Research Article

Effects of *Cordyceps militaris* Roots Supplementation in Diet on Laying Hen Performance, Egg Quality, and Yolk Lipid Oxidation

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Abstract | The nutritional composition of poultry feed plays a pivotal role in determining the quality and functional value of chicken eggs. This study investigated the effects of supplementing laying hen diets with *Cordyceps militaris* roots, a bioactive-rich by-product of fungal cultivation on productive performance, egg quality, and yolk lipid peroxidation. A completely randomized design was employed with five dietary treatments containing increasing levels of *Cordyceps militaris* roots powder, administered over an 8-week period. Hen productivity parameters were recorded daily, while egg quality traits were assessed weekly. Antioxidant activity in egg yolks was evaluated at baseline, week 4, and week 8 using the TBARS assay. The findings revealed that *Cordyceps militaris* roots supplementation significantly improved key reproductive performance indicators, including egg number, laying rate, egg mass, and feed conversion efficiency. Notably, yolk pigmentation was enhanced dose-dependently, with the greatest improvement observed at 15–20 g/kg supplementation levels. Furthermore, elevated levels of *Cordyceps militaris* roots in the feed were associated with a significant reduction in malondialdehyde (MDA) levels ($P < 0.05$), indicating enhanced antioxidant protection in egg yolks. In summary, incorporating *Cordyceps militaris* roots into the diets of laying hens not only improved egg production and yolk pigmentation but also fortified the antioxidant defences of the yolk. These outcomes suggest promising applications of this natural supplement in poultry nutrition strategies aimed at producing value-added, health-enhancing eggs for human consumption.

Keywords | *Cordyceps militaris*, Performance, Egg quality, Yolk pigmentation, Antioxidant activity, Laying hens

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INTRODUCTION

Eggs are one of the most widely consumed and nutritionally dense foods, providing key nutrients to meet daily dietary requirements for humans. In recent years, with improvements in living standards and increasing consumer demand not only for quantity but also for high-quality and functional foods, the production of nutritionally enriched eggs has garnered significant attention from both researchers and producers. Particularly, the enrichment of eggs through dietary supplementation in laying hens has become a focal point in efforts to enhance egg quality. Numerous studies have demonstrated that the inclusion of various functional supplements in the diets of laying hens can significantly improve the nutritional value of eggs, aligning with the specific benefits of the supplements used (Hu *et al*., 2011; Abou-Elkhair *et al*., 2018; Vui *et al*., 2024). As a result, the nutritional composition of eggs is closely influenced by the nutritional profile of the hens' diet.

Cordyceps militaris, a fungus belonging to the class Ascomycetes, has long been utilized in traditional East

Asian medicine and functional foods (Paterson, 2008). It is rich in biologically active compounds such as cordycepin, polysaccharides, adenosine, glycolipids (cerebrosides), glycoproteins (lectins), D-mannitol (also known as cordycepic acid), xanthophylls including carotenoids (lutein and zeaxanthin), sterols (ergosterol), statins (lovastatin), phenolic compounds (including phenolic acids and flavonoids), as well as essential vitamins and minerals like magnesium, potassium, selenium, and sulfur (Jędrejko *et al*., 2021).

Among these, cordycepin, a nucleoside analogy, has been widely studied for its broad-spectrum biological activities, including anti-tumor (Jin *et al*., 2018), antibacterial and antifungal (Ahn *et al*., 2000; Wang *et al*., 2021), anti-inflammatory (Tan *et al*., 2020), and antioxidant properties (Ramesh *et al*., 2012; Tong He *et al*., 2019). Polysaccharides derived from *Cordyceps militaris* have also demonstrated antioxidant, anti-tumor, anti-inflammatory, and lipid-lowering effects (Miao *et al*., 2022). Moreover, adenosine has been recognized for its cardioprotective effects and therapeutic potential in treating chronic heart failure (Asakura *et al*., 2007). Additionally, xanthophylls, including lutein and zeaxanthin, not only contribute to enhanced yolk pigmentation but also boost antioxidant capacity in eggs (Zhao *et al*., 2021; Chou *et al*., 2024).

In contemporary production, *Cordyceps militaris* is widely cultivated on artificial nutrient media, enabling high-yield output to meet growing nutritional demands. Typically, only the fruiting bodies are harvested for commercial purposes, while the fungal roots embedded in the substrate are treated as waste or by-products. These roots, therefore, represent an inexpensive and readily available resource. However, emerging research has explored the application of these by-products, including *Cordyceps militaris* polysaccharides, spent substrates, and waste culture media, as dietary supplements for laying hens. These studies have shown beneficial effects on egg productivity and quality (Wang *et al*., 2015; Chen *et al*., 2020). Given these findings, there is promising potential in utilizing the root portion of *Cordyceps militaris* currently an underused by-product as a feed additive to improve laying hen performance and egg quality. Therefore, the objective of this study is to evaluate the effects of dietary supplementation with *Cordyceps militaris* roots on egg production performance, egg quality characteristics, and the antioxidant capacity of egg yolks.

MATERIALS AND METHODS

Animals

This study was conducted on 120 commercial Egyptian laying hens, all 28 weeks old and with uniform body weights to ensure consistency across experimental groups. Before the trial began, all hens were vaccinated according to standard poultry health protocols to protect against common infectious diseases. All animal procedures were approved by the Animal Care and Use Committee of Tra Vinh University, Vietnam (License No. 44/2024/HD-HDKH&DT-DHTV), and complied with institutional and national guidelines for animal welfare. The hens were housed in well-managed, welfare-oriented facilities designed to maintain optimal bioclimatic conditions, including controlled temperature, relative humidity, appropriate ventilation, and a consistent lighting program. To minimize stress during handling, all vaccinations and blood collections were performed by trained personnel using gentle restraint techniques. Post-procedures, hens were given vitamin C supplemented drinking water to promote physiological recovery and to help mitigate potential stress responses. Throughout the experimental period, all management practices strictly adhered to animal welfare standards to ensure the well-being of the birds.

Experimental design

The experimental design employed in this study was a completely randomized design (CRD) structured to assess the effects of varying levels of dietary *Cordyceps militaris* roots supplementation on the performance of laying hens. A total of five treatment groups were established, each corresponding to a different inclusion level of *Cordyceps militaris* roots in the feed. Specifically, the dietary treatments consisted of 0 g/kg (T1 - control group), 5 g/kg (T2), 10 g/kg (T3), 15 g/kg (T4), and 20 g/kg (T5) of *Cordyceps militaris* roots added per kilogram of feed, equivalent to 0%, 0.5%, 1%, 1.5%, and 2% of the diet, respectively. Each treatment group was further subdivided into four replicates to strengthen statistical validity and ensure reliable comparisons among treatments. Each replicate consisted of 6 individual hens, resulting in 24 hens per treatment group and a total of 120 hens used in the experiment. Birds were randomly assigned to treatment groups and replicates to reduce potential bias and ensure uniform distribution of individual variation across all groups. The trial was conducted over an 8-week period, beginning when the hens were 28 weeks of age and continuing until they reached 35 weeks.

Preparation of *Cordyceps militaris* roots

The *Cordyceps militaris* roots refers to the base of the *Cordyceps militaris* fungus remaining after the fruiting body has been harvested. This by-product was obtained from the Center for Applied Biology, Faculty of Agriculture and Aquaculture, Tra Vinh University, Vietnam. The base consists of two components: the *Cordyceps militaris* roots and the cultivation substrate. After harvesting, the *Cordyceps militaris* roots was carefully separated from the substrate. The roots were then dried intermittently at a temperature of 50 °C for 18 hours, with periodic intervals to allow for better moisture control and prevent overheating. Once completely dried, the roots were finely ground into powder and stored at 4°C until further use.

Animal management

The hens were acclimatized for 4 weeks prior to the start of the experiment. They were housed in cages, with three hens per cage. Throughout the experimental period, all hens were raised and managed under identical conditions, including a lighting schedule of 16 hours per day. Water was provided ad libitum, and the quantity was kept consistent across all experimental groups. A commercial layer diet was supplemented with different levels of *Cordyceps militaris* roots and provided twice daily. Drinking water was also replaced twice a day. Feed and water intake were monitored and recorded daily. Feeders and drinking systems were cleaned thoroughly every day. Body weights of the hens were measured at the beginning and at the end of the experimental period. All hens used in the experiment had already been laying eggs for four weeks prior to the trial. The nutritional composition of the experimental diets is presented in Table 1.

Table 1: Proximate chemical composition of feed supplemented with *Cordyceps militaris* roots.

Items	T1	T2	T3	T4	T5
DM (%)	88.80	87.60	87.00	86.50	88.60
Crude protein (%)	16.80	16.70	16.50	16.50	16.70
Ether extract (%)	3.66	3.66	3.67	3.76	3.43
Crude fiber (%)	6.03	6.42	6.62	6.61	6.30
Calcium (%)	4.06	4.07	4.05	4.11	3.85
Phosphorus (%)	0.60	0.60	0.58	0.57	0.56
Ash (%)	13.50	13.40	13.30	13.70	13.60

Data are presented as mean ± standard deviation. T1 represents the control group, while T2, T3, T4, and T5 correspond to 5, 10, 15, and 20 g of *Cordyceps* per kg of feed, respectively.

Productive performance evaluation

Each hen was weighed individually at both the start (28 weeks of age) and end (35 weeks of age) of the experiment to track body weight changes. During the entire duration of the trial, daily data collection was conducted to accurately record several key production parameters. These included the amount of feed consumed per hen per day, measured in grams; the average egg weight, also recorded in grams; the total number of eggs laid per hen; and the percentage of egg production, which reflects the proportion of hens laying eggs on a given day. Egg mass was calculated as the product of the average egg weight and the percentage of egg production, providing a comprehensive indicator of overall egg output. Furthermore, the feed conversion ratio (FCR) was determined by dividing the total feed intake, in grams, by the total egg mass produced, also in grams, thus indicating the efficiency with which feed was converted into egg output. All of these production parameters were analysed based on replicate groups within the experimental design, and the data were subsequently summarized and presented as bi-weekly averages to account for variation over time and to enhance the reliability of the performance assessment.

Egg quality evaluation

Over the 8-week experimental period, weekly egg quality assessments were carried out to examine the effects of different treatments. Each week, 24 eggs were randomly selected from each treatment group, with 6 eggs collected per replicate, ensuring a representative sample for analysis. The eggs were evaluated for both internal and external quality characteristics using standardized procedures described by Kirubakaran *et al.* (2011). The measured parameters included yolk weight, albumin weight, and shell weight to determine the egg's compositional balance. Quality indicators such as albumin index and yolk index were used to assess egg freshness and content consistency. The shape index was recorded to evaluate egg form, and shell thickness was measured to determine structural integrity. Haugh units served as a key indicator of overall egg quality, while yolk colour was assessed using a yolk colour fan scale.

Egg yolk lipid peroxidation evaluation

Lipid peroxidation in egg yolk was evaluated using the thiobarbituric acid reactive substances (TBARS) assay to determine malondialdehyde (MDA) levels, based on the protocol established by Siu and Draper (1978). For this purpose, eight eggs per treatment group (two eggs per replicate) were collected at three time points: the start (28th week of age), the midpoint (31st week), and the end (35th week) of the experimental period. Prior to analysis, all eggs were kept at room temperature for 10 days. To prepare for MDA quantification, egg yolks were homogenized with distilled water in a 1:5 ratio. Lipid peroxidation was then initiated by adding 0.24 mM ferric sulfate and incubating the mixture at 38°C for 15 minutes. Afterward, 0.5 ml of the sample was mixed with 1 ml of TBA reagent (comprising 0.375% TBA, 15% trichloroacetic acid, and 0.25N HCl), boiled for 20 minutes, cooled, and centrifuged. The absorbance of the supernatant was measured at 535 nm using a microplate spectrophotometer. MDA concentrations were calculated by comparing the absorbance values to a standard curve and expressed as nmol MDA per 0.5 g of egg yolk.

Statistical analysis

Statistical analysis of the experimental data was performed using a one-way analysis of variance (ANOVA) to determine whether there were significant differences among the treatment groups. Prior to conducting ANOVA, data normality was assessed using the Shapiro–Wilk test. As all data were normally distributed, no data transformations were applied. The analysis was conducted using IBM SPSS Statistics software, version 22. Following the ANOVA, the Tukey test was applied as a post-hoc analysis to identify specific differences between treatment means. The level of statistical significance was set at P<0.05.

RESULTS

Cordyceps militaris roots supplementation and productive performance

The impact of supplementing laying hen diets with *Cordyceps militaris* roots on reproductive performance is presented in Table 2. The findings indicate that although there was a slight increase in body weight across all treatments, these differences were not statistically significant when comparing the supplemented groups to the control (P>0.05). Similarly, egg weight remained unaffected by the treatments, with no significant variation among the groups (P>0.05).

In contrast, feed intake showed a notable difference. Between weeks 30-35, hens fed 20 g/kg *Cordyceps militaris* roots had significantly higher feed intake than those in other groups (P<0.05). Regarding egg number, a positive correlation was observed between supplementation level and egg production, with the 20 g/kg group producing the highest number of eggs, significantly exceeding the control (P<0.05).

Table 2: Impact of dietary *Cordyceps militaris* roots supplementation on laying hen productivity.

Parameters	Periods	Treatments					P value
		T1	T2	T3	T4	T5	
Body weight (kg)	Initial BW	1.43±0.01	1.43±0.02	1.45±0.03	1.42±0.01	1.46±0.01	0.081
	Final BW	1.51±0.01	1.50±0.02	1.53±0.03	1.51±0.02	1.54±0.01	0.099
	Weight gain	0.08±0.01	0.08±0.01	0.08±0.01	0.09±0.01	0.09±0.01	0.135
Feed intake (g/hen/day)	Week 28-29	91.41±1.10	90.77±1.06	91.26±1.87	90.59±1.62	92.00±0.39	0.588
	Week 30-31	94.79±0.15^{b}	94.56±0.11^{b}	94.42±0.26^{b}	94.76±0.47^{b}	95.93±0.16^{a}	<0.001
	Week 32-33	94.48±0.13^{b}	94.63±0.17^{b}	94.75±0.24^{b}	94.80±0.13^{b}	95.53±0.16^{a}	<0.001
	Week 34-35	94.35±0.09^{c}	94.32±0.04^{c}	94.44±0.15^{bc}	94.63±0.11^{b}	95.19±0.09^{a}	<0.001
Egg weight (g)	Week 28-29	52.09±0.86	52.90±0.98	52.47±0.83	52.19±0.68	52.48±0.78	0.577
	Week 30-31	54.97±1.41	54.34±0.86	54.72±0.43	54.12±0.71	53.68±0.80	0.340
	Week 32-33	54.56±0.96	54.91±0.50	55.47±0.73	55.06±1.04	53.84±0.71	0.113
	Week 34-35	55.40±0.18	54.97±0.64	55.94±0.72	55.69±0.83	54.44±0.94	0.067
Egg number (egg/hen/week)	Week 28-29	5.20±0.08^{b}	5.95±0.24^{a}	6.00±0.31^{a}	6.01±0.31^{a}	6.06±0.20^{a}	0.001
	Week 30-31	5.16±0.12^{b}	5.91±0.35^{a}	5.93±0.18^{a}	5.97±0.09^{a}	6.04±0.25^{a}	<0.001
	Week 32-33	5.15±0.20^{b}	5.73±0.23^{a}	5.85±0.17^{a}	5.96±0.14^{a}	6.08±0.31^{a}	<0.001
	Week 34-35	5.32±0.16^{b}	5.84±0.23^{a}	5.86±0.10^{a}	5.93±0.24^{a}	6.03±0.28^{a}	0.003
Egg production (%)	Week 28-29	74.35±1.19^{b}	85.00±3.46^{a}	85.71±4.45^{a}	85.96±4.46^{a}	86.72±2.94^{a}	0.001
	Week 30-31	73.74±1.78^{b}	84.53±5.08^{a}	84.76±2.61^{a}	85.32±1.38^{a}	86.37±3.70^{a}	<0.001
	Week 32-33	73.63±2.86^{b}	82.00±3.31^{a}	83.69±2.53^{a}	85.20±2.07^{a}	86.84±4.30^{a}	<0.001
	Week 34-35	76.00±2.30^{b}	83.53±3.34^{a}	83.73±1.51^{a}	84.73±3.52^{a}	86.20±4.12^{a}	0.003
Egg mass (g)	Week 28-29	38.73±0.55^{b}	44.95±1.30^{a}	44.97±2.40^{a}	44.87±2.59^{a}	45.75±1.69^{a}	<0.001
	Week 30-31	40.52±0.65^{b}	45.91±2.15^{a}	46.37±1.15^{a}	46.18±1.24^{a}	46.39±2.62^{a}	0.001
	Week 32-33	40.17±1.84^{b}	45.01±1.63^{a}	46.41±1.41^{a}	46.91±1.40^{a}	46.74±1.82^{a}	<0.001
	Week 34-35	42.11±1.35^{b}	45.93±2.26^{ab}	46.85±1.41^{a}	47.17±1.72^{a}	46.94±2.79^{a}	0.013
FCR (g feed/g egg)	Week 28-29	2.36±0.05^{a}	2.02±0.05^{b}	2.03±0.11^{b}	2.02±0.15^{b}	2.01±0.08^{b}	0.001
	Week 30-31	2.34±0.03^{a}	2.06±0.09^{b}	2.03±0.04^{b}	2.05±0.05^{b}	2.07±0.11^{b}	<0.001
	Week 32-33	2.35±0.11^{a}	2.10±0.07^{b}	2.04±0.06^{b}	2.02±0.06^{b}	2.04±0.07^{b}	<0.001
	Week 34-35	2.24±0.07^{a}	2.05±0.10^{ab}	2.01±0.06^{b}	2.00±0.07^{b}	2.03±0.12^{b}	0.014

Data are presented as mean ± standard deviation. Different superscript letters (a, b, or c) within the same row denote statistically significant differences between treatments (P<0.05). T1 represents the control group, while T2, T3, T4, and T5 correspond to 5, 10, 15, and 20 g of *Cordyceps* per kg of feed, respectively.

Table 3: Impact of dietary *Cordyceps militaris* roots supplementation on egg quality in laying hens.

Parameters	Periods	Treatments					P-value
		T1	T2	T3	T4	T5	
Albumen weight (%)	Week 28-29	61.03±0.75	61.08±0.70	61.94±0.49	61.54±0.94	61.61±0.54	0.362
	Week 30-31	62.09±0.22	62.27±1.39	62.26±1.08	61.30±1.85	61.31±1.66	0.714
	Week 32-33	61.03±0.58	60.88±1.21	60.52±1.12	60.64±0.54	60.69±1.06	0.944
	Week 34-35	61.06±0.98	60.87±1.02	60.55±0.37	60.31±0.30	61.10±0.46	0.474
Yolk weight (%)	Week 28-29	26.39±0.82	26.58±0.79	26.38±0.81	26.22±0.54	26.32±0.56	0.968
	Week 30-31	26.17±0.49	26.21±0.33	26.12±0.57	26.22±1.41	26.36±1.74	0.998
	Week 32-33	26.41±0.75	26.49±0.89	26.93±0.65	26.84±0.30	26.83±0.67	0.784
	Week 34-35	26.71±0.77	26.44±0.72	26.85±0.61	26.94±0.23	26.50±0.51	0.729
Shell weight (%)	Week 28-29	12.34±0.19	12.35±0.68	12.23±0.15	12.22±0.55	12.18±0.24	0.979
	Week 30-31	12.27±0.15	12.26±0.28	12.22±0.56	12.17±0.34	12.19±0.48	0.994
	Week 32-33	12.30±0.26	12.37±0.17	12.37±0.39	12.42±0.15	12.38±0.39	0.987
	Week 34-35	12.35±0.42	12.43±0.28	12.34±0.24	12.36±0.38	12.35±0.16	0.993
Egg shape index	Week 28-29	0.770±0.006	0.764±0.010	0.773±0.006	0.765±0.016	0.771±0.008	0.683
	Week 30-31	0.766±0.006	0.759±0.012	0.769±0.004	0.765±0.004	0.761±0.008	0.483
	Week 32-33	0.775±0.011	0.769±0.012	0.768±0.012	0.763±0.006	0.763±0.004	0.226
	Week 34-35	0.762±0.012	0.752±0.008	0.753±0.009	0.747±0.017	0.749±0.005	0.401
Albumin index	Week 28-29	0.029±0.001	0.030±0.002	0.030±0.001	0.031±0.003	0.031±0.002	0.837
	Week 30-31	0.031±0.001	0.031±0.001	0.031±0.002	0.029±0.001	0.030±0.001	0.205
	Week 32-33	0.030±0.002	0.030±0.001	0.029±0.001	0.030±0.001	0.029±0.001	0.726
	Week 34-35	0.028±0.001	0.027±0.001	0.026±0.001	0.027±0.002	0.028±0.000	0.619
Yolk index	Week 28-29	0.343±0.008	0.346±0.021	0.331±0.018	0.335±0.008	0.340±0.009	0.616
	Week 30-31	0.340±0.014	0.342±0.010	0.335±0.004	0.340±0.010	0.343±0.005	0.820
	Week 32-33	0.343±0.014	0.344±0.009	0.333±0.017	0.333±0.011	0.334±0.018	0.701
	Week 34-35	0.362±0.007	0.363±0.010	0.352±0.003	0.357±0.011	0.364±0.011	0.339
Haugh units	Week 28-29	90.64±1.14	89.59±2.80	91.00±1.91	91.33±2.86	92.55±2.42	0.508
	Week 30-31	92.62±0.36	92.60±2.11	92.90±2.67	91.45±0.79	92.12±0.80	0.739
	Week 32-33	89.88±2.67	90.25±1.04	89.72±0.85	90.88±0.91	90.46±1.13	0.815
	Week 34-35	88.85±2.27	89.06±1.34	88.69±0.78	88.90±2.33	89.22±0.77	0.992
Shell thickness (mm)	Week 28-29	0.318±0.002	0.320±0.004	0.321±0.007	0.321±0.002	0.321±0.002	0.891
	Week 30-31	0.315±0.004	0.312±0.005	0.316±0.002	0.315±0.004	0.313±0.004	0.761
	Week 32-33	0.317±0.002	0.318±0.002	0.320±0.005	0.321±0.002	0.322±0.002	0.332
	Week 34-35	0.320±0.004	0.321±0.002	0.322±0.002	0.322±0.002	0.322±0.005	0.815
Yolk color core	Week 28-29	12.40±0.37	12.41±0.15	12.40±0.31	12.59±0.12	12.56±0.07	0.643
	Week 30-31	$12.81±0.07^{c}$	$13.18±0.23^{b}$	$13.50±0.25^{ab}$	$13.62±0.10^{a}$	$13.66±0.06^{a}$	<0.001
	Week 32-33	$12.62±0.14^{c}$	$13.22±0.15^{b}$	$13.31±0.12^{b}$	$13.87±0.14^{a}$	$14.00±0.20^{a}$	<0.001
	Week 34-35	$12.84±0.12^{d}$	$13.31±0.37^{cd}$	$13.56±0.23^{bc}$	$14.00±0.35^{ab}$	$14.37±0.14^{a}$	<0.001

Data are presented as mean ± standard deviation. Different superscript letters (a, b, c, or d) within the same row denote statistically significant differences between treatments (P<0.05). T1 represents the control group, while T2, T3, T4, and T5 correspond to 5, 10, 15, and 20 g of *Cordyceps* per kg of feed, respectively.

Additionally, both egg production rate and egg mass were significantly improved in the supplemented groups (P<0.05), showing a progressive increase with higher supplementation levels. Notably, the feed conversion ratio (FCR) was significantly lower in the groups receiving *Cordyceps militaris* roots compared to the control (P<0.05), indicating better feed efficiency. However, no significant differences in FCR were observed among the *Cordyceps*-supplemented treatments themselves (P>0.05).

Table 4: Impact of dietary *Cordyceps militaris* roots supplementation on malondialdehyde (MDA) levels in egg yolk of laying hens (nmol MDA/0.5 g yolk).

Periods	Treatments					P-value
	T1	T2	T3	T4	T5	
Initial phase (Week 28)	134.05±1.21^{a}	131.61±0.18^{b}	131.40±1.15^{b}	131.34±0.59^{b}	130.23±0.59^{b}	0.003
Intermediate phase (Week 31)	135.16±1.21^{a}	127.16±2.36^{b}	121.04±2.39^{c}	71.40±1.98^{d}	64.00±2.35^{e}	<0.001
Final phase (Week 35)	135.57±1.33^{a}	123.55±0.56^{b}	118.22±0.32^{c}	68.96±2.99^{d}	59.16±1.65^{e}	<0.001

Data are presented as mean ± standard deviation. Different superscript letters (a, b, c, d, or e) within the same row denote statistically significant differences between treatments (P<0.05). T1 represents the control group, while T2, T3, T4, and T5 correspond to 5, 10, 15, and 20 g of *Cordyceps* per kg of feed, respectively.

Cordyceps militaris roots supplementation and egg quality

Table 3 illustrates the influence of dietary supplementation with *Cordyceps militaris* roots on egg quality over an 8-week period. The findings revealed that most egg quality traits including yolk weight, albumin weight, shell weight, yolk index, albumin index, shape index, shell thickness, and Haugh unit were not significantly affected by the treatments (P>0.05). Nonetheless, the addition of *Cordyceps militaris* roots did impact yolk pigmentation. The results demonstrated a clear trend where increasing levels of *Cordyceps* supplementation led to more intense yellow coloration in the yolks, whereas lower supplementation levels produced paler yolks. Particularly, the hens receiving 15 g/kg and 20 g/kg of *Cordyceps militaris* roots showed the most pronounced yolk colour enhancement, with scores significantly higher than those of the control group (P<0.05).

Egg yolk lipid peroxidation

Table 4 outlines the malondialdehyde (MDA) levels found in egg yolks following a 10-day storage period at room temperature. During the first week of the study, no significant differences in antioxidant capacity were detected among the groups receiving *Cordyceps militaris* roots supplementation (P>0.05). However, when compared to the control group, these supplemented groups showed significantly lower MDA levels (P<0.05). As the experiment progressed into the mid and final phases, MDA concentrations showed a declining trend relative to the initial phase. Significant reductions in MDA levels were observed in the *Cordyceps*-supplemented groups compared to the control group (P<0.05). Importantly, a dose-dependent effect was evident, with higher inclusion levels of *Cordyceps militaris* roots resulting in lower MDA values. Conversely, the control group consistently demonstrated the highest MDA concentrations, with statistically significant differences noted when compared to the supplemented groups at both the middle and final stages of the trial (P<0.05).

DISCUSSION

The present study investigated the effects of dietary supplementation with *Cordyceps militaris* roots on the productive performance, egg quality, and oxidative stability of egg yolks in laying hens. Overall, the findings suggest that inclusion of *Cordyceps militaris* roots in the diet can beneficially influence several performance and quality parameters, although some traits remained unaffected.

Laying performance

Body weight and egg weight were unaffected by supplementation, aligning with previous studies where the inclusion of herbal or fungal by-products in poultry diets did not markedly affect growth or egg weight (Kirubakaran *et al.*, 2011; Karageorgou *et al.*, 2024). This suggests that *Cordyceps* roots may not significantly influence growth at these doses. However, feed intake was significantly increased in the group supplemented with 20 g/kg *Cordyceps militaris* roots. This may suggest either an improvement in feed palatability, as bioactive compounds from *Cordyceps* could enhance flavour and aroma, or a compensatory response due to slight alterations in nutrient density from the inclusion of the root powder. Notably, this finding contrasts with the report by Wang *et al.* (2015), who observed reduced feed intake in laying hens supplemented with *Cordyceps militaris* waste medium. The discrepancy between studies could be attributed to differences in the source and composition of *Cordyceps* by-products used, as waste culture media may differ substantially in bioactive and nutritional profiles compared to root material. Further research is needed to clarify the mechanisms underlying these intake variations.

More importantly, egg production metrics such as egg number, egg production rate, and egg mass were significantly enhanced in the supplemented groups, particularly at higher inclusion levels. These results indicate a dose-dependent improvement in reproductive performance. Such findings may be attributed to the immunomodulatory and metabolic-enhancing properties of *Cordyceps*, which are known to support reproductive functions and overall vitality in poultry (Cheng *et al.*, 2019). The improved feed conversion ratio (FCR) in the supplemented groups also supports the hypothesis that *Cordyceps militaris* roots enhance nutrient utilization efficiency and stimulate appetite, thereby contributing to increased egg production. This result is consistent with previous studies on the use of *Cordyceps* mushrooms to improve reproductive performance in laying hens. Chen *et al.* (2020) used *Cordyceps militaris* polysaccharides, and Wang *et al.* (2015) utilized *Cordyceps militaris* waste medium as dietary supplements for hens, which led to improvements in egg number, laying rate, and feed conversion ratio.

In our study, dietary supplementation with *Cordyceps militaris* roots did not significantly improve egg weight across treatments. This finding aligns with previous research demonstrating that while *Cordyceps militaris* supplementation can enhance laying performance, feed efficiency, and egg quality traits, its impact on individual egg weight is often limited or nonsignificant (Wang *et al.*, 2015; Cheng *et al.*, 2019; Chen *et al.*, 2020). The bioactive compounds present in *Cordyceps militaris* roots, such as cordycepin, polysaccharides, and carotenoids, are primarily recognized for their antioxidant, immunomodulatory, and metabolic-enhancing effects rather than for providing substantial anabolic nutrients like high levels of protein or amino acids necessary for egg mass accumulation. As a result, supplementation likely supported improved hen health, oxidative stability, and reproductive efficiency without directly stimulating greater deposition of yolk or albumen material needed to increase egg size.

Egg quality

Regarding egg quality traits, most parameters including yolk weight, albumin weight, shell weight, yolk and albumin indices, shell thickness, and Haugh unit were not significantly affected by the treatments. These findings suggest that *Cordyceps militaris* roots do not adversely affect the structural and internal quality of eggs. The findings obtained from the present study align closely with those reported by Chen *et al.* (2020), who investigated the effects of dietary supplementation with *Cordyceps militaris* polysaccharides in laying hens. Their research demonstrated that the addition of this bioactive compound did not produce any statistically significant changes in key egg quality traits. These traits included albumen height, shape index, Haugh units, eggshell breaking strength, and eggshell thickness parameters that are widely recognized as reliable indicators of egg quality. In contrast, the study conducted by Wang *et al.* (2015) presented a somewhat different perspective. They observed that incorporating *Cordyceps militaris* waste medium into the diet of laying hens led to measurable improvements in specific egg quality parameters, particularly in egg white weight and eggshell strength. The discrepancy in results between the current study and previous research could be attributed to a multitude of contributing factors. These may include variations in the dosage or concentration of *Cordyceps militaris* used, differences in the form or method of administration (e.g., powder, extract, or fermented waste medium), and the specific type or source of *Cordyceps* by-product utilized in the feed. Moreover, other influential elements such as differences in animal husbandry practices, the design and conditions of the housing environment, the nutritional composition of the basal diet, as well as the genetic background, breed, or age of the laying hens involved in each study, may have played significant roles in shaping the observed outcomes. Furthermore, it is important to note that the *Cordyceps militaris* waste medium, which likely contained residual nutrients from the cultivation substrate, including higher levels of minerals such as calcium, phosphorus, and trace elements essential for shell formation. In contrast, the *Cordyceps militaris* roots used in our study were focused more on delivering bioactive compounds such as cordycepin, adenosine, and polysaccharides, with relatively lower mineral content. Given these complexities, our findings underscore the importance of standardizing experimental conditions in future research to more accurately assess the impact of *Cordyceps* supplementation on poultry performance and egg quality. By considering such variables, we can better understand the mechanisms underlying the effects of *Cordyceps* and refine its practical applications in poultry nutrition.

In the present study, a significant enhancement in yolk pigmentation was observed, particularly in the groups supplemented with 15 g/kg and 20 g/kg of *Cordyceps militaris*. Yolk colour is a crucial attribute influencing consumer preference and marketability of eggs. This coloration is primarily due to the accumulation of carotenoids in the yolk, as hens themselves are incapable of synthesizing pigment compounds (Kljak *et al.*, 2021). Instead, they acquire these pigments through their diet. The carotenoids consumed are absorbed in the intestinal tract, transferred to the bloodstream, and ultimately deposited into the developing yolk. Once in the bloodstream, carotenoids are transported to the liver in proto-micelle form, where they bind to lipoproteins before being delivered to the ovary for yolk deposition (Matache *et al.*, 2024). Among the various factors affecting carotenoid assimilation, intestinal absorption plays a key role in determining the efficiency of yolk pigmentation (Kojima *et al.*, 2022). The dominant natural pigments contributing to yolk colour are xanthophylls, which belong to the oxycarotenoid group. These oxycarotenoids not only influence the pigmentation of the yolk but also contribute to the coloration of skin, legs, beak, comb, and feathers. *Cordyceps militaris* fruiting bodies are known to be rich in carotenoids and thus present a valuable source of natural pigments for dietary enrichment. The principal carotenoids in *Cordyceps militaris* have been identified as xanthophylls, specifically termed cordyxanthin. Cordyxanthin accounts for approximately 86.7% of the total carotenoid content in *Cordyceps militaris* and is notable for its highwater solubility, which facilitates its absorption in the body (Dong *et al*., 2013). This makes cordyxanthin a potentially superior pigment or carotenoid supplement compared to conventional sources such as β-carotene, lycopene, lutein, and zeaxanthin, both in the food industry and in functional food applications. The findings of this study are in agreement with those of earlier researchers, including Wang *et al*. (2015) and Chen *et al*. (2020), who also reported improved yolk coloration following dietary inclusion of *Cordyceps militaris* supplements.

Egg yolk lipid peroxidation

The enhancement of egg yolk antioxidant capacity through *Cordyceps militaris* roots supplementation was further substantiated by measuring malondialdehyde (MDA) levels, an indicator of lipid peroxidation. While no significant differences were observed in MDA content during the initial week of storage, prolonged storage revealed a marked reduction in MDA concentrations among the supplemented groups compared to the control. This suggests improved oxidative stability, particularly at higher supplementation levels, indicating a dose-dependent antioxidant effect of *Cordyceps militaris* by-products. The consistent decline in MDA levels over time reflects the efficacy of *Cordyceps*-derived bioactive compounds in inhibiting lipid peroxidation, thereby enhancing the shelf life and preserving the quality of eggs. These findings align with previous research demonstrating the antioxidative potential of *Cordyceps* extracts in animal models (Tang *et al*., 2019).

The growing interest in incorporating *Cordyceps militaris* into laying hen diets stems from its diverse profile of antioxidant-rich bioactive compounds, including cordycepin, polysaccharides, ergosterol, flavonoids, phenolic acids, and xanthophyll carotenoids (Jędrejko *et al*., 2021). Once ingested, these compounds contribute to systemic improvements in oxidative status in hens, ultimately increasing the antioxidant potential of their eggs especially in the yolk, where lipids are particularly vulnerable to oxidative damage.

Among these compounds, cordycepin (3′-deoxyadenosine) plays a pivotal role. It acts as a potent scavenger of reactive oxygen species (ROS) and enhances the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), all of which are crucial for mitigating oxidative stress and preventing lipid oxidation in yolk membranes (Ramesh *et al*., 2012; Tong He *et al*., 2019). In addition, *Cordyceps militaris* polysaccharides have been shown to activate the nuclear factor erythroid 2–related factor 2 (Nrf2) signalling pathway, which regulates the expression of various antioxidant and phase II detoxifying enzymes. Activation of this pathway leads to the upregulation of SOD and GSH-Px and a reduction in MDA levels, further supporting the role of *Cordyceps militaris* in oxidative protection (Song and Zhu, 2020). This mechanism may explain our observed MDA reductions, though further research is needed to confirm pathway activation in laying hens.

Furthermore, the presence of carotenoids particularly xanthophylls such as lutein and zeaxanthin in *Cordyceps militaris* offers additional antioxidant advantages. These pigments, which contribute to the fungus's characteristic yellow-orange coloration, are well-known for their lipid-soluble antioxidant properties (Chou *et al*., 2024). In avian species, dietary carotenoids are absorbed and preferentially deposited into egg yolks, where they bolster antioxidant defences. Xanthophylls, due to their polar nature, are more efficiently absorbed and retained in egg yolks than nonpolar carotenoids like β-carotene (Dansou *et al*., 2023). Although direct studies on the transfer of *Cordyceps militaris* derived carotenoids to egg yolks are limited, the established presence of these compounds in the fungus, alongside well-documented deposition mechanisms in poultry, supports the likelihood of their contribution to yolk antioxidant capacity (Zhao *et al*., 2021).

This study is limited in that it did not assess the long-term effects of *Cordyceps militaris* root supplementation beyond the 8-week trial period. Additionally, the stability and bioavailability of active compounds such as cordycepin and cordyxanthin during feed processing and storage were not evaluated. These factors may influence the efficacy of supplementation under commercial conditions. Future research should address these aspects to ensure the practical applicability and consistency of the observed benefits.

CONCLUSION

In conclusion, the incorporation of *Cordyceps militaris* roots into the diets of laying hens at inclusion levels of 15 to 20 g/kg feed appears to be optimal for enhancing yolk pigmentation and improving antioxidant capacity, without adversely affecting reproductive performance. These findings underscore the potential of *Cordyceps militaris* roots as a functional feed additive for promoting hen health and improving egg quality, thereby offering nutritional benefits to consumers. To further validate and expand upon these outcomes, future studies should investigate the impact of *Cordyceps militaris* supplementation on the nutritional composition of eggs particularly with regard to antioxidant content as well as on the systemic oxidative status of laying hens, including serum biomarkers. Additionally, evaluating the cost-effectiveness of such supplementation strategies will be essential for practical adoption in commercial poultry production.

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NOVELTY STATEMENT

The novelty of this study lies in the use of *Cordyceps militaris* roots as a dietary supplement for laying hens to enhance egg production and quality. The results of this study have a positive impact on productivity, improve yolk colour and the antioxidant capacity of the egg yolk. These findings suggest that *Cordyceps militaris* roots hold considerable promise for practical application in the poultry industry.

AUTHOR'S CONSTRIBUTION

NVV: Conceived and designed the experiments.
NVV and NQAQ: Performed the experiments and analysed the data.
NNT: Contributed materials.
NVV, NQAQ and NNT: Wrote the paper.
All authors reviewed and approved the final manuscript.

Generative AI and AI-assisted technology statement

The authors acknowledge that ChatGPT was utilized to refine the manuscript's English grammar. All material was thoroughly examined, edited, and confirmed by the authors to ensure its accuracy and originality.

Conflict of interest

The authors have declared no conflict of interest.

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