg is made up of the yolk (30%–33%), albumen (about 60%), and the shell (9%–12%). The Haugh Units were calculated using the thickness of the albumen and the weight of an egg (Haugh, 1937). This was a key indicator of egg albumen quality and related to shelf life. In the present study, dietary 25–50 mg/kg Bopu powder supplementation significantly increased egg Haugh Units. We speculated that it might be attributed to the antioxidant activity of protopine. Meanwhile, similar results were found in a previous study, which demonstrated that dietary antioxidant tea polyphenol

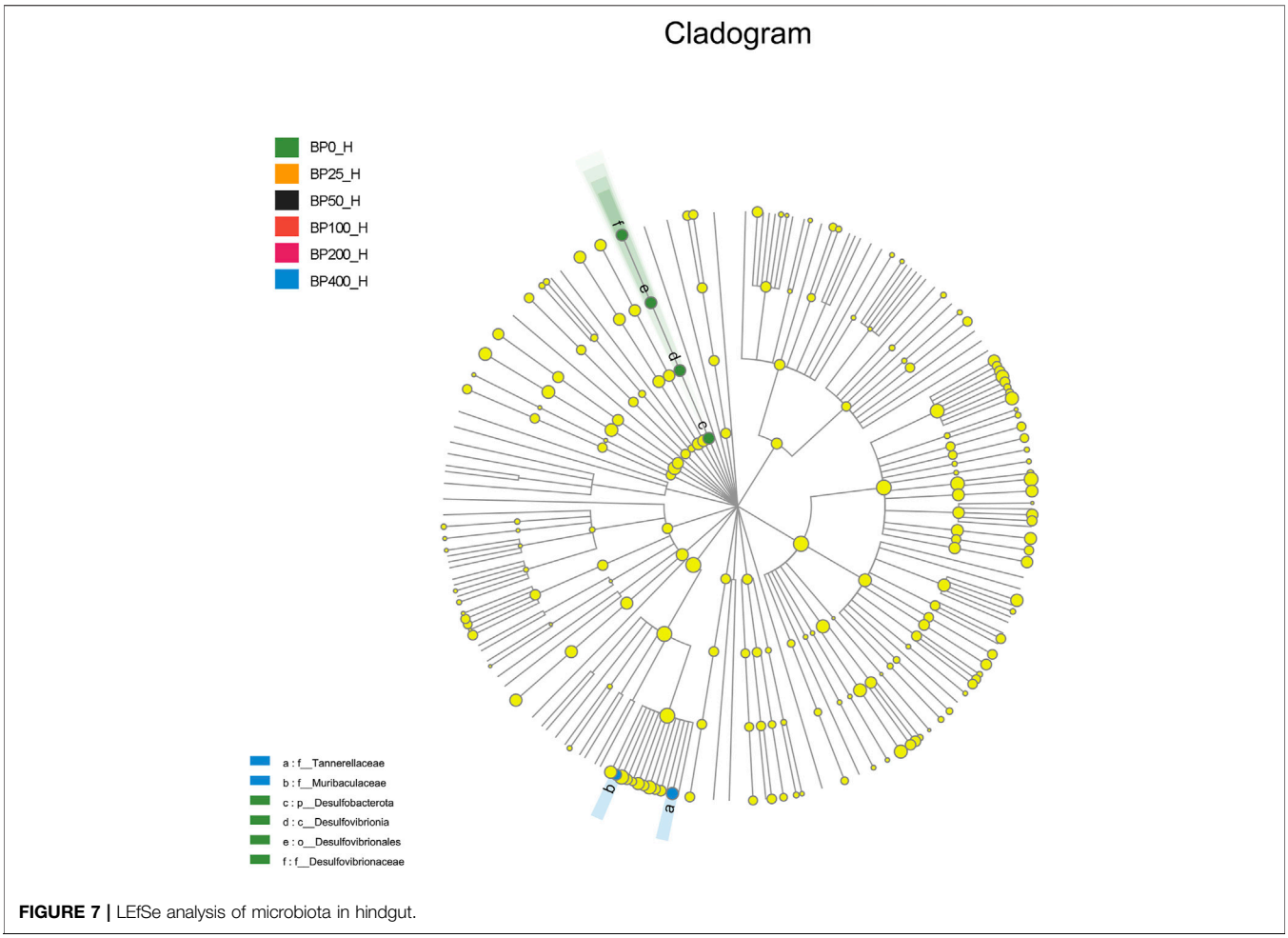

FIGURE 7 | LEfSe analysis of microbiota in hindgut.

supplementation increased the egg HU in hens during the late laying period (Wang et al., 2018). The eggshell contained mainly calcium carbonate, which was formed in the shell gland pouch for more than 15 h. In this study, dietary Bopu powder increased eggshell thickness considerably. Propine administration may have improved the health of the gut and shell gland pouch through anti-inflammatory and antioxidant processes. Similarly, Guo et al. (2021) also demonstrated that dietary *Macleaya cordata* extract (consisting of 7.5% sanguinarine) significantly increased eggshell thickness in Xuefeng black-bone chicken. Hen eggs are healthy foods with balanced nutrition, which contain high-quality proteins and lipids, trace elements, and vitamins. Thus, eggs are easily oxidized by a series of oxidative reactions during storage, resulting in a negative effect on egg nutritional values. In this study, dietary Bopu powder enhanced the antioxidant capacity of the yolk, hence improving egg quality and shelf life. We also discovered that serum antioxidant capacity was positively correlated with yolk antioxidant capacity, implying that Bopu powder supplementation could minimize oxidative stress by regulating laying hen metabolism. A recent study found that dietary antioxidants like selenium-enriched yeast or natural astaxanthin improved the antioxidant capacity of the yolk (Gao et al., 2020; Lin et al., 2020). As a result, nutritional supplementation with Bopu powder may increase egg quality by enhancing laying hen antioxidant capacity.

The gut microbiome has been identified as one of the primary elements influencing laying hen productivity and health. Previous research has linked greater gut microbiota richness and diversity to improved health and productivity (Stanley et al., 2012; Yan et al., 2017). The current study found that dietary Bopu powder supplementation improved the diversity of microbiota in the foregut, implying that supplemented Bopu power may improve the health and productivity of laying hens. Similarly, we discovered that 50 mg/kg Bopu powder administration increased the diversity of microbiota in the foregut, which was consistent with increased serum antioxidant capacity and egg quality in laying hens. We also discovered that the distribution of microbial communities differed significantly between the foregut and hindgut in this investigation. Xiao et al. (2021) discovered that the dominating bacteria in the duodenum were *Lactobacillus*, however, the dominant microorganisms in the cecum and colorectum were more complex, primarily comprising *Bacteroides*, *Odoribacter*, and *Clostridiales vadin BB60* group in laying hens. At the phylum level, *Firmicutes* was the main flora in the foregut, while *Firmicutes* and *Bacteroidota* were the dominant flora in the hindgut

in this study. The addition of 50–400 mg/kg of Bopu powder to the diet increased the relative abundance of *Bacteroidota* in the foregut. Interestingly, *Bacteroidota* is commonly seen as a "generalist" degrader of dietary fiber, implying that supplementing with Bopu powder may boost fiber fermentation and short-chain fatty acid (SCFA) production. At genus level, dietary 25–100 mg/kg Bopu powder supplementation enriched *Enterococcus* in the foregut, Bacteroidaceae, Fusobacteriaceae, and Lachnospiraceae in the hindgut. *Enterococcus* was assumed to be a natural antibacterial probiotic capable of preventing diarrhea, improving feed efficiency, and promoting animal production growth (Franz et al., 2011). Bacteroidaceae was considered as a probiotic for its ability to degrade complex polysaccharides and produce acetate, propionate, or succinate (Polansky et al., 2016; Medvecky et al., 2018). Fusobacteriaceae could modulate the growth of other bacterial species through metabolic by-products, which was helpful for the maintenance of an overall microbial structure. Similarly, Wang *et al.* (2020) found that dietary probiotic *Bacillus subtilis* supplementation increased the abundances of *Fusobacteria* phylum, *Fusobacteriia* class, Fusobacteriaceae family, and *Fusobacterium* genus and improved the performance of breeding geese during the laying period. Lachnospiraceae was involved in the production of SCFAs (butyrate, propionate, and acetate), which were important energy sources for intestinal epithelial cells (Rychlik, 2020). In recent studies, Lachnospiraceae have been shown to be enriched in long-living Italian and Chinese populations (Kong et al., 2016). Given that egg layers have a 1-year lifespan of production, enrichment with Lachnospiraceae could have a significant favorable impact on health and productivity. In addition, LEfSe analysis showed that the relative abundance of Tannerellaceae and Muribaculaceae were significantly enriched in the BP50 group, while the BP0 group significantly enriched the abundance of Desulfovibrionaceae in the hindgut. Previous studies found that the Tannerellaceae were enriched in certain gastrointestinal disorders, such as Crohn's disease, indicating that Tannerellaceae might be related to intestinal immunity (Hernández et al., 2019). Furthermore, Zhao et al. (2020) demonstrated that Tannerellaceae was negatively correlated with immune traits, whereas Muribaculaceae was positively correlated with immune traits. In the present study, Tannerellaceae and Muribaculaceae were both enriched in hindgut in the BP50 group and positively correlated with improving egg quality and antioxidant capacity of laying hens. Thus, we speculated that the enrichment abundance of Tannerellaceae and *Muribaculacea*e might improve the gut health of laying hens. Chang et al. (2022) discovered that orange corn (high carotenoids) diet significantly enriched Tannerellaceae in laying hens compared to white corn (low carotenoids) diet. Desulfovibrionaceae could release sulfate in the gut and produce hydrogen sulfide. A larger concentration of hydrogen sulfide, on the other hand, was hazardous and could cause inflammatory bowel disease in the colon (Guo et al., 2016). In laying hens with an average age of 64 weeks (Joat et al., 2021), the abundance of Desulfovibrionaceae increased significantly, showing that Desulfovibrionaceae was negatively correlated with intestinal health.

Surprisingly, dietary low-dose 25–50 mg/kg Bopu powder supplementation had a greater impact on egg quality and antioxidant status in laying hens but high-dose, 100–400 mg/kg, groups had no additive effects. The effects of Bopu powder supplementation were connected with the diversity of gut microbiota in the current investigation. It is possible that low-dose Bopu powder supplementation improved egg quality and antioxidant capacity of layers by increasing the diversity of gut microbiota and the abundance of beneficial bacteria, whereas high doses reduced the diversity of gut microbiota due to Bopu powder's antibacterial effect. Furthermore, excessive Bopu powder supplementation may raise drug metabolism stress in laying hens, thereby counteracting the synergistic effects of Bopu powder.

CONCLUSION

In the 56-day trial, dietary Bopu power supplementation of 25–400 mg/kg had no negative effects on laying hens. Furthermore, supplementing laying hens with 50 mg/kg Bopu powder improved egg quality and antioxidant capacity, which could be attributed to an increase in intestinal microbiota richness and changes in microbial composition, particularly the enrichment of *Enterococcus* in the foregut and *Bacteroidaceae*, *Fusobacteriaceae*, and *Lachnospiraceae* in the hindgut. Thus, Bopu powder may be utilized in laying hens to improve egg quality and intestinal health, and we proposed that the ideal dietary dose of Bopu powder in laying hens was 25–50 mg/kg.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The investigation plan and methods have been authorized with the guidelines of the Animal Care and Use Committee of Hunan Agricultural University. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

The research was designed and conducted by HL, QL, XbL, JZ, and JH. The animal experiment was conducted by HL, ML, XyL, and QL. The detection and analysis works were conducted by HL, QL, XbL, PH, ZY, and MC. HL and QL prepared the manuscript draft. All authors participated in the discussion and editing of the manuscript.

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Effects of *Artemisia argyi* Powder on Egg Quality, Antioxidant Capacity, and Intestinal Development of Roman Laying Hens

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This study was conducted to evaluate the effect of dietary supplementation with *Artemisia argyi* (*A. argyi*) on egg quality, serum biochemical, antioxidant capacity, and intestinal development in Roman laying hens. A total of 432 (34-week-old) Roman hens were randomly divided into control group and three experimental groups. The control group was fed a basal diet, and the experimental group was fed a basal diet with 1%, 2%, and 3% *A. argyi* powder, respectively. The results showed that dietary supplementation of 2% *A. argyi* to the diet increased egg weight and egg white weight, and the daturic acid (C17:0), stearic acid (C18:0), eicosadienoic acid (C20:2), docosahexaenoic acid (C22:6n-3), α-linolenic acid (C18:3n-3), linoleic acid (C18:2n-6c), and polyunsaturated fatty acid (PUFA) in egg yolk. Meanwhile, the addition of 1~3% *A. argyi* decreased serum urea. Moreover, dietary supplementation of 1% *A. argyi* promoted the antioxidative capacity of the hens by increasing hepatic T-SOD and CAT activities, as well as GSH-Px content. However, the addition of 3% *A. argyi* to the diet significantly increased the content of malondialdehyde in serum and liver and destroyed the intestinal morphology by increasing duodenal crypt depth. In conclusion, the addition level of *A. argyi* promoting egg quality and antioxidant capacity was at 2% and 1%, respectively.

Keywords: Artemisia argyi, laying hens, egg quality, antioxidative capacity, intestinal development

INTRODUCTION

Artemisia argyi (*A. argyi*) is a traditional Chinese herb with a history of use spanning over 2000 years. It is a perennial herb or small shrub with strong aroma. *Artemisia* belongs to the family Compositae, with more than 500 species (Abad et al., 2012). *A. argyi* contains a variety of bioactive chemicals such as polysaccharides, flavonoids, essential oils, and triterpenoids (Zhang et al., 2013). Increasing experiments have proved that *A. argyi* has many biological activities, such as antibacterial, anti-tumor, anti-oxidation, and immune regulation (Bao et al., 2013; Zhang et al., 2014). Some studies have suggested that the active components of *A. argyi* may exert anti-inflammatory effects through toll-like receptor 4 (TLR4)/nuclear factor-kappa B (NF-κB) signaling pathway (Zeng et al., 2014; Shin et al., 2017). As a plant homologous to medicine and food (Yamamoto et al., 2011; Li et al., 2018), owing to its antioxidant, antibacterial, hypoglycemic, hypolipidemic, and liver protection properties (Batiha et al., 2020), *A. argyi* can be applied in health care products and skin care products (Huang

et al., 2012). Its own nutrients and bioactive ingredients gradually saw it applied in feed additives as well. Recently, studies have shown that *A. argyi* in diets can promote organic metabolism and improve animal digestion and absorption rate and production performance (Kim et al., 2012). Research indicated that 1% addition of *Artemisia* powder increased the weight gain of broilers by 217.9 g (Kaab et al., 2022). The addition of 2% *A. argyi* powder was reported to increase the activities of catalase (CAT) and glutathione peroxidase (GSH-Px) of the liver in broilers on d 21 as well as hepatic total superoxide dismutase (T-SOD) activity on d 42 and decrease hepatic malondialdehyde (MDA) content on d 42. Adding 1% *A. argyi* powder to the diet was superior for enhancing intestinal anti-oxidative capacity, represented by increased T-SOD activity of duodenum and total antioxidant capability (T-AOC) and CAT activity of jejunum, as well as decreased MDA in duodenum and ileum (Zhang et al., 2020a). When *A. argyi* extract was added to the diet, the optimal addition for improving intestinal antioxidant systems (SOD-CAT enzyme mechanism) of broilers at 21 d and 42 d was 0.5% and 1%, respectively (Zhao et al., 2016). It shows that *A. argyi* may have potential applications as antioxidants and production enhancers.

At present, there are more studies of *A. argyi* on broiler chickens, with relatively few focusing on laying hens. However, finding a natural feed additive which can effectively improve egg quality and release antioxidant stress contributes to the production of characteristic brand eggs, in line with people's pursuit of healthy food. Therefore, the present study was conducted to investigate the effect of different levels of *A. argyi* on egg quality, serum biochemical indexes, antioxidant function, and intestinal mucosa morphological structure in Roman layers and its reasonable level of addition, so as to provide an experimental basis for the application of *A. argyi* in poultry production.

MATERIALS AND METHODS

Preparation of *Artemisia argyi* powder

Fresh green *A. argyi* was collected from Hunan in July. Plant materials were washed with distilled water and dried in the shade at room temperature. The dry material was then cut into 1–2 cm pieces, crushed by a grinder, and sieved through an 80-mesh sieve to obtain the powder, which was then stored at ambient temperature (22–25°C) pending its use. The chemical constituents of A. argyi powder (analyzed value) are: genal energy 19.19 MJ/kg, dry matter 91.56%, crude protein 17.71%, crude fat 4.41%, crude ash 10.47%, crude fiber 16.64%, calcium 1.19%, and total phosphorus 0.28%.

Animals and experimental details

A total of 432 (34-week-old) Roman hens were randomly divided into control group and three experimental groups with six replicates and 18 birds each. The control group was fed a basal diet, and the experimental group was fed a basal diet with 1%, 2%, and 3% *A. argyi* powder, respectively. The trial lasted for 8 weeks including the 2-week adaptation period. The basic diet was formulated to contain similar levels of CP and to meet recommendations of the National Research Council (NRC) for laying hens (1994), whose composition and nutritional level are shown in **Table 1**.

TABLE 1 | Composition and nutrient levels of basal diets (air-dry basis, %).

Items	Diets			
	0.00%	1.00%	2.00%	3.00%
Ingredients				
Corn	57.57	55.49	53.32	51.19
Soybean meal	30.01	30.37	30.77	31.15
Artemisia argyi powder	0.00	1.00	2.00	3.00
Oil	0.97	1.69	2.46	3.21
Limestone	8.45	8.45	8.45	8.45
3% Premix[a]	3.00	3.00	3.00	3.00
Total	100.00	100.00	100.00	100.00
Nutrient levels[b]				
ME/(Mcal/kg)	2.75	2.75	2.75	2.75
Crude protein	17.00	17.00	17.00	17.00
Crude fiber	3.04	3.04	3.05	3.05
Calcium	3.50	3.50	3.50	3.50
Total phosphorus	0.54	0.53	0.53	0.52
Available phosphorus	0.32	0.32	0.32	0.32
Lysine	0.95	0.95	0.96	0.96
Methionine	0.36	0.36	0.36	0.36
Methionine + Cystine	0.65	0.65	0.65	0.64

[a]*The premix provided the following (per kilogram of complete diet) micronutrients: VA, 6 000 IU, VD_3 2 500 IU, VE, 25 mg, VK_3 2.25 mg, VB_1 1.8 mg, VB_2 7 mg, VB_6 4 mg, VB_{12} 0.2 mg, D-pantothenic acid 12 mg, nicotinic acid 35 mg, biotin 0.14 mg, folic acid 0.8 mg, Cu (as copper sulfate) 11 mg, Zn (as zinc sulfate) 70 mg, Fe (as ferrous sulfate) 60 mg, Mn (as manganese sulfate) 115 mg, Se (as sodium selenite) 0.30 mg, and I (as potassium iodide) 0.4 mg.*

[b]*Nutrient levels are calculated values.*

The experiment was carried out in two-layer full-step type chicken cages (1.2 m × 1.2 m × 0.5 m), with one cage for each repetition, and each group was evenly distributed in 1–2 layers. Chickens were fed twice a day (07:00 and 15:30) freely and drunk water through a nipple-type dispenser, and were provided with natural lighting and artificial lighting in the morning and evening to ensure the constant 16 h-lighting time every day. Infrared heating devices were used to maintain the temperature automatically. Humidity was controlled at about 65%. All eggs were collected on time at 12:00 and 17:00. The immunization procedure was carried out in accordance with the provisions of chicken farm epidemic prevention.

At the end of the experiment, blood samples were taken from a vein in the hen's wing (two birds/replicate, 12 birds/treatment). The whole blood was coagulated in a test tube at room temperature and centrifuged at 3,500 rpm for 15 min. Serum samples were separated and stored at −20 °C until it was used to measure antioxidant and biochemical indicators. After blood collection, the hens were euthanized by carbon inhalation. After the abdominal cavity was opened, the tissues of small intestine (duodenum, jejunum, and ileum) were cut into 3–5 cm pieces and fixed in 10% neutral formalin solution. The liver tissue samples were taken at 2 g, kept in the centrifuge tube, and frozen at −20 °C for later analysis.

Egg quality and yolk fatty acid measurements

The egg quality and yolk fatty acid was measured as previously described (Liu et al., 2021). Egg shape index was calculated as the ratio of the vertical and horizontal diameter of the egg. Eggshell strength was measured by the eggshell strength tester (EFR-01, Orka Co., Ltd.). Eggshell thickness (no shell membrane) was measured through the average values of three different sites (top, middle, and bottom of egg) using the eggshell thickness tester (NFN-380, Japan FHK Co., Ltd.). Haugh unit and yolk color were determined by SONOVA automatic egg quality analyzer (Orka Food Technology Ltd, Ramat Hasharon, Israel). After weighing the eggs on an electronic balance, the yolks and whites were separated and weighed to calculate the ratio of yolks and egg whites.

After been frozen, 5 g of egg yolk sample was put into the filter cartridge with 20 g sea sand. The filter cartridge was placed in the Sodel extractor and then refluxed in 65°C anhydrous ether bath until completely extracted. Then 3 ml of boron trifluoride-methanol was esteraped at 90°C for 7 min. The fatty acid content was detected using a HP-7890 gas chromatograph (HP, Uited States), a hydrogen flame ionization detector (FID), and a HPINNOWAX capillary column (30 m × 0.25 mm × 0.25μm).

Serum biochemical indices

The contents of total protein (TP), glucose (Glu), albumin (ALB), globulin (GLB), and uric acid (UA) in serum were detected by Mindray BS-200 automatic biochemical analyzer (Shenzhen Mindray Biomedical Electronics Co., Ltd.) and kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The determination method was operated in strict accordance with the instructions of the kit.

Antioxidant indices determination

T-SOD, T-AOC, GSH-Px, CAT, and MDA of the serum and liver were measured using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's recommendations. The total protein content in the liver was determined by Coomassie brilliant blue method.

Intestinal morphology analysis

Intestine samples (duodenum, jejunum, and ileum) were collected and fixed by 4% paraformaldehyde, dehydrated by alcohol, embedded by paraffin, and made into continuous sections for hematoxylin and eosin (H&E) staining. Then images were collected by Image-Pro Plus 4.5 software (Media Cybernetics Inc. Shanghai, China) for measuring the villus height and crypt depth to calculate the ratio of villus height to crypt depth.

Statistical analysis

The test data were expressed as mean and standard error of mean (SEM). The four treatments means were compared by one-way analysis of variance (ANOVA) and tested by Duncan's multiple range. $p < 0.05$ was regarded as statistically significant. Statistical model is as follows:

$Y_{ij} = \mu + T_i + e_{ijk}$, where, Y_{ij} means individual observed value, μ is the overall mean, T_i represents the treatmenI (i = 1, 2, 3, 4) effect, and e_{ijk} indicates a random error.

RESULTS

The addition of *A. argyi* increased egg weight, egg white, and polyunsaturated fatty acid (PUFA) in yolk, especially 2% *A. argyi* addition

The effect of dietary *A. argyi* on egg parameters in laying hens is shown in **Table 2**. Inclusion of *A. argyi* in the diet had no effect on any egg parameters, except egg weight and egg white weight. The addition of 2% and 3% *A. argyi* to the diet significantly increased egg weight and reduced shell thickness ($p < 0.05$). In addition, 2% *A. argyi* in the diet significantly increased the egg white weight ($p < 0.05$).

The effect of dietary *A. argyi* on fatty acid composition in egg yolk is shown in **Table 3**. The proportion of heptadecanoic acid (C17:0) ($p < 0.01$), eicosadienoic acid (C20:2) ($p < 0.01$), docosahexaenoic acid (C22:6n-3) ($p < 0.01$), α-linolenic acid (C18:3n-3) ($p < 0.05$), linoleic acid (C18:2n-6c) ($p < 0.05$), and polyunsaturated fatty acid (PUFA) ($p < 0.05$) significantly increased as the *A. argyi* addition in the diet increased from 0% to 2%, but when it increased to 3%, these acids no longer increased but decreased. The stearic acid (C18:0) proportion of 2% *A. argyi* addition group was higher than other three groups ($p < 0.05$). The C18:1n-9t proportion of 2% *A. argyi* addition was significantly higher than that of 1% and 3% *A. argyi* addition ($p < 0.01$). No obvious effect of diet was observed on other fatty acids ($p > 0.05$).

The addition of *A. argyi* significantly decreased the amount of UA in serum

As shown in **Table 4**, inclusion of *A. argyi* in the diet had no apparent effect on GLU, TP, ALB, and GLB in serum ($p > 0.05$). However, the addition of *A. argyi* significantly decreased the amount of UA in serum ($p < 0.05$).

The addition of 2% *A. argyi* enhanced the antioxidant capacity in body, but 3% *A. argyi* addition had the opposite effect

Table 5 demonstrated the effect of dietary *A. argyi* on serum antioxidant indices in laying hens. Composed to other three groups, the GSH-Px of 2% *A. argyi* addition in the diet was remarkably raised ($p < 0.05$). And for the group of 3% *A. argyi* addition, its serum GSH-Px was significantly lower than other three groups ($p < 0.05$), while its serum MDA was significantly higher than the other three groups ($p < 0.05$).

The addition of 1% *A. argyi* had the most beneficial impact on liver antioxidant indices, 2% addition secondly, 3% addition inversely

As shown in **Table 6**, the CAT amount in liver of 1% *A. argyi* addition ($p < 0.05$) group and the MDA amount in liver of 3% *A.*

TABLE 2 | Effects of Artemisia argyi Meal on Fatty Acid Composition in Egg Yolk of Roman Laying Hens (%).

actItems	Artemisia argyi powder level				SEM	*p*-value
	0%	1%	2%	3%		
Average egg weight/g	57.51b	57.99b	63.23a	63.46a	0.88	<0.01
Shell strength/(kg/cm^2)	4.59	5.04	4.82	4.86	0.12	0.66
Shell thickness/mm	0.38	0.37	0.37	0.36	0.00	0.41
Haugh unit	69.06	74.41	66.30	72.43	1.35	0.15
Shape index	1.33	1.34	1.31	1.32	0.01	0.36
Egg yolk color	4.38	4.50	4.20	4.00	0.09	0.20
Egg yolk weight/g	15.97	14.95	16.47	15.71	0.27	0.32
Egg white weight/g	35.60b	37.28b	49.03a	39.25b	1.56	<0.01
Egg yolk relative weight/%	28.44	25.77	25.99	25.82	0.43	0.07
Egg white relative weight/%	63.15	64.29	64.08	64.25	0.50	0.85

TABLE 3 | Effects of Artemisia argyi Meal on Fatty Acid Composition in Egg Yolk of Roman Laying Hens (%).

Items	Artemisia argyi powder level				SEM	*p*-value
	0%	1%	2%	3%		
Saturated fatty acid (SFA), %						
C12:0	0.00	0.00	0.01	0.00	0.01	0.51
C14:0	0.34	0.34	0.33	0.28	0.04	0.37
C15:0	0.04	0.05	0.04	0.05	0.01	0.11
C16:0	25.60	24.77	24.40	24.39	1.21	0.67
C17:0	0.13c	0.16ab	0.17a	0.15bc	0.02	0.01
C18:0	8.57b	8.29b	9.62a	8.21b	0.60	0.01
C20:0	0.03	0.00	0.01	0.01	0.01	0.11
C22:0	0.05	0.05	0.05	0.02	0.02	0.08
Total SFA	34.71	33.55	31.32	33.08	0.64	0.28
Monounsaturated fatty acid (MUFA), %						
C14:1	0.09	0.08	0.07	0.05	0.03	0.61
C16:1	3.11	3.53	2.72	2.64	1.07	0.88
C20:1	0.23	0.20	0.21	0.21	0.02	0.61
C18:1n-9t	0.14ab	0.12bc	0.16a	0.10c	0.02	<0.01
C18:1n-9c	40.81	38.70	37.51	37.67	2.05	0.19
Total MUFA	43.34	41.46	40.61	40.66	0.86	0.70
Polyunsaturated fatty acid (PUFA), %						
C20:2	0.15c	0.19bc	0.23a	0.20b	0.03	<0.01
C22:6n-3	1.13c	1.34b	1.60a	1.43ab	0.20	<0.01
C18:3n-3	0.54c	0.66bc	0.97a	0.79ab	0.18	0.01
C18:3n-6	0.15	0.14	0.12	0.14	0.03	0.4
C18:2n-6c	14.84b	18.48ab	22.17a	20.45a	3.28	0.03
C20:3n-6	0.33	0.25	0.26	0.27	0.05	0.12
C20:4n-6	2.97	3.02	2.71	2.97	0.18	0.15
Total PUFA	20.12b	23.57ab	28.10a	26.24a	1.13	0.02

TABLE 4 | Effects of Artemisia argyi on Serum Biochemical Indices of Roman Laying Hens.

Items	Artemisia argyi powder level				SEM	*p*-value
	0%	1%	2%	3%		
GLU (mmol/L)	12.04	12.60	10.53	11.47	0.56	0.76
TP (g/L)	72.44	72.39	72.85	77.79	2.24	0.91
ALB (g/L)	23.88	23.28	23.52	23.68	0.56	0.99
GLB (g/L)	52.97	54.97	52.55	53.32	2.08	0.98
UA (μmol/L)	93.99a	67.81b	66.97b	63.54b	4.99b	0.04

TABLE 5 | Effects of Artemisia argyi on Serum Antioxidant Indices of Roman Laying Hens.

Items	Artemisia argyi powder level				SEM	*p*-value
	0%	1%	2%	3%		
T-AOC/(U/ml)	0.41	0.44	0.49	0.53	0.02	0.20
T-SOD/(U/ml)	22.73	26.50	44.37	37.87	4.00	0.22
GSH-Px/(U/ml)	282.04b	258.20b	389.27a	144.43c	33.14	<0.01
CAT/(U/ml)	15.51	13.91	14.39	14.69	0.39	0.56
MDA/(nmol/ml)	1.42b	1.42b	1.42b	2.99a	0.20	<0.01

TABLE 6 | Effects of Artemisia argyi on liver Antioxidant Indices of Roman Laying Hens.

Items	Artemisia argyi powder level				SEM	*p*-value
	0%	1%	2%	3%		
T-AOC/(U/ml)	0.48	0.44	0.43	0.45	0.02	0.85
T-SOD/(U/g)	190.63ab	267.63a	265.63a	140.85b	19.71	0.05
GSH-Px/(U/g)	15.40bc	22.71a	20.22ab	11.75c	1.57	0.02
CAT/(U/g)	23.90b	31.95a	24.43b	23.93b	1.49	0.05
MDA/(nmol/g)	36.31b	45.31b	44.75b	79.01a	5.51	<0.01

argyi addition group ($p < 0.05$) were the highest among the four groups, respectively. The T-SOD and GSH-Px amounts in liver of 3% *A. argyi* addition were both below those of 1% and 2% *A. argyi* addition ($p < 0.05$), and the GSH-Px of 0% *A. argyi* addition was also significantly lower than that of 1% *A. argyi* addition ($p < 0.05$).

The addition of 3% *A. argyi* impaired the morphological structure of the intestine

Figure 1 showed the morphological structure of the intestine. As shown in **Table 7**, dietary *A. argyi* significantly increased crypt depth of duodenum in laying hens ($p < 0.05$). Composed to the addition of 1% and 2% *A. argyi*, villus height in jejunum of 3% *A. argyi* addition was significantly reduced ($p < 0.05$). In ileum, villus height of 2% *A. argyi* addition was significantly higher than that of 1% and 3% *A. argyi* addition ($p < 0.05$).

TABLE 7 | Effects of Artemisia argyi on Intestinal Histological Morphology of Roman Laying Hens (μm).

Items	Artemisia argyi powder level				SEM	*p*-value
	0%	1%	2%	3%		
Duodenum						
Villus height	515.20	551.30	553.51	642.30	21.07	0.15
Crypt depth	57.61b	78.46a	72.79a	75.18a	3.30	0.05
Villus height/crypt depth	8.94	7.02	7.65	8.56	0.34	0.16
Jejunum						
Villus height	514.33ab	585.57a	575.49a	448.46b	22.12	0.03
Crypt depth	76.23	65.88	63.76	71.30	2.37	0.30
Villus height/crypt depth	6.76	6.95	9.02	6.29	0.53	0.37
Ileum						
Villus height	306.10ab	260.81b	383.62a	241.79b	21.19	0.05
Crypt depth	42.18	45.71	47.26	43.92	1.28	0.57
Villus height/crypt depth	7.26	5.70	8.11	5.68	0.43	0.92

DISCUSSION

Egg weight is a key indicator reflecting production performance of hens. Total egg weight is influenced by egg production rate, average egg weight, egg breakage rate, and other indicators, thus it is a comprehensive indicator reflecting the overall production performance of hens. The results of this experiment showed that the addition of 2% and 3% *A. argyi* to the diet increased egg weight and egg white weight to different degrees. Fatty acids are important lipid molecules in cells, which participate in many biological processes *in vivo*. Studies have shown that the dietary fatty acids composition is an important factor affecting human health, for instance, excessive intake of saturated fatty acids (SFAs) and cholesterol will cause inflammation and insulin resistance (Grundy, 1987), whereas PUFAs have positive biological functions. PUFAs are the main fatty acids in *A. argyi*, accounting for about 52.1%, followed by SFAs and monounsaturated fatty acids (MUFAs) (40.8% and 7.1% respectively) (Song et al., 2019). In the present study, additive 2% and 3% *A. argyi* significantly increased the eicosadienoic acid (C20:2), docosahexaenoic acid (C22:6n-3), α-linolenic acid (C18:3n-3), linoleic acid (C18:2n-6c), and PUFA contents in egg yolk, particularly the 2% group which increased the most. Kim et al. (2002) also found dietary wormwood increased omega-3 fatty acid contents of loin in beef cattle. According to Kim et al. (2015), linolenic acid (C18:3) is the most abundant fatty acid in *A. argyi*, with a relative percentage content of 36.36%. It was followed by palmitic acid (C16:0) and linoleic acid (C18:2) with 18.82% and 15.73%, respectively. The increased proportion of unsaturated fatty acids in egg yolks indicated the possibility that *A. argyi* favorably regulated the nutrient composition of egg yolks to some extent, pointing out a direction for subsequent studies to improve the fatty acid composition of eggs.

Early studies have shown that liver and kidney are the main organs of uric acid production in animals, and the production capacity of liver is higher than that of kidney in poultry (McFarland and Coon, 1984). UA is the end-product of amino acid metabolism in poultry, mainly generated from the degradation of proteins and nucleic acids and excreted by the kidneys. The UA content in serum reflects the balance of amino acids in diet and the level of protein catabolism in poultry kidney. Lower UA content means lower nitrogen excretion and more nitrogen deposition, which is beneficial in improving laying performance and protecting the liver and kidneys. In this experiment, the downward content of UA in each group after adding *A. argyi* indicated that *A. argyi* supplement can improve the utilization rate of protein and amino acids and promotes protein synthesis in laying hens, which may account for enhanced egg weight and egg white weight.

Redox homeostasis is of vital importance to cells, tissues, and organs of the body, and its maintenance depends mainly on the dynamic balance between oxidative and antioxidant systems. Once this dynamic balance is disrupted, oxidative stress occurs due to excessive reactive oxygen species (ROS) production or insufficient antioxidant capacity of the body (Yin et al., 2014). ROS can cause peroxidative damage to cell membrane lipids and produce MDA to attack polyunsaturated fatty acids in biological cell membranes, which can further trigger lipid peroxidation and lead to more severe oxidative stress damage (Shi et al., 2006). Therefore, MDA is often regarded as a marker of lipid oxidative damage. CAT and SOD play a crucial role in protecting the body from oxidative damage, with SOD converting ROS to H_2O_2 and CAT converting H_2O_2 to O_2 and H_2O (Finkel and Holbrook, 2000). GSH-Px has been found to scavenge superoxide and lipid hydroperoxide radicals (Arthur, 2001). The present study revealed that the addition of 1% of *A. argyi* to the diet increased hepatic CAT and GSH-Px content. Zhang et al. (2020a) also observed the same results in broiler chickens when adding 2% *A. argyi* powder to the diet. They also found T-SOD increased and MDA decreased in liver. This benefit might be attributed to the high antioxidant content of *A. argyi*, such as total phenolic compounds, which is positively associated with antioxidant activity (Song et al., 2019). Researchers found that the bioactive components (polysaccharides, flavonoids, and polyphenols) contained in *A. argyi* have a strong ability to scavenge free radicals (Lan et al., 2010; Melguizo-Melguizo et al., 2014; Han et al., 2017). Nevertheless, when the addition amount reached 3% in the present study, MDA levels in liver and serum were significantly increased while serum GSH-Px levels were significantly reduced. However, there was a report that 1%

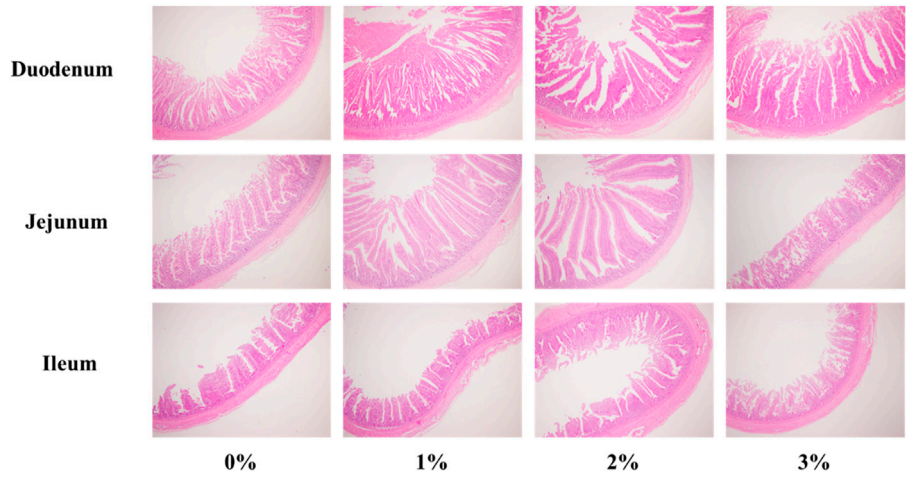

FIGURE 1 | Effects of Artemisia argyi on intestinal morphology of duodenum, jejunum and ileum of Romance laying hens. HE 4 × 10.

A. argyi extract supplementation tended to increase the activities of serum GSH-Px and CAT, but significantly reduced serum MDA level in broilers (Zhang et al., 2020b). These results illustrated that laying hens may have a greater sensitivity to *A. argyi* than broilers and a 1% addition helps to enhance the antioxidant function, but when increased to 3%, this function is impaired. The above results showed that the *A. argyi* addition of 1% is superior to 2% and 3% on improving the antioxidant capacity of laying hens.

The villi height and crypt depth of the intestine play an important role in nutrient absorption and providing a protective barrier (Liu et al., 2012). The villi is an important component of the intestinal tract, whose height reflects the absorption capacity of the small intestine (Pluske et al., 1997). Therefore, villi height, crypt depth, and the ratio of villi height to crypt depth are key indicators for assessing intestinal health and function (Liu et al., 2012). In the current trial, compared with the control group, the addition of 3% *A. argyi* to the diet significantly increased the crypt depth of the duodenum and tended to decrease the villus height of the jejunum and ileum. Chu and Song (2012) reported that more than 8% addition of wormwood decreased growth performance of rats on account of decreased nutrient digestibility. Thus, *A. argyi* seems to be potentially toxic. The results showed that when the addition of *A. argyi* reaches 3%, it destroys the morphological structure of the intestinal tract, which is not conducive to the absorption of nutrients.

CONCLUSION

In conclusion, dietary supplementation with 2% *A. argyi* was beneficial for improving egg quality and increasing polyunsaturated fatty acids in egg yolk. As far as enhancing the antioxidant function, the 1% addition was better. The addition of 3% reduced the antioxidant capacity of the organism and damaged the morphological structure of the intestine.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

All the experiment procedures were reviewed and approved by the Animal Care Committee of the Institute, Changsha Medical University, Changsha, China.

AUTHOR CONTRIBUTIONS

Conceptualization and validation, JC, SP, and YO; formal analysis, SP and YO; investigation and data curation, JC and FC; resources, QL, YL, and BH; writing—original draft preparation, JC and FC; writing—review and editing and project administration, QL and YL; funding acquisition, QL, YL, and BH. All authors have read and agreed to the published version of the manuscript.

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