



Impacts of Chive (*Allium schoenoprasum*) Extract on Performance, Egg Quality, Blood Biochemistry, and Visceral Organ Characteristics in Laying Hens

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ABSTRACT

The overuse of antibiotics in poultry production has intensified the studies for plant-derived antioxidants that can sustain productive performance while improving product quality and bird health status. Chive (*Allium schoenoprasum*) bulbs are sources of quercetin and sulfur compounds with documented immunomodulatory effects in broiler chickens, but evidence regarding their effects in laying hens is limited. This study assessed the impact of dietary chive bulb extract (CBE) on productive performance (egg production, egg weight, egg mass, feed intake, feed conversion ratio), egg quality (Haugh unit, yolk color, shell performance), serum biochemistry (lipid profile, liver enzymes, protein fractions, minerals, immunoglobulins), immune-related organs (spleen, liver) and selected visceral organ characteristics (heart, crop, jejunum, ileum, caeca, abdominal fat) in laying hens. Seventy-two 50-week-old CP Hy-Line Brown laying hens (1.91 ± 0.18 kg) were randomly assigned to four dietary treatments containing 0, 0.5, 1.0, or 1.5% CBE (dry matter basis). Each treatment consisted of three replicates with six hens per replicate. The feeding trial lasted eight weeks after a one-week adaptation period. Egg production, egg weight, egg mass, and feed conversion ratio were not affected by CBE, although feed intake increased and was highest at 1.5% CBE. Supplementation with 1.5% CBE significantly increased the Haugh unit of freshly laid eggs without altering yolk color, eggshell color, thickness, or breaking strength compared to the control group. CBE at all levels did not influence immunoglobulin concentrations, visceral organ weights, or intestinal pH and length, indicating an absence of adverse effects on health status. In contrast, CBE improved aspects of the serum lipid profile by reducing low-density lipoprotein (LDL) plus very low-density lipoprotein (VLDL) at 1.0% inclusion and decreasing high-density lipoprotein (HDL) cholesterol at 1.5% inclusion. Additionally, CBE lowered alanine aminotransferase (ALT) activity in the 1.0% and 1.5% groups compared to the control, and elevated serum phosphate concentration in all supplemented groups relative to the control. Overall, dietary inclusion of up to 1.5% CBE in laying hens enhanced egg albumen quality and modulated some serum lipid and mineral profiles without compromising productive performance or organ integrity in laying hens.

Keywords: Chive, Egg production, Egg quality, Laying hen

INTRODUCTION

Eggs are affordable, nutrient-dense foods, and their consumption is rising globally, particularly in low and middle-income countries where eggs support nutrition and food security (Morris et al., 2018). This growing demand has been supported by intensive layer production systems, which are often accompanied by a higher incidence of infectious and zoonotic diseases (Bissong et al., 2022). Antibiotics in feed have long been used to control disease,

boost egg output, and enhance feed utilization efficiency (Abreu et al., 2023). However, concerns about antimicrobial resistance and residues in poultry products have led many countries to restrict or ban antibiotic growth promoters (Wallinga et al., 2022). Under these constraints, the identification of safe and effective alternatives that maintain performance and product quality has become a priority, and phytogetic feed additives have received increasing attention.

To bridge the previous context, plant-derived alternatives can address both disease control and oxidative challenges in poultry. Oxidative stress constrains poultry production by compromising health, performance, and product quality (Oke et al., 2024). Dietary antioxidants are therefore added to neutralize free radicals and limit oxidative damage (Surai, 2020). Synthetic antioxidants (for example, butylated hydroxytoluene and tertiary butyl hydroquinone) are effective, but their long-term safety has been debated due to potential toxicological concerns (Nabavi et al., 2014). This has stimulated interest in plant-derived antioxidants as substitutes for synthetic compounds (Pitino et al., 2021). Quercetin, a flavanol abundant in fruits and vegetables, shows strong antioxidant activity, delays lipid oxidation in meat and other products (Pool et al., 2012), and together with related flavanols from *Allium* species modulates inflammatory and metabolic pathways in animals, supporting the potential utility of a functional feed additive (Kothari et al., 2019).

Chive (*Allium schoenoprasum*), a low-cost specialty crop in central Vietnam, is rich in quercetin and other flavonoids, as well as sulfur-containing compounds (Phan et al., 2020). Chive bulb extracts have improved immune Newcastle virus and growth performance of broiler chickens (Hai and Hoa, 2020), indicating their potential as locally available antioxidant and immunomodulatory feed ingredients. However, information on chive-based products in laying hens is scarce. Layers differ from broilers in lifespan, physiology, and production objectives, so responses to feed additives cannot be assumed to be the same across poultry types. In particular, additional data are needed to clarify whether dietary chive-derived products can influence laying performance, egg quality traits, and physiological indicators such as serum biochemical and immune indices, without adverse effects on organ development under antibiotic-restricted production conditions.

Given these knowledge gaps, this study aimed to evaluate the effects of a chive bulb extract (CBE) on productive performance, egg quality and preservation, serum biochemical and immune indices, and organ traits in laying hens.

MATERIALS AND METHODS

Ethical approval

All procedures involving animals complied with Article 72 of the Vietnamese Law on Animal Husbandry (No. 32/2018/QH14). The research protocol was reviewed

and approved by the institutional authority at Hue University, Vietnam (Approval No.: HUVNO28).

Chive bulb extract

Organic chive bulbs (*Allium schoenoprasum*) were obtained from Dien Mon, Phong Dien, and Hue (Central Vietnam), cleaned and sorted to remove defective bulbs. Juice was extracted using a juicer and filtered through a double-layer mesh. The chive suspension was mixed with soybean powder (6:4, weight/weight), dried at 45°C for 38 hours in a freeze dryer, and ground to obtain chive bulb powder (CBP; Phan et al., 2020). Proximate composition was analyzed according to AOAC (2006), and total polyphenols and quercetin were quantified using the Folin-Ciocalteu assay (Kavalcová et al., 2014; Table 1). Diets were prepared by mixing the required CBP level into the basal diet for 30 min in a feed mixer before bagging.

Table 1. Approximate chemical composition of the chive bulb extract

Ingredient	Content (g/100 g)
Crude protein	9.98
Crude fat	6.97
Crude fiber	8.05
Ash	2.01
Calcium	0.07
Phosphorus	0.17
Total polyphenol	0.38
Quercetin	0.36

Hens, housing, and experimental design

Seventy-two brown CP-Hy-Line laying hens (CP Vietnam Corporation) were randomly assigned to four dietary treatments with three replicates of six hens each. Hens were housed in two-tier stainless-steel cages (6 hens/cage; 98 × 45 × 42 cm). A corn-soybean basal diet was formulated to meet or exceed the Vietnamese National Feed Standards for Poultry (Code: TCVN 2265:2020; Table 2). Feed was provided twice daily (07:00 and 15:00 h), and water was available *ad libitum* via nipple drinkers. House temperature and relative humidity were maintained at 24 ± 2°C and 60–70%, respectively. A 16-hour light and 8-hour dark lighting program was applied (05:00-21:00 hours), with light intensity maintained at approximately 10–15 lux. Hens followed a commercial vaccination program, including vaccines against Marek's disease (Boehringer Ingelheim, USA; subcutaneous at 1 day old, no booster), Newcastle

disease (AVAC, Vietnam; ocular at 5 days old, boosters every 3 months), fowl pox (Ceva, France; subcutaneous at 7 days old, no booster), and avian encephalomyelitis (Ceva, France; ocular at 10 weeks old). Chive bulb powder (dry matter basis) was incorporated into the corn–soybean basal diet at 0, 0.5, 1.0, or 1.5% (w/w) by replacing an equivalent proportion of the basal diet. The calculated amount of powder was first premixed with a small portion of basal feed and then mixed thoroughly for 5 min in a vertical feed mixer to ensure homogeneity throughout the feed. After a 1-week adaptation period, the feeding trial lasted 8 weeks.

Table 2. Ingredients and chemical composition of the basal diet for hens

Ingredient (%)	Content
Corn	50.57
Dried distillers' soluble corn grains	24.54
Limestone	9.94
Soybean flour	8.03
Rapeseed meal	0.32
Sesame seed powder	2.12
Feather meal	1.6
Animal fat	0.8
Dicalcium phosphate	0.62
Synthetic lysine sulfate	0.43
Synthetic methionine (liquid)	0.18
Synthetic threonine	0.04
Salt	0.25
Mineral premix ¹	0.22
Vitamin mix ²	0.13
Sodium bicarbonate	0.15
Choline-chloride	0.06
Calculated value	
True metabolizable energy (kcal/kg)	2.800
Crude protein (%)	17.00
Crude fat (%)	5.40
Crude fiber (%)	3.60
Calcium (%)	4.00
Lysin (%)	0.84
Total sulfur amino acids (%)	0.71
Available phosphor (%)	0.28

¹Mineral Premix/ per kilogram of the diet; Fe: 35 mg; Zn: 20 mg; Mn: 25 mg; Cu: 15 mg; Co: 0.2 mg; Se: 0.2 mg; ²Vitamin/kilogram of the diet; A: 45,000 IU; D₃: 9,000 IU; E: 15 IU; Nicotinic acid, 60 mg; Pantothenic acid: 20 mg; VB₁, 5 mg; B₂: 13 mg; B₆, 5 mg; K₃: 4 mg; Folic acid: 2 mg; B₁₂: 0.03 mg

Productive performance

Eggs (including broken ones) were collected and recorded daily per replicate. Hen-day egg production (%), average egg weight (g), daily egg mass (g/hen/day), weekly feed intake (g/hen/day), and feed conversion ratio (FCR; g feed/g egg mass) were calculated using standard procedures (Bell and Weaver, 2002).

Egg quality

Each week, 20 eggs per treatment (5 per replicate) were randomly selected for analysis. Egg weight (g) was measured using a digital scale (Ohaus, USA); eggshell breaking strength (kg/cm²) was assessed with an Imada EG001 tester (Imada, Japan); albumen height (mm) was determined using a digital micrometer (Mitutoyo, Japan), and Haugh unit (HU) was calculated as $100 \times \log(H + 7.58 - 1.7W^{0.38})$, where H is albumen height (mm) and W is egg weight (g, Eisen et al., 1962). Eggshell colour was evaluated using a Samyang color fan (Samyang, South Korea), yolk color with a Roche color fan (Roche, Switzerland), and shell thickness (mm) at the equator with a digital gauge (Mitutoyo, Japan) according to the equipment manufacturer's instructions.

Egg storage stability

At the end of the trial, normal eggs collected during four consecutive days were stored at 20°C (room temperature simulation) to assess storage stability. A total of 75 eggs of similar weight were used to determine HU after 1, 3, and 5 weeks of storage.

Blood sampling and analyses

After 8 weeks, five hens per treatment (1.91 ± 0.18 kg) were selected for blood sampling. Approximately 10 mL of blood was taken from the jugular vein into serum separator tubes (BD Vacutainer, USA). Serum was separated by centrifugation (2,000 rpm, 15 min) and stored at -20°C. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TG), total cholesterol (TC), albumin, globulin, creatinine, calcium, phosphate, and amylase were determined using an automated analyser and commercial kits (Roche Diagnostics, Germany). High-density lipoprotein (HDL) cholesterol (mg/dL) was measured with a specific HDL kit (Roche Diagnostics, Germany). Low-density lipoprotein plus very low-density lipoprotein (LDL+VLDL) cholesterol was calculated as TC - HDL after precipitation with phosphotungstic acid and dextran sulfate (Friedewald et al., 1972). Serum IgA, IgM, and IgG were quantified by ELISA (MyBioSource, USA) from standard curves (450 nm).

Organ characteristics and intestinal pH

Following blood collection, selected hens were euthanized humanely by cervical dislocation. Visceral organs (liver, spleen [immune organ], heart, crop, jejunum, ileum, caeca, abdominal fat, and ovaries) were excised and weighed (g). Relative organ weights (g/100 g body weight) and intestinal lengths (cm/100 g body weight) were calculated. After removing the mesentery, jejunal and ileal lengths were measured, and digesta pH in both segments was determined using a digital pH meter (Hanna Instruments, USA).

Statistical analysis

All data were analyzed using IBM SPSS Statistics software (version 21.0; IBM). The experiment was arranged as a completely randomized design with four dietary treatments. Treatment effects were evaluated by one-way analysis of variance (ANOVA). Results are presented as treatment means \pm standard deviation (SD), and statistical significance was indicated at $p < 0.05$.

RESULTS

Productive performance

Dietary CBP did not affect hen-day egg production, egg weight, egg mass, or FCR during the time of experiment ($p > 0.05$; Table 3). In contrast, feed intake increased with rising CBP inclusion ($p < 0.05$), and hens fed 1.5% CBP consumed more feed than the CON group (124.08 vs. 120.97 g/hen per day; $p < 0.05$).

Egg quality

The supplementation of CBP affected albumen quality but not external egg traits (Table 4). Haugh unit differed

among treatments and was higher in hens receiving 1.5% CBP (92.20 ± 8.05) compared to the control (89.65 ± 8.95) and 0.5% CBP groups (87.12 ± 10.59 ; $p < 0.05$). Eggshell colour, yolk colour, shell breaking strength, and shell thickness were not altered by CBP inclusion ($p > 0.05$).

Serum biochemical and immune parameters

Most serum biochemical variables (TG, TC, AST, creatinine, calcium, albumin, globulin, and amylase) were unaffected by CBP ($p > 0.05$; Table 5). However, CBP modified components of the lipid profile, including HDL and LDL+VLDL cholesterol differed among treatments ($p < 0.05$).

Specifically, LDL+VLDL cholesterol was lowest in the 1.0% CBP group (90.26 ± 5.37 mg/dL) compared to the control (105.09 ± 7.13 mg/dL; $p < 0.05$), while HDL cholesterol decreased in the 1.5% CBP group (9.01 ± 2.73 mg/dL) relative to the control (11.58 ± 1.57 mg/dL; $p < 0.05$). Alanine aminotransferase (ALT) activity was lower in hens fed 1.0% and 1.5% CBP compared to the control ($p < 0.05$). Serum phosphate concentration was higher in all CBP-fed groups than in the control ($p < 0.05$). Concentrations of IgA, IgG, and IgM did not differ among dietary treatments ($p > 0.05$; Table 6).

Organ characteristics and intestinal traits

Relative weights of the liver, spleen, heart, crop, jejunum, ileum, caeca, and abdominal fat were not significantly affected by CBP ($p > 0.05$), although liver weight tended to be lower in supplemented groups ($p > 0.05$; Table 7). Jejunal and ileal pH, absolute length, and length relative to body weight also remained unchanged across treatments ($p > 0.05$; Table 8).

Table 3. Effects of chive bulb powder supplementation on productive performance in laying hens over 8 weeks

Parameters	CON	CBP (0.5%)	CBP (1.0 %)	CBP (1.5%)	p -value
Egg production (%)	79.21 \pm 5.95	82.52 \pm 4.22	81.52 \pm 4.19	83.01 \pm 7.04	0.13
Egg weight (g)	65.05 \pm 1.55	65.11 \pm 0.56	65.16 \pm 0.43	64.34 \pm 0.96	0.89
Egg weight (g/hen/day)	51.21 \pm 5.11	55.46 \pm 0.88	55.42 \pm 0.89	54.11 \pm 4.43	0.16
Feed intake (g/hen/day)	120.97 \pm 1.65 ^b	122.03 \pm 1.15 ^{ab}	122.53 \pm 1.25 ^{ab}	124.08 \pm 0.67 ^a	0.04
FCR (g feed/egg)	2.31 \pm 0.24	2.20 \pm 0.05	2.21 \pm 0.05	2.29 \pm 0.30	0.18

^{a,b} Different superscript letters in the same row show statistically significant difference at $p < 0.05$. FCR: Feed conversion ratio. Hens in the CON, CBP (0.5%), CBP (1.0%), and CBP (1.5%) groups were fed the basal diet supplemented with 0, 0.5%, 1.0%, and 1.5% chive bulb powder, respectively.

Table 4. Effects of chive bulb powder supplementation on egg quality in laying hens over 8 weeks

Parameters	CON	CBP (0.5%)	CBP (1.0%)	CBP (1.5%)	p-value
Haugh unit	89.65 \pm 8.95 ^{ab}	87.12 \pm 10.59 ^b	89.62 \pm 1.22 ^{ab}	92.20 \pm 8.05 ^a	0.012
Eggshell color	11.77 \pm 1.47	11.65 \pm 1.39	11.68 \pm 1.48	11.84 \pm 1.35	0.69
Egg yolk color	7.82 \pm 0.57	8.01 \pm 0.61	8.03 \pm 0.57	7.96 \pm 0.61	0.27
Eggshell thickness (mm)	0.41 \pm 0.05	0.41 \pm 0.06	2.18 \pm 0.05	0.81 \pm 0.06	0.38
Egg breakage resistance (kg/cm ²)	2.91 \pm 0.82	3.18 \pm 0.79	2.91 \pm 0.82	2.98 \pm 0.86	0.08

^{a,b} Different superscript letters in the same row show statistically significant difference at $p < 0.05$. Hens in the CON, CBP (0.5%), CBP (1.0%), and CBP (1.5%) groups were fed the basal diet supplemented with 0, 0.5%, 1.0%, and 1.5% chive bulb powder, respectively.

Table 5. Effects of chive bulb powder supplementation on blood characteristics in laying hens over 8 weeks

Parameters	CON	CBP (0.5%)	CBP (1.0 %)	CBP (1.5%)	p-value
Blood lipid					
TG (mg/dL)	1.209.37±96.17	893.04 ± 54.75	991.00 ± 61.72	1.026.40 ± 78.73	0.092
TC (mg/dL)	120.66 ± 21.66	108.19 ± 18.36	111.29 ± 18.32	116.00 ± 6.95	0.552
HDL (mg/dL)	11.58 ^a ± 1.57	11.75 ^a ± 2.46	11.05 ^a ± 2.14	9.01 ^b ± 2.73	0.043
LDL+VLDL (mg/dL)	105.09 ^a ± 7.13	101.26 ^a ± 6.35	90.26 ^b ± 5.37	104.92 ^a ± 6.55	0.032
Serum protein					
Albumin (g/dL)	2.61 ± 0.18	2.59 ± 0.24	2.76 ± 0.28	2.61 ± 0.24	0.764
Globulin (g/dL)	6.67 ± 0.32	3.12 ± 0.29	3.72 ± 0.27	2.84 ± 0.43	0.355
Mineral (Mineral metabolism)					
Calcium (mg/dL)	20.48 ± 2.18	21.56 ± 1.61	20.51 ± 1.51	20.60 ± 1.56	0.625
Phosphate (mg/dL)	5.31 ± 0.82 ^b	6.26 ± 0.69 ^{ab}	5.91 ± 0.62 ^{ab}	6.91 ± 0.95 ^a	0.034
Liver enzyme index					
AST (U/L)	151.03 ± 7.38	152.77 ± 9.75	154.15 ± 8.72	162.40 ± 11.25	0.182
ALT (U/L)	4.38 ^a ± 0.47	3.75 ^{ab} ± 0.85	3.45 ^b ± 0.75	3.33 ^b ± 0.74	0.031
Kidney function					
Creatinine (mg/dL)	0.26 ± 0.09	0.28 ± 0.07	0.27 ± 0.06	0.25 ± 0.08	0.856
Pancreatic enzyme					
Amylase (U/L)	290.20 ± 61.38	292.2 ± 59.88	290.1 ± 47.28	278.92 ± 51.92	0.726

^{a,b} Different superscript letters in the same row show statistically significant difference at $p < 0.05$. Abbreviations: TG: Triglycerides; TC: Total cholesterol; HDL: High-density lipoprotein; LDL+VLDL: Low-density lipoprotein plus very low-density lipoprotein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase. CBP (0.5%), CBP (1.0%), and CBP (1.5%) groups fed the basal diet supplemented with 0, 0.5%, 1.0%, and 1.5% chive bulb powder, respectively.

Table 6. Effects of chive bulb powder supplementation on immunoglobulin levels (optical density at 450 nm) in laying hens over 8 weeks

Parameter	CON	CBP (0.5%)	CBP (1.0%)	CBP (1.5%)	p-value
IgA	183.21 ± 9.45	179.35 ± 10.76	192.46 ± 13.73	185.21 ± 10.65	0.772
IgG	35.23 ± 13.33	32.66 ± 11.17	33.29 ± 11.13	29.71 ± 13.25	0.682
IgM	33.91 ± 5.11	44.21 ± 8.83	41.19 ± 7.85	42.12 ± 14.33	0.261

IgA: Immunoglobulin A; IgG: Immunoglobulin G; IgM: Immunoglobulin M. Hens in the CON, CBP (0.5%), CBP (1.0%), and CBP (1.5%) groups were fed the basal diet supplemented with 0, 0.5%, 1.0%, and 1.5% chive bulb powder, respectively.

Table 7. Effects of chive bulb powder supplementation on the relative organ weight (g) in CP Hy-Line laying hens over 8 weeks

Organ	CON	CBP (0.5%)	CBP (1.0%)	CBP (1.5%)	p-value
Heart	0.43 ± 0.05	0.42 ± 0.05	0.47 ± 0.05	0.45 ± 0.05	0.66
Spleen	0.12 ± 0.05	0.09 ± 0.04	0.12 ± 0.04	0.10 ± 0.04	0.96
Liver	1.59 ± 0.27	1.62 ± 0.15	1.51 ± 0.16	1.42 ± 0.25	0.08
Crop	1.52 ± 0.13	1.61 ± 0.24	1.60 ± 0.24	1.59 ± 0.14	0.47
Jejunum	0.71 ± 0.12	0.61 ± 0.16	0.68 ± 0.16	0.66 ± 0.13	0.35
Ileal	0.58 ± 0.14	0.56 ± 0.15	0.58 ± 0.15	0.51 ± 0.06	0.45
Cecum	0.29 ± 0.06	0.27 ± 0.05	0.28 ± 0.06	0.27 ± 0.07	0.73
Abdominal fat	4.63 ± 1.25	3.77 ± 1.04	3.88 ± 1.05	4.35 ± 0.85	0.26

Hens in the CON, CBP (0.5%), CBP (1.0%), and CBP (1.5%) groups fed the basal diet supplemented with 0, 0.5%, 1.0% and 1.5% chive bulb powder, respectively.

Table 8. Effects of chive bulb powder supplementation on intestinal traits in laying hens over 8 weeks

Parameter	CON	CBP (0.5%)	CBP (1.0%)	CBP (1.5%)	p-value
Length of jejunum (cm)	58.79 ± 2.44	57.43 ± 3.37	58.12 ± 4.34	56.885 ± 5.23	0.13
Length of jejunum/100 g body weight	3.22 ± 0.33	3.14 ± 0.22	3.13 ± 0.35	3.13 ± 0.41	0.41
Length of ileum (cm)	52.31 ± 5.21	50.88 ± 4.03	53.63 ± 5.75	54.23 ± 4.14	0.76
Ileal length/100 g body weight	2.88 ± 0.22	3.01 ± 0.26	3.10 ± 0.31	2.87 ± 0.31	0.87
pH of jejunum	5.12 ± 0.26	5.38 ± 0.21	5.31 ± 0.18	5.22 ± 0.26	0.43
pH of the ileal	5.88 ± 0.27	5.86 ± 0.42	5.78 ± 0.29	5.44 ± 0.45	0.59

Hens in the CONCBP (0.5%), CBP (1.0%), and CBP (1.5%) groups fed the basal diet supplemented with 0, 0.5%, 1.0% and 1.5% chive bulb powder, respectively.

DISCUSSION

This study evaluated the effects of CBP for laying hens under antibiotic-free conditions. Overall, dietary CBP up to 1.5% maintained productive performance, improved albumen quality, and modulated selected blood variables without adverse effects on organ characteristics.

Chive bulb powder had no influence on hen-day egg production, egg weight, egg mass, or feed conversion ratio, suggesting that the tested levels neither hindered nor boosted these metrics. This is consistent with reports in broilers supplemented with similar *Allium*-derived extracts, onion powder, which also maintained production parameters without significant alterations (Kim et al., 2013; Goodarzi et al., 2014). The higher feed intake observed at increasing CBP inclusions may be linked to improved palatability and aroma, in line with findings for other *Allium*-derived additives, particularly onion (*Allium cepa*) powders in broilers (Goodarzi et al., 2014), and suggests that CBP is well accepted by laying hens.

Chive bulb powder did not influence yolk colour or eggshell traits, corroborating earlier work with phytochemical mixtures and herbal powders like garlic (*Allium sativum*) powder, another *Allium*-based phytochemical additive in layer hens (Kim and Paik, 2008; Li et al., 2016). In contrast, the 1.5% CBP diet increased the Haugh unit, indicating improved albumen quality. This enhancement is plausibly related to the antioxidant activity of chive polyphenols and quercetin, which could protect albumen proteins from oxidative damage (Radwan et al., 2008). However, CBP did not prevent the decline in HU during storage, implying limited efficacy against age-related albumen thinning under ambient conditions.

Serum biochemical data show that CBP selectively affected lipid and liver-related indices while leaving most parameters unchanged. Triglycerides, total cholesterol, AST, creatinine, calcium, albumin, globulin, and amylase were similar among treatments, indicating preserved metabolic and hepatic function. At 1.0% CBP, LDL and VLDL cholesterol were reduced, whereas HDL decreased

at 1.5% compared to the control, indicating a dose-dependent but not uniformly favorable modulation of lipoproteins. These modulations were due to *Allium*-based supplements, which can influence cholesterol pathways via quercetin-mediated inhibition of hepatic lipid synthesis (Goodarzi et al., 2014; Pitino et al., 2021). Alanine aminotransferase activity declined in the 1.0% and 1.5% groups relative to the control, potentially indicating reduced hepatic stress, although this enzyme alone is not liver-specific and requires corroboration with additional markers like aspartate aminotransferase (Kim and Lillehoj, 2019). Serum phosphate rose across all CBE groups compared to the control, which holds value for mineral balance in laying hens, possibly aiding eggshell calcification and bone integrity (Miles et al., 1983).

Unchanged relative weights of major organs and intestinal segments, as well as stable jejunal and ileal pH and length, indicate that CBP did not induce morphological adaptations or toxicity in the gastrointestinal tract or viscera. Similarly, the lack of effect on circulating IgA, IgG, and IgM suggests that no humoral immune stimulation under non-challenged conditions CBP in hens. This contrasts with previous work showing immune enhancement by *Allium schoenoprasum* in broilers (Hai and Hoa, 2020), likely reflecting differences in bird type, age, environmental challenge, and possibly the need for higher doses or longer exposure to elicit measurable immunomodulation.

CONCLUSION

Dietary supplementation of CBP up to 1.5% maintained productive performance in laying hens, including egg production and feed efficiency, while eliciting a modest increase in feed intake, and significantly enhancing albumen quality. Furthermore, CBP positively modulated selected serum indices (lipoprotein fractions, alanine aminotransferase, and phosphate) without measurable changes in organ characteristics or circulating immunoglobulins. These findings support CBP as a viable

phytogenic additive for sustainable egg production in industry. Future studies should investigate its long-term impact on egg oxidative stability and efficacy under commercial challenge conditions.

DECLARATIONS

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Availability of data and materials

The raw data supporting the conclusions of this article is kept by the project and will be made available by the authors on request.

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Authors' contribution

Phan Vu Hai authored the original text, contributed to the experimental design, reviewed and approved the final version of the manuscript for publication. Nguyen Dinh Vinh and Hoang Van Son contributed to both the statistical analysis and the design of the experiments. All authors have reviewed and approved the final version of the manuscript for publication.

Competing interests

The authors have declared that no competing interests exist.

Ethical considerations

The authors affirm that they have adhered to ethical research practices, avoiding plagiarism, misconduct, data fabrication or falsification, and duplicate submission or publication, and have provided their consent for this article's publication. No AI tools were used to conduct and prepare this study.

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