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Effects of chili meal supplementation on productive performance, intestinal health, and liver lipid metabolism of laying hens fed low-protein diets

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ABSTRACT

This study aimed to explore the effects of chili meal (CM), a by-product of chili pepper oil extraction, on the productive performance, intestinal health, and lipid metabolism of laying hens fed low-protein (LP) diets. A total of 384 Hy-Line brown laying hens (32 weeks old) were divided into six groups: control (CON) diet with 16.5 % crude protein (CP), LP diet with 15 % CP, and LP diets supplemented with 3 %, 5 %, 7 %, and 9 % CM. Results showed that dietary CM supplementation of up to 5 % did not negatively affect the productive performance of laying hens fed LP diets. However, the groups receiving 7 % and 9 % CM exhibited a significant increase in the feed-to-egg ratio ($P < 0.05$). Additionally, dietary CM supplementation effectively enhanced egg yolk color in a dose-dependent manner ($P < 0.05$). Intestinal morphology analysis indicated that the 5 % CM group had a higher villus height-to-crypt depth ratio than the LP and 9 % CM groups ($P < 0.05$), with no significant differences among the other groups. Dietary supplementation with 3 %–7 % CM did not significantly affect serum and jejunal antioxidant capacity, and the 9 % CM group exhibited the highest levels of serum and jejunal malondialdehyde among the groups ($P < 0.05$). Dietary CM supplementation significantly increased anti-inflammatory cytokines (IL-4 and IL-10) and decreased pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) in the serum and jejunal tissue of laying hens ($P < 0.05$). Moreover, CM supplementation significantly altered the cecal microbiota composition in laying hens, increasing the abundance of beneficial bacteria, such as *Desulfovibrio* and *Megamonas*. Furthermore, dietary CM supplementation significantly decreased serum triglyceride levels; downregulated liver mRNA levels of *ACC, FAS*, and *SREBP-1C/2*; and upregulated the mRNA levels of *ACOX1, PPAR-α, Apob*, and *CPT* in laying hens fed LP diets. In conclusion, CM supplementation should not exceed 5 % to avoid negative impacts on performance while supporting intestinal health and lipid metabolism.

Introduction

Over the past three decades, the production of poultry eggs increased significantly by 150 % (Bist et al., 2024). According to 2021 data from the Food and Agriculture Organization, China accounted for 38 % of global poultry egg production. The global population is projected to reach approximately 9.7 billion by the year 2050, leading to a significant increase in the demand for poultry products. The expansion of poultry products presents considerable challenges and risks for the sustainable development of poultry production. These challenges include the scarcity of traditional feed resources and the competition between animal feed and essential human food staples (Govoni et al., 2021; Henchion et al., 2021). Low-protein (**LP**) feeding offers several benefits for the sustainable development of poultry farming, including reduced nitrogen excretion; decreased dependence on traditional ingredients, such as soybeans; and potential cost savings (Liu et al., 2021; Tan et al., 2023). Additionally, there is a growing interest in sustainable and innovative alternative feed sources for poultry farming aimed at reducing feed costs and alleviating competition between animal feed and human food supplies.

Chili pepper (*Capsicum annuum* L.) is a globally cultivated and consumed vegetable, primarily utilized for its fresh and cooked fruits. It is also used as a natural feed additive in poultry to improve the growth performance and health of animals (Abd El-Hack et al., 2022; Munglang and Vidyarthi, 2019). Capsaicin (8-methyl-N-vanilla base-6-nonene amide) is the primary bioactive component of chili pepper, accounting

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for 0.1 %–0.2 % of its dry weight (Govindarajan and Sathyanarayana, 1991). Studies have demonstrated that capsaicin possesses antioxidant, anti-inflammatory, and antimicrobial properties and regulates lipid metabolism and the gut microbiome (Aasvang et al., 2008; Lee et al., 2003; Liang et al., 2023; Prakash and Srinivasan, 2010). Chili meal (**CM**), a by-product of the extraction of red pigments, chili oil, and capsaicin from chili peppers, is a promising alternative to traditional feed resources. When capsaicin, chili oil, and pigments were extracted from chili peppers, more than 80 % of the CM remained.

China is the world's largest producer of fresh chili peppers, with an annual production of 20 million tons (Zhang et al., 2022). Unfortunately, a significant portion of the CM is often discarded, resulting in considerable resource waste and environmental pollution. Thiamhirunsopit et al. (2014) demonstrated that dietary supplementation with CM at levels of up to 7.8 % did not negatively affect ileal nutrient digestibility and growth performance of broiler chickens. Fan et al. (2017) demonstrated that CM possesses moderate energy density and nutrient digestibility when incorporated into pig diets. Additionally, the inclusion of 5 % CM as a dietary supplement did not significantly affect the growth performance of growing pigs. Although CM has been used in animal production, research on its effects on the performance and health of laying hens, particularly those fed LP diets, is limited. Therefore, in this study, we evaluated the effects of dietary supplementation with varying levels of CM on the productive performance, egg quality, antioxidant and anti-inflammatory responses, intestinal morphology, cecum microbiome composition, and lipid metabolism of laying hens fed LP diets.

Materials and methods

Animals, diets, and sample collection

Animal protocols were conducted in accordance with the Guidelines for Animal Care and Use Committee of Qingdao Agricultural University (No. DKY20231105). CM was purchased from Chenguang Biotech Group Co. Ltd. (Handan, China). The nutrient profiles of the CM (Table 1) were determined before conducting the study. We randomly divided three hundred and eighty-four 32-week-old Hy-Line brown laying hens (with similar productive performance) into six groups, with eight replicates per group and eight hens per replicate. The control (**CON**) group received a basal diet containing 16.5 % crude protein, formulated to fulfill the nutritional requirements of the National Research Council (NRC, 1994; Table 2). The LP group was fed a diet containing 15.5 % crude protein, and the levels of lysine, methionine, threonine, and tryptophan were consistent with those in the CON diet. The CM supplementation groups received LP diets supplemented with 3 %, 5 %, 7 %, and 9 % CM. The birds were housed in a room with a temperature maintained at 22°C and received 16 h of light per day. The experiment lasted 12 weeks, including 1 week for acclimation and 11 weeks for experimentation, during which diet and water were provided ad libitum.

Table 1
Chemical composition of CM (g/kg DM).

Items	Content	Items	Content
AME[1], MJ/kg	11.8	Serine, %	0.51
Crude protein	168.4	Leucine, %	0.71
Crude fat	13.5	Isoleucine, %	0.39
Crude fiber	265.0	Valine, %	0.57
Ash	90.5	Arginine, %	0.63
Calcium,	10.7	Glutamic acid, %	1.36
Phosphorus	2.4	Aspartic acid, %	2.81
Capsaicin[1]	0.43	Cystine, %	0.11
Lysine, %	0.64	Tyrosine, %	0.28
Methionine, %	0.08	Phenylalanine, %	0.54
Threonine, %	0.52	Histidine, %	0.25
Glycine, %	0.54	Proline, %	1.20
Alanine, %	0.58		

[1] The value of AME and capsaicin were obtained from Thiamhirunsopit et al. (2014).

Table 2
Composition and nutrient levels of the basal diet (air-dry basis).

Items (%)	CON	LP	Chili meal			
			3 %	5 %	7 %	9 %
Ingredients						
Corn	63	66.4	62.99	60.79	58.56	56.34
Soybean meal	26	23	22.6	22.3	22.03	21.75
Capsicum meal	0	0	3	5	7	9
Soybean oil	1	0.5	1.3	1.8	2.3	2.8
Ground limestone	8	8	8	8	8	8
Methionine	0	0.02	0.02	0.03	0.03	0.03
Lysine	0	0.04	0.05	0.04	0.04	0.04
Threonine	0	0.04	0.04	0.04	0.04	0.04
Premix[1]	2	2	2	2	2	2
Total	100	100	100	100	100	100
Nutrient level[2]						
Metabolizable energy MJ/kg	11.33	11.31	11.31	11.31	11.31	11.30
Crude protein	16.53	15.55	15.52	15.51	15.53	15.56
Crude fiber	2.99	2.92	3.41	3.83	4.25	4.67
Methionine	0.38	0.38	0.38	0.38	0.38	0.38
Lysine	0.9	0.9	0.9	0.9	0.9	0.9
Threonine	0.63	0.63	0.63	0.63	0.63	0.63
Tryptophan	0.18	0.18	0.18	0.18	0.18	0.18
Ca	3.89	3.93	3.88	4.02	3.95	3.85
Total phosphorus	0.75	0.73	0.71	0.79	0.72	0.77

[1] The premix provided the following per kg of diets: Vitamin A (trans-retinyl acetate) 10000 IU; Vitamin D3 (cholecalciferol) 2500 IU; Vitamin E (dl-α-tocopherol acetate) 30 IU; Vitamin K3 0.75 mg; Vitamin B1 (thiamin) 1.5 mg; Vitamin B2 (riboflavin) 3.8 mg; Vitamin B6 (pyridoxine HCl) 4.5 mg; Vitamin B12 (cobalamin) 0.01 mg; biotin 0.15 mg; folic acid 0.5 mg; D-pantothenic acid 10 mg; nicotinic acid 30 mg; choline (as choline chloride) 500 mg; Cu (as copper sulfate) 10 mg; Fe (as ferrous sulfate) 80 mg; Mn (as manganese sulfate) 80 mg; Zn (as zinc sulfate) 90 mg; I (as potassium iodide) 0.40 mg; Se (as sodium selenite) 0.30 mg; Met (as dl-Met) 0.12 %; available P (as CaHPO4) 2.8 %; NaCl 0.15 %. [2] The nutrient levels were calculated values (except for CP, Ca, and phosphorus) according to data obtained from China Feed Data (https://www.chinafeeddata.org.cn/admin/Login/index.html). The value of capsaicin was obtained from Thiamhirunsopit et al. (2014).

At the end of the experiment, after fasting for 12 h, one hen from each replicate was selected for blood sample collection from the wing veins. After being centrifuged for 30 min (3,000 g at 4°C), serum samples were collected and stored at −20°C for further analysis. Subsequently, the laying hens from each group were euthanized by exsanguination. The jejuna were collected and preserved in 4 % paraformaldehyde for intestinal histomorphological analysis. Additionally, the mucosa of the jejunum and liver tissues were collected, frozen in liquid nitrogen, and stored at −80°C for antioxidant and immune analysis. For RNA extraction, samples were preserved in RNAlater (Thermo Fisher Scientific, USA) immediately after collection to stabilize RNA and prevent degradation. The cecum contents were collected in sterile cryotubes, immediately frozen in liquid nitrogen, and subsequently stored at −80°C until analysis.

Chemical analysis of CM

The basal diet and CM contents of crude protein, crude fat, crude fiber, ash, calcium, and phosphorus were determined using the standard procedures of the AOAC (AOAC, 2007). The amino acid content of the CM was determined using the National Standard of PR China (GB/T 18246-2019).

Productive performance and egg quality

Eggs were collected and weighed daily to calculate the average egg weight (**AEW**) and laying rate. Feed intake was recorded during weeks 4,

8, and 11, respectively, and used to calculate the **ADFI** and feed-to-egg ratio (**F:E**). The weeks 4 and 11 were after the experiment, two eggs from each replicate were selected for quality analysis. Albumen height, yolk color, and Haugh unit were measured using an egg analyzer (EMT-5200, Robotmation, Tokyo, Japan). Eggshell thickness and strength were assessed using eggshell thickness testers (ETG-1601A; Robotmation, Tokyo, Japan) and eggshell strength testers (EFG-0503; Robotmation), respectively.

Intestinal morphology analysis

Fixed jejunum tissue was embedded in paraffin and sectioned into 5 μm slices for hematoxylin-eosin staining, as per our previous report. Villus height (**VH**) and crypt depth (**CD**) were measured using Olympus CellSens Entry software and analyzed using the HMIAS-2000 image analysis system. For each laying hen, VH and CD were measured thrice, and the ratio of VH to CD (**VH/CD**) was calculated.

Serum and jejunum antioxidant and cytokines analysis

A total of 100 mg of jejunum mucosa was homogenized in 0.9 mL of saline using a grinder (SCIENTZ-24, Ningbo Scientz Biotechnology Co., Ltd) equipped with zirconium beads (60 Hz, three cycles of 60 s each, with ice bath intervals between cycles). The homogenate was then subsequently centrifuged at 12,000 × g for 10 min at 4°C. The resulting supernatant was collected for further analysis. The activities of catalase (**CAT**), glutathione peroxidase (**GSH-Px**), and superoxide dismutase (**SOD**), as well as the levels of malondialdehyde (**MDA**) in both the serum and jejunum, were measured using a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Serum concentrations of triglyceride (**TG**), total cholesterol (**T-CHO**), high density lipoprotein cholesterol (**HDL-C**), and **LDL-C** were determined using a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Jejunum levels of immunoglobulin A (**IgA**), **IgG, IgM**, interleukin-1β (**IL-1β**), **IL-4, IL-6, IL-10**, interferon-γ (**IFN-γ**), and tumor necrosis factor-α (**TNF-α**) were measured using commercial ELISA kits (Enzyme-linked Biotechnology Co., Ltd., Shanghai, China), per the manufacturer's instructions.

Quantitative real-time PCR

Total RNA was isolated from the liver tissue using TRIzol reagent (Tiangen Biotech Co., Ltd., Beijing, China). Reverse transcription and real-time quantitative PCR were performed with the CFX96 PCR System (Bio-Rad, Hercules, CA, USA) using commercial kits (TaKaRa, Kusatsu, Japan) (Liu et al., 2022). Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method, and primers are listed in Table S1.

Cecum microbiome analysis

Cecal microbiome analysis was conducted as described previously, with some modifications (Liu et al., 2022). In brief, microbial genomic DNA was isolated from the cecum by using a TIANamp stool DNA kit. A bead-beating step using 0.1 mm glass beads for 5 min at 30 Hz was incorporated to improve lysis efficiency for gram-positive bacteria. The volume of the elution buffer was reduced to 50 μL to maximize DNA concentration. The purified DNA was sequenced using an Illumina HiSeq 2500 PE250 system (Illumina Inc., San Diego, CA, USA). High-quality clean tags were filtered using QIIME2 (min length: 200 bp, max ambiguous bases: 0, min quality score: 25) with chimera removal by uchime-denovo. Operational taxonomic units were clustered at 97 % sequence identity by using UCLUST. Taxonomy was assigned to representative sequences by using the SILVA database. Alpha and beta diversities were calculated using QIIME2. Linear discriminant analysis effect size (LEfSe) was performed using tools available in OmicStudio.

Statistical analysis

The effects of CM supplementation on productive performance, egg quality, liver lipid metabolism, jejunal morphology, antioxidant levels, and cytokine profiles in laying hens were analyzed using orthogonal polynomial contrast to compare the levels of CM, and the other treatments, not included in the serial inclusion levels of CM, was compared using pair-wise contrasts with SPSS version 26.0 software (SPSS Inc., Chicago, IL, USA). Data are presented as the mean ± standard error. Graphs were generated using GraphPad Prism 8.0 software (GraphPad Software Inc., La Jolla, CA, USA). A P value < 0.05 was considered statistically significant. For cecum microbiome analysis, the alpha and beta diversities of the cecum microbiota were assessed using the Kruskal-Wallis test. LEfSe analysis applied filters for p values below 0.05 and an LDA above 3.

Results

Effects of CM supplementation on productive performance and egg quality of laying hens fed LP diets

As presented in Table 3, no statistically significant differences in productive performance (laying rate, ADFI, AEW, and F:E) were observed between the CON and LP groups. Compared with the LP group, no significant differences in productive performance were observed in laying hens supplemented with 3 %, 5 %, 7 %, and 9 % CM, except that the diet supplemented with 9 % CM significantly increased ($P < 0.05$) the ADFI of laying hens during the 5 to 8-week period. From weeks 9 to 11, LP diets supplemented with 7 % and 9 % CM significantly decreased the laying rate and increased the F:E ratio of laying hens compared to the CON group ($P < 0.05$). Laying hens in the 7 % and 9 % CM groups exhibited higher F:E ratios than those in the CON group throughout the experimental period ($P < 0.05$). Furthermore, 9 % CM supplementation significantly increased the ADFI of laying hens compared with that of the CON group ($P < 0.05$). The effects of dietary supplementation with CM on the egg quality of laying hens fed LP diets are presented in Table 4. There were no significant differences in eggshell thickness, eggshell strength, albumin height, or Haugh unit among the six groups of laying hens at 4 weeks of the experiment. However, dietary supplementation with 3 %, 5 %, 7 %, and 9 % CM significantly increased the yolk color of eggs compared with that of the CON and LP groups ($P < 0.05$). Furthermore, yolk color was significantly correlated with the level of dietary CM supplementation ($P < 0.05$).

Effects of CM supplementation on intestinal morphology of laying hens fed LP diets

Histological analysis of the jejunum revealed no significant differences in VH or CD among the six groups (Fig. 1 and Table 5). However, dietary supplementation with 5 % CM significantly increased the ratio of VH to CD compared with that in the CON, LP, and 9 % CM groups ($P < 0.05$).

Effects of CM supplementation on serum and jejunum antioxidant capacity of laying hens fed LP diets

As shown in Table 6, there were no significant differences in serum and jejunum GSH-Px, SOD, and CAT activities or MDA levels in laying hens between the LP and CON groups. Compared with the LP group, dietary supplementation with 5 % CM significantly increased the jejunal activities of GSH-Px and SOD in laying hens ($P < 0.05$). However, laying hens in the 9 % CM group exhibited higher serum and jejunal MDA levels and lower serum SOD activity than those in the CON group ($P < 0.05$). Serum and jejunal CAT activity showed no significant differences among the CON, LP, and 3 %–9 % CM groups. Additionally, dietary supplementation with 3 % and 7 % CM did not result in significant

Table 3
Effects of chili meal on productive performance of laying hens fed low-protein diets ($n = 8$).

Item	CON	LP	Chili meal				SEM	*P*-value		
			3 %	5 %	7 %	9 %		Treatment[1]	Linear[2]	Quadratic[3]
Week 1 to 4										
Laying rate, %	97.2	95.1	95.3	97.6	94.9	95.7	0.525	0.534	0.815	0.455
ADFI, g	127	128	131	130	130	131	0.620	0.246	0.174	0.434
AEW, g	60.6	61.2	61.9	61.6	61.6	61.5	0.148	0.129	0.782	0.378
F:E	2.17	2.22	2.25	2.17	2.25	2.22	0.015	0.433	0.637	0.580
Week 5 to 8										
Laying rate, %	96.6	94.9	95.6	96.6	95.1	94.1	0.399	0.407	0.555	0.140
ADFI, g	128^{B}	128^{b}	130^{ab}	131^{Aab}	131^{ab}	132^{Aa}	0.418	0.018	0.003	0.657
AEW,g	64.4	64.9	65.6	64.7	64.9	65.1	0.172	0.470	0.694	0.979
F:E	2.06^{B}	2.08^{ab}	2.08^{b}	2.09^{ab}	2.12^{ab}	2.16^{Aa}	0.011	0.008	0.031	0.306
Week 9 to 11										
Laying rate, %	96.1^{A}	93.8	94.0	94.4	92.4^{B}	92.1^{B}	0.431	0.014	0.111	0.353
ADFI, g	130	131	133	134	133	131	0.579	0.527	0.841	0.095
AEW, g	64.1	64.4	64.8	63.7	64.2	63.6	0.175	0.458	0.164	0.787
F:E	2.12^{B}	2.17	2.18	2.24	2.26^{A}	2.25^{A}	0.016	0.014	0.075	0.652
Week 1 to 11										
Laying rate, %	96.6	94.6	94.9	96.2	94.1	93.9	0.376	0.202	0.502	0.217
ADFI, g	128^{B}	129	131	131	131	132^{A}	0.413	0.025	0.068	0.144
AEW, g	63.0	63.5	64.0	63.3	63.6	63.4	0.150	0.486	0.546	0.697
F:E	2.12^{B}	2.16	2.17	2.17	2.21^{A}	2.22^{A}	0.011	0.009	0.082	0.701

CON: basal diet; LP:basal diet with a 1 % reduction in protein level;1 % CM:LP diet supplementation with 1 % chili meal; 3 % CM:LP diet supplementation with 3 % chili meal; 5 % CM:LP diet supplementation with 5 % chili meal; 7 % CM:LP diet supplementation with 7 % chili meal; 9 % CM:LP diet supplementation with 9 % chili meal. "n" indicating the number of replicates and number of birds per replicate. [1]Compared with the CON group, different capital superscript letters in the same row indicate significant differences ($P < 0.05$). [2,3] Compared among LP and 1 %~9 %CM groups, different lowercase superscript letters in the same row indicate significant differences. ($P < 0.05$).

Table 4
Effects of chili meal on egg quality of laying hens fed low-protein diets ($n = 8$).

Item	CON	LP	Chili Meal				SEM	*P*-value		
			3 %	5 %	7 %	9 %		Treatment[1]	Linear[2]	Quadratic[3]
Week 4										
Yolk color	8.16^{B}	8.20^{c}	8.93^{Ab}	9.52^{Ab}	10.63^{Aa}	10.88^{Aa}	0.176	0.001	0.001	0.575
Eggshell thickness, mm	0.42	0.41	0.41	0.41	0.42	0.42	0.001	0.290	0.22	0.411
Eggshell strength, N/m^2	4.97	4.48	4.54	4.34	4.56	4.64	0.091	0.495	0.651	0.614
Albumen height, mm	8.78	8.65	9.15	8.66	8.50	8.57	0.127	0.761	0.462	0.682
Haugh unit	92.98	92.17	94.33	92.38	91.57	91.81	0.639	0.857	0.527	0.677
Week 11										
Yolk color	7.77^{B}	7.85^{d}	8.87^{Ac}	9.57^{Ab}	9.47^{Ab}	10.13^{Aa}	0.147	0.001	0.001	0.035
Eggshell thickness, mm	0.42	0.41	0.41	0.40	0.40	0.409	0.002	0.057	0.250	0.230
Eggshell strength, N/m^2	4.39	4.23	4.54	4.68	4.64	4.76	0.085	0.479	0.073	0.454
Albumen height, mm	11.57	11.71	11.92	11.72	11.61	11.31	0.129	0.859	0.275	0.435
Haugh unit	105.48	105.55	106.27	105.78	105.41	105.30	0.471	0.912	0.373	0.424

CON: basal diet; LP:basal diet with a 1 % reduction in protein level;1 % CM:LP diet supplementation with 1 % chili meal; 3 % CM:LP diet supplementation with 3 % chili meal; 5 % CM:LP diet supplementation with 5 % chili meal; 7 % CM:LP diet supplementation with 7 % chili meal; 9 % CM:LP diet supplementation with 9 % chili meal. "n" indicating the number of replicates and number of birds per replicate. [1]Compared with the CON group, different capital superscript letters in the same row indicate significant differences ($P < 0.05$). [2,3] Compared among LP and 1 %~9 %CM groups, different lowercase superscript letters in the same row indicate significant differences. ($P < 0.05$).

differences in the serum and jejunal antioxidant capacity of laying hens compared with those of the CON and LP groups.

Effects of CM supplementation on serum and jejunum Cytokines of laying hens fed LP diets

The effects of CM supplementation on cytokine levels in laying hens fed LP diets are illustrated in Table 7. In serum, there were no significant differences in the concentrations of IgA, IgG, IgM, IL-1β, IL-6, IL-10, TNF-α, and IFN-γ between the CON and LP groups. However, compared with the LP group, dietary supplementation with 3 %–9 % CM significantly increased serum IL-4 levels and decreased IL-6 concentrations ($P < 0.05$). Serum IgM concentrations in the 7 % and 9 % CM groups were higher than those in the CON and LP groups ($P < 0.05$). Additionally, dietary supplementation with 5 % to 9 % CM significantly increased serum IL-10 levels compared with those of the CON group ($P < 0.05$) and reduced TNF-α levels compared with the LP group ($P < 0.05$). In the jejunal mucosa, there were no significant differences in the concentrations of IL-4 and TNF-α among the six groups. The concentrations of IL-1β and IFN-γ in the 3 % CM group were significantly lower than those in the CON and 5 % to 9 % CM groups ($P < 0.05$). The IL-10 concentration in the LP group was lower than that in the CON group, and CM supplementation increased IL-10 levels in laying hens fed LP diets. Compared with the CON and LP groups, supplementation with 3 %, 5 %, and 9 % CM significantly decreased jejunal IL-6 concentrations ($P < 0.05$).

Effects of CM supplementation on cecum microbiome composition of laying hens fed LP diets

The Chao1, Shannon, Simpson, and OUT indices were utilized to assess the α-diversity of cecum microbiome composition (Fig. 2 and Table 8). The results indicated that dietary supplementation with CM did not result in a significant difference in the α-diversity of cecum

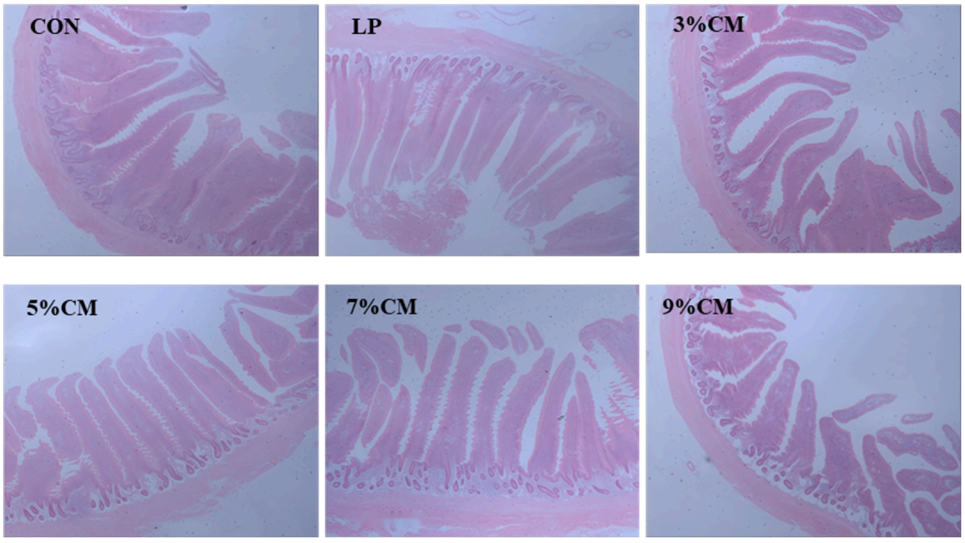

Fig. 1. Effects of Effects of chili meal on the jejunal morphology of laying hens fed low protein diets ($n = 8$). CON: control diet; LP: diets with crude protein reduced by 1 %; 3 % CM: LP diets +3 % chili meal; 5 % CM: LP diets +5 % chili meal; 7 % CM: LP diets +7 % chili meal; 9 % CM: LP diets +9 % chili meal. Different lowercase letters of peer shoulder notes indicate significant differences ($P < 0.05$).

Table 5
Effects of chili meal on the jejunal morphology of laying hens fed low protein diets ($n = 8$).

Items	CON	LP	Chili Meal				SEM	*P*-value		
			3 %	5 %	7 %	9 %		Treatment[1]	Linear[2]	Quadratic[3]
Jejunum										
VH um	46.3	895.68	897.77	927.87	904.26	836.54	12.85	0.481	0.280	0.119
CD um	8.23	156.18	149.03	134.92	143.10	157.18	3.36	0.414	0.889	0.061
VH/CD	5.73^{B}	5.85^{b}	6.23^{ab}	7.01^{Aa}	6.38^{ab}	5.45^{b}	0.15	0.048	0.558	0.005

CON: basal diet; LP:basal diet with a 1 % reduction in protein level;1 % CM:LP diet supplementation with 1 % chili meal; 3 % CM:LP diet supplementation with 3 % chili meal; 5 % CM:LP diet supplementation with 5 % chili meal; 7 % CM:LP diet supplementation with 7 % chili meal; 9 % CM:LP diet supplementation with 9 % chili meal. "n" indicating the number of replicates and number of birds per replicate. [1]Compared with the CON group, different capital superscript letters in the same row indicate significant differences ($P < 0.05$). [2,3] Compared among LP and 1 %~9 %CM groups, different lowercase superscript letters in the same row indicate significant differences. ($P < 0.05$).

Table 6
Effects of chili meal on serum and jejunal antioxidant status in laying hens fed low protein diets ($n = 8$).

Item	CON	LP	Chili Meal				SEM	*P*-value		
			3 %	5 %	7 %	9 %		Treatment[1]	Linear[2]	Quadratic[3]
Serum										
GSH-Px U/ml	956^{A}	929^{ab}	967^{a}	972^{a}	906^{ab}	871^{Bb}	13.4	0.040	0.031	0.027
SOD U/ml	13.9^{A}	13.9^{a}	14.2^{a}	14.2^{a}	13.2^{ab}	11.9^{Bb}	0.233	0.047	0.012	0.080
MDA nmol/ml	5.01^{B}	5.04^{b}	4.95^{b}	4.71^{b}	4.71^{b}	5.52^{Aa}	0.062	0.005	0.139	0.001
CAT U/ml	16.6	16.3	16.7	16.6	16.9	15.1	0.246	0.537	0.327	0.155
Jejunal										
GSH-Px U/mg prot	234	219^{b}	251^{ab}	273^{a}	228^{b}	210^{b}	5.61	0.058	0.307	0.002
SOD U/mg prot	541	518^{b}	583^{ab}	604^{a}	538^{ab}	515^{b}	8.95	0.053	0.492	0.005
MDA U/mg prot	1.04^{B}	1.15^{ab}	0.81^{b}	0.67^{b}	1.19^{ab}	1.80^{Aa}	0.991	0.015	0.055	0.015
CAT nmol/mg prot	14.5	14.4	14.7	14.6	14.9	13.1	0.215	0.521	0.224	0.147

CON: basal diet; LP:basal diet with a 1 % reduction in protein level;1 % CM:LP diet supplementation with 1 % chili meal; 3 % CM:LP diet supplementation with 3 % chili meal; 5 % CM:LP diet supplementation with 5 % chili meal; 7 % CM:LP diet supplementation with 7 % chili meal; 9 % CM:LP diet supplementation with 9 % chili meal. "n" indicating the number of replicates and number of birds per replicate. [1]Compared with the CON group, different capital superscript letters in the same row indicate significant differences ($P < 0.05$). [2,3] Compared among LP and 1 %~9 %CM groups, different lowercase superscript letters in the same row indicate significant differences. ($P < 0.05$).

microbiome composition in laying hens fed LP diets. PCoA analysis demonstrated that the β-diversity of the cecum microbiota composition in the 3 % to 9 % CM groups was significantly different from that in the CON and LP groups ($P < 0.05$). According to the results of the cecal microbiome analysis at the phylum level, the 5 %–9 % CM group exhibited an increased abundance of *Firmicutes* and a decreased relative abundance of *Bacteroidetes* compared with the CON group ($P < 0.05$). Additionally, the abundance of *Desulfobacteriota* in the 7 % CM group was significantly higher than that in the CON, LP, 3 % CM, and 5 % CM groups ($P < 0.05$). Furthermore, the relative abundance of *Actinobacteriota* in the 9 % CM group was significantly higher than that in the other groups ($P < 0.05$). At the genus level, dietary supplementation with 5 % to 9 % CM significantly decreased the abundance of *Bacteroides* compared with the CON and LP groups ($P < 0.05$). By contrast, the LP

Table 7
Effects of chili meal on serum and jejunal cytokines levels in laying hens fed low protein diets ($n = 8$).

Item	CON	LP	Chili Meal				SEM	*P*-value		
			3 %	5 %	7 %	9 %		Treatment[1]	Linear[2]	Quadratic[3]
Serum										
IgA ng/ml	1366	1275	1315	1246	1262	1281	19.4	0.544	0.779	0.805
IgG ng/ml	18.8	16.1	18.9	19.2	19.2	18.7	0.452	0.329	0.134	0.102
IgM ng/ml	947^{B}	928^{b}	995^{b}	958^{b}	1072^{a}	1125^{Aa}	14.7	0.001	0.001	0.163
IL-1β ng/l	23.3	25.8	25.3	25.1	25.8	25.8	0.345	0.247	0.888	0.561
IL-4 ng/l	30.7^{A}	24.1^{Bb}	31.1^{a}	31.6^{a}	30.8^{a}	29.6^{a}	0.616	0.001	0.008	0.001
IL-6 ng/l	6.12^{A}	5.92^{a}	5.08^{Bbc}	4.78^{Bc}	5.22^{Bb}	5.17^{Bbc}	0.102	0.001	0.004	0.001
IL-10 ng/l	7.98^{B}	8.56^{bc}	8.95^{Ab}	9.17^{Aab}	9.87^{Aa}	9.13^{Aab}	0.138	0.001	0.024	0.094
IFN-γ pg/ml	31.5	32.1	31.8	32.4	33.1	34.4	0.484	0.587	0.135	0.501
TNF-α ng/l	10.0^{A}	10.3^{a}	9.9^{ab}	9.4^{bc}	9.6^{bc}	9.1^{Bc}	0.111	0.009	0.001	0.521
Jejunal										
IL-1β ng/mg prot	28.8^{A}	26.5^{ab}	24.2^{Bb}	27.1^{a}	28.9^{a}	27.2^{a}	0.439	0.012	0.039	0.969
IL-4 ng/mg prot	29.1	25.5	26.6	27.1	26.9	27.5	0.481	0.467	0.171	0.666
IL-6 ng/mg prot	6.65^{A}	6.55^{a}	5.62^{Bb}	5.58^{Bb}	5.95^{b}	5.51^{Bb}	0.122	0.005	0.011	0.077
IL-10 ng/mg prot	9.14^{A}	7.80^{Bb}	8.15^{ab}	8.17^{ab}	8.72^{a}	8.45^{ab}	0.134	0.046	0.032	0.481
IFN-γ pg/mg prot	27.9^{A}	22.9^{Bcd}	21.2^{Bd}	24.7^{Bbc}	27.0^{ab}	28.0^{a}	0.538	0.001	0.001	0.164
TNF-α ng/mg prot	11.16	9.71	9.69	9.86	10.80	10.91	0.200	0.083	0.059	0.493

CON: basal diet; LP:basal diet with a 1 % reduction in protein level;1 % CM:LP diet supplementation with 1 % chili meal; 3 % CM:LP diet supplementation with 3 % chili meal; 5 % CM:LP diet supplementation with 5 % chili meal; 7 % CM:LP diet supplementation with 7 % chili meal; 9 % CM:LP diet supplementation with 9 % chili meal. "n" indicating the number of replicates and number of birds per replicate. [1]Compared with the CON group, different capital superscript letters in the same row indicate significant differences ($P < 0.05$). [2,3] Compared among LP and 1 %~9 %CM groups, different lowercase superscript letters in the same row indicate significant differences. ($P < 0.05$).

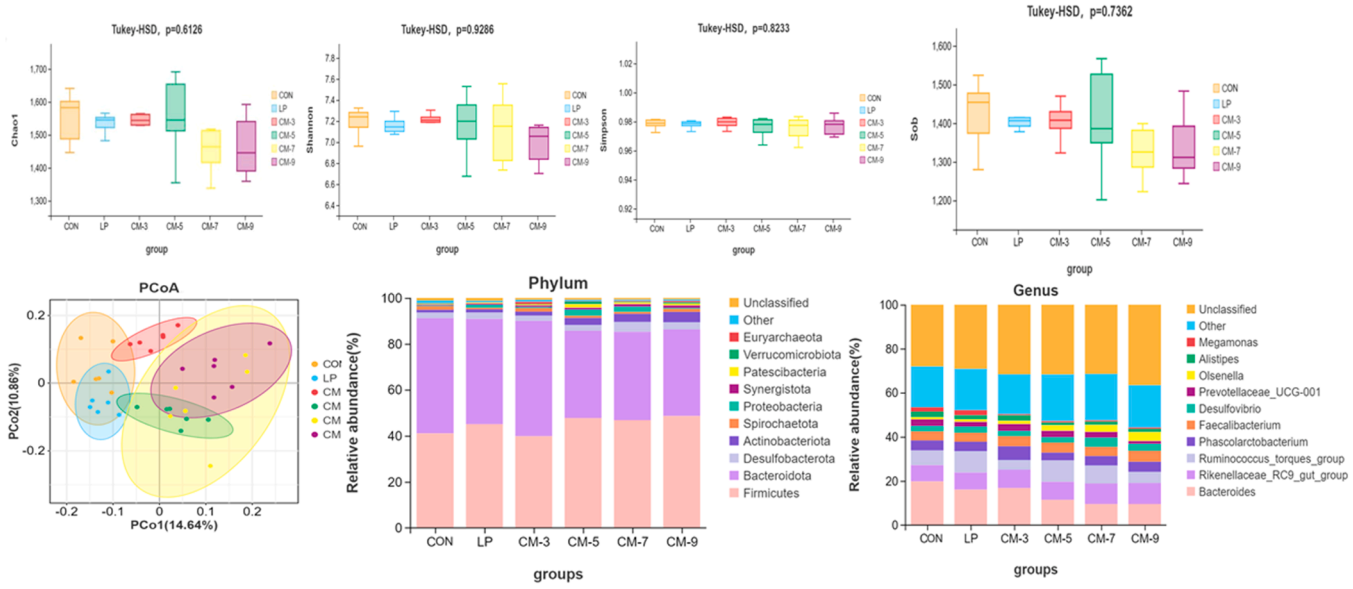

Fig. 2. Effects of chili meal on α-diversity, β-diversity, abundance of cecum microbiota in laying hens fed low protein diets ($n = 8$). CON: control diet; LP: diets with crude protein reduced by 1 %; 3 % CM: LP diets +3 % chili meal; 5 % CM: LP diets +5 % chili meal; 7 % CM: LP diets +7 % chili meal; 9 % CM: LP diets +9 % chili meal.

and 5 % CM groups exhibited an increased abundance of *Ruminococcus_torques_group* compared with the CON group ($P< 0.05$). Additionally, the abundances of *Alistipes* and *Megamonas* were lower in the 5 %–9 % CM group than in the CON and LP groups ($P < 0.05$). The 9 % CM group significantly increased the relative abundance of *Olsenella* when compared with the CON and LP groups ($P < 0.05$). The relative abundance of *Phascolarctobacterium* was significantly higher in the 3 % CM group (6.26 %) than in the other groups ($P < 0.05$). Furthermore, the relative abundances of *Desulfovibrio* and *Lactobacillus* were the highest in the 7 % CM group. Compared with the CON and LP groups, the 7 % CM and 9 % CM groups showed an increased abundance of *Synergistes* and a reduced relative abundance of *Parabacteroides* ($P < 0.05$).

Effects of CM supplementation on lipid metabolism of laying hens fed LP diets

The effects of CM supplementation on serum lipid metabolism-related indicators and liver lipid metabolism-related mRNA levels in laying hens fed LP diets are shown in Table 9. There were no significant differences in serum HDL-C, LDL-C, or TCHO levels among the six groups. However, serum TG concentrations in the 5 %–9 % CM groups were significantly lower than those in the LP group ($P < 0.05$). The LP group exhibited a significantly higher liver mRNA abundance of fatty acid synthase (*FAS*) than the CON group ($P < 0.05$). Dietary supplementation with CM significantly reduced *FAS* mRNA levels in the LP group ($P < 0.05$). Moreover, the LP group exhibited lower levels of carnitine palmitoyltransferase 1 B (*CPT*) and acyl-CoA oxidase 1 (*ACOX-*

Table 8
Effects of chili meal on α-diversity, β-diversity, abundance of cecum microbiota in laying hens fed low protein diets ($n = 8$).

Item	CON	LP	Chili Meal				SEM	*P*-value		
			3 %	5 %	7 %	9 %		Treatment[1]	Linear[2]	Quadratic[3]
Phylum										
Firmicutes	41.1^{B}	45.1^{a}	39.8^{b}	47.6^{Aa}	46.7^{Aa}	48.5^{Aa}	0.870	0.005	0.006	0.351
Bacteroidota	50.2^{A}	45.9^{a}	50.2^{a}	38.1^{Bb}	38.5^{Bb}	37.7^{Bb}	1.21	0.001	0.001	1.936
Desulfobacterota	2.40^{B}	2.76^{b}	2.28^{b}	2.45^{b}	4.20^{Aa}	3.08^{ab}	0.198	0.039	0.092	0.023
Actinobacteriota	1.21^{B}	1.37^{b}	1.73^{b}	3.09^{ab}	3.58^{Aab}	4.53^{Aa}	0.341	0.012	0.003	0.912
Spirochaetota	1.63	0.715^{b}	1.59^{a}	1.01^{ab}	0.903^{ab}	1.35^{ab}	0.149	0.390	0.479	0.021
Synergistota	0.417^{B}	0.599^{b}	0.787^{ab}	0.869^{ab}	1.21^{Aa}	1.13^{Aab}	0.078	0.015	0.014	0.697
Genus										
Bacteroides	19.7^{A}	16.0^{a}	16.7^{a}	11.4^{Bb}	9.48^{Bb}	9.51^{Bb}	0.869	0.001	0.001	0.723
Ruminococcus_torques_group	6.66^{B}	9.63^{Aa}	4.31^{b}	9.65^{Aa}	8.06^{a}	4.99^{b}	0.501	0.001	0.072	0.001
Desulfovibrio	2.35^{B}	2.71^{b}	2.22^{b}	2.36^{b}	4.08^{Aa}	3.01^{ab}	0.191	0.038	0.089	0.023
Olsenella	1.01^{B}	1.15^{b}	1.47^{b}	2.58^{ab}	3.21^{ab}	4.11^{Aa}	1.38	0.019	0.004	0.758
Alistipes	2.51^{A}	1.83^{ab}	2.47^{a}	1.17^{Bb}	1.46^{Bb}	1.44^{Bb}	0.199	0.006	0.037	0.784
Megamonas	1.93^{A}	2.25^{a}	0.472^{Bb}	0.580^{Bb}	0.514^{Bb}	0.552^{Bb}	0.155	0.008	0.004	0.011
Lactobacillus	0.679	0.563^{ab}	0.984^{ab}	0.444^{b}	1.32^{a}	1.07^{ab}	0.105	0.121	0.109	0.033
Synergistes	0.411^{B}	0.588^{b}	0.785^{ab}	0.864^{ab}	1.21^{Aa}	1.13^{Aab}	0.077	0.013	0.013	0.680
Parabacteroides	1.35^{A}	0.943^{a}	0.753^{ab}	0.671^{ab}	0.568^{Bb}	0.498^{Bb}	0.065	0.001	0.006	0.622
Erysipelatoclostridium	0.751^{A}	0.858^{a}	0.393^{Bb}	0.763^{a}	0.496^{ab}	0.499^{Bab}	0.051	0.033	0.105	0.021

CON: basal diet; LP:basal diet with a 1 % reduction in protein level;1 % CM:LP diet supplementation with 1 % chili meal; 3 % CM:LP diet supplementation with 3 % chili meal; 5 % CM:LP diet supplementation with 5 % chili meal; 7 % CM:LP diet supplementation with 7 % chili meal; 9 % CM:LP diet supplementation with 9 % chili meal. "n" indicating the number of replicates and number of birds per replicate. [1]Compared with the CON group, different capital superscript letters in the same row indicate significant differences ($P < 0.05$). [2,3] Compared among LP and 1 %~9 %CM groups, different lowercase superscript letters in the same row indicate significant differences. ($P < 0.05$).

Table 9
Effects of chili meal on lipid metabolism-related biochemical parameters levels and mRNA expression in the serum and jejunal mucosa of laying hens fed low protein diets ($n = 8$).

Item	CON	LP	Chili Meal				SEM	*P*-value		
			3 %	5 %	7 %	9 %		Treatment[1]	Linear[2]	Quadratic[3]
Serum										
HDL-C mmol/L	1.08	1.26	1.55	0.94	1.12	1.03	0.077	0.337	0.137	0.994
LDL-C mmol/L	0.413	0.372	0.621	0.422	0.475	0.414	0.037	0.534	0.823	0.378
T-CHO mmol/L	1.15	1.22	1.12	1.07	1.15	1.11	0.031	0.925	0.410	0.428
TG mmol/L	3.17	3.97^{a}	3.10^{ab}	2.67^{b}	2.68^{b}	2.62^{b}	0.138	0.065	0.007	0.113
Liver										
ACC	1.01^{A}	0.992^{a}	0.817^{a}	0.874^{a}	0.523^{Bb}	0.565^{Bb}	0.034	0.001	0.001	0.918
ACOX-1	1.00^{A}	0.773^{Bc}	1.10^{a}	1.18^{a}	1.12^{a}	0.852^{bc}	0.029	0.001	0.362	0.001
FAS	1.00^{B}	1.39^{Aa}	0.817^{bc}	0.932^{b}	0.804^{Cbc}	0.621^{c}	0.031	0.001	0.001	0.068
PPAR-α	1.01	0.822^{bc}	1.07^{abc}	1.11^{ab}	1.31^{a}	0.784^{c}	0.039	0.075	0.642	0.001
Apob	1.01	0.955^{b}	1.22^{a}	1.01^{b}	1.04^{ab}	0.853^{b}	0.030	0.076	0.108	0.017
CPT	1.00^{A}	0.66^{Bd}	1.29^{Ba}	1.11^{ab}	0.903^{bcd}	0.815^{cd}	0.036	0.001	0.792	0.001
SREPB-1C	1.00^{A}	1.14^{a}	0.903^{bc}	0.761^{bc}	0.717^{Bbc}	0.583^{Bc}	0.034	0.001	0.001	0.082
SREPB-2	1.00	1.08^{a}	0.852^{b}	0.815^{b}	0.815^{b}	0.886^{ab}	0.027	0.092	0.054	0.016

CON: basal diet; LP:basal diet with a 1 % reduction in protein level;1 % CM:LP diet supplementation with 1 % chili meal; 3 % CM:LP diet supplementation with 3 % chili meal; 5 % CM:LP diet supplementation with 5 % chili meal; 7 % CM:LP diet supplementation with 7 % chili meal; 9 % CM:LP diet supplementation with 9 % chili meal. "n" indicating the number of replicates and number of birds per replicate. [1]Compared with the CON group, different capital superscript letters in the same row indicate significant differences ($P < 0.05$). [2,3] Compared among LP and 1 %~9 %CM groups, different lowercase superscript letters in the same row indicate significant differences. ($P < 0.05$).

1) mRNA than the CON group ($P < 0.05$). By contrast, dietary supplementation with CM (3 % and 5 % CM groups) significantly elevated *CPT* and *ACOX-1* mRNA levels ($P < 0.05$), restoring them to the CON levels. Compared with the LP group, dietary supplementation with CM significantly decreased ($P < 0.05$) the mRNA levels of carboxylase (*ACC*) (7 % and 9 % CM groups), sterol regulatory element-binding protein 1C (*SREBP-1C*) (5 % to 9 % CM groups), and *SREBP-2* (3 % to 7 % CM groups) and upregulated ($P < 0.05$) the mRNA levels of apolipoprotein B (*Apob*) (3 % CM group) and peroxisome proliferator-activated receptor alpha (*PPAR-α*) (7 % and 9 % CM group) in the liver of laying hens.

Discussion

The chemical composition of CM is usually affected by the chili pepper species, processing method, and extraction solvent. The chemical composition of CM in this study exhibited notable variations compared to previous reports. Specifically, crude protein, crude fiber, ash, Ca, and P were higher than values reported by Thiamhirunsopit et al. (2014), whereas crude fat content was markedly lower. In contrast, Fan et al. (2017) documented higher crude protein, ash, and P in CM compared to our findings, while their crude fat content was significantly lower than both our study and Thiamhirunsopit et al. (2014). These discrepancies likely arise from differences in chili pepper cultivars, agronomic conditions, and processing methods. This discrepancy may be attributed to the extraction of the capsicum pigment or oleoresin processing method.

Birds, unlike mammals, are insensitive to the tingling and burning pain of chili pepper, capsaicin, and natural capsicum extracts (Jordt and Julius, 2002). Consequently, poultry can consume chili pepper powder or natural extracts without any adverse effects on growth performance. Thiamhirunsopit et al. (2014) reported that capsaicin in CM (0.43 g/kg DM) represents 23 % of the concentration found in chili powder. Given the established safety of chili pepper powder and capsicum extract as

antibiotic alternatives in poultry (Abd El-Hack et al., 2022), CM's lower capsaicin content suggests potential applicability. However, its specific effects on laying hens require further investigation. Therefore, in this study, we systematically evaluated the effects of CM on the production performance, antioxidant activity, anti-inflammatory responses, intestinal health, and lipid metabolism of laying hens fed LP diets.

Thiamhirunsopit et al. (2014) suggested that dietary supplementation with up to 7.89 % CM had no significant adverse effects on the growth performance of broilers under high stocking density conditions. A diet supplemented with 5 % CM for 28 d did not significantly affect the growth performance of growing pigs; however, dietary supplementation with 10 % CM also resulted in decreased ADG and feed efficiency in growing pigs (Fan et al., 2017). Similarly, in our study, during the initial period (1–4 weeks), dietary supplementation with 3 %–9 % CM did not have a significant adverse effect on the productive performance of laying hens fed LP diets. However, adverse effects were observed in the 7 % and 9 % CM groups, which exhibited higher ADFI and F:E ratios. This suggests that CM levels should not exceed 5 % of the LP diet of laying hens during long-term supplementation. The increased F:E ratio in laying hens was attributed to the higher ADFI in the 7 % and 9 % CM groups. Liu et al. (2021a) demonstrated that dietary supplementation with capsaicin significantly improved the ADFI in aged laying ducks. Therefore, the elevated ADFI in the 7 % and 9 % CM groups may be linked to the higher capsaicin content of their diets. Moreover, during the 9–11-week period, the 7 % and 9 % CM groups exhibited lower laying rates than the CON group. Studies have indicated that high doses of capsaicin have detrimental effects on ovarian follicular development, and lower doses promote follicular development (Alatriste et al., 2013; Zik et al., 2012). Therefore, in our study, the reduced laying rates observed in laying hens receiving high levels of CM supplementation may result from the synergistic interaction between the low-protein dietary regimen and CM inclusion, rather than capsaicin dose alone. Furthermore, we observed a significant increase in egg yolk color in the groups supplemented with CM, and other egg quality parameters were not significantly affected by dietary CM supplementation. This finding is consistent with those reported by Lokaewmanee et al. (2013). The color of egg yolks is determined by the deposition and pigmentation capacity of dietary carotenoids. Studies have revealed that capsaicin imparts a deep red color to egg yolks (Bocanegra et al., 2004; Hamilton et al., 1990). Furthermore, we observed that egg yolk color in the CM supplementation groups was significantly increased, and other egg quality parameters were not significantly affected by dietary CM supplementation. This finding is consistent with those of Lokaewmanee et al. (2013). The increased egg yolk color may be explained by the increased concentrations of capsaicin in the diets from the 3 % to 9 % CM groups.

Changes in the VH, CD, and their ratios are key indicators of intestinal health and affect nutrient digestion and absorption. In this study, supplementation with 5 % CM resulted in an increased ratio of VH to CD in laying hens fed LP diets. However, no significant differences were observed between the CM supplementation and the CON groups. Li et al. (2022) reported that dietary capsaicin supplementation enhanced jejunal VH, width, and surface area in broilers at 21 d of age; however, no significant differences were observed in jejunal morphology at 42 d. Thiamhirunsopit et al. (2014) found that dietary supplementation with CM did not significantly affect intestinal nutrient digestibility. These results suggested that the lack of pronounced effects of CM supplementation on intestinal morphology, as well as the beneficial effects of capsaicin on intestinal morphology, may be correlated with bird age.

Studies have shown that chili peppers, especially capsaicin, enhance antioxidant properties in broilers and pigs (Abd El-Hack et al., 2022; Cervantes et al., 2024). Antioxidant enzymes, such as GSH-Px, CAT, and SOD, protect against oxidative damage, and MDA serves as a biomarker of redox status. Thiamhirunsopit et al. (2014) found that dietary CM supplementation reduced serum MDA levels in broilers under high stocking density conditions. Fan et al. (2017) reported no significant effects on serum MDA, SOD, or T-AOC in growing pigs. In our study, 3 %–7 % CM supplementation did not affect serum SOD, CAT, or GSH-Px activity, but 9 % CM significantly decreased GSH-Px activity and increased MDA levels compared with those of the CON group. These results suggest that the effects of chili meal on antioxidant parameters may vary depending on the animal species and rearing conditions. In our present study, the 5 % CM group showed higher SOD and CAT activities in jejunal tissues than the LP group, and 9 % CM increased jejunal MDA concentrations. These differences may be due to variations in animal models. The negative effects of 9 % CM on antioxidant status may result from the high soybean oil levels in the diet of this group, which can oxidize and impair antioxidant function.

The anti-inflammatory properties of capsaicin have been confirmed in in vitro and in vivo studies, particularly in rats (Srinivasan, 2016). Similarly, in this study, a beneficial effect of CM supplementation was observed in the serum and jejunum of laying hens fed the LP diet. Results indicated that serum levels of IL-6 and TNF-α, as well as jejunal levels of IL-6, IL-1β, and IFN-γ, were decreased in the groups receiving CM supplementation. By contrast, serum levels of IL-10 and IgM were elevated in these groups. Spiller et al. (2008) reported that chili pepper could reduce serum levels of TNF-α and IL-1β in mice. Additionally, it has been shown that capsaicin decreased the mRNA expression levels of TNF-α, IL-1β, and IL-6 in rats with acetylsalicylic acid-induced gastritis, reducing inflammatory cell infiltration (Mendivil et al., 2019). Therefore, the beneficial anti-inflammatory effects of CM in laying hens may be attributed to the presence of capsaicin residues.

Capsaicin, a bioactive component in chili meal, has been shown to reduce pathogenic gut bacteria and enhance intestinal barrier integrity (Periferakis et al., 2023). The gut microbiota is crucial for sustaining host health and has emerged as a prominent focus of research (Yeoman et al., 2012). In this study, supplementation with 5 %–9 % CM enhanced the abundance of Firmicutes while concurrently reducing the abundance of Bacteroidetes. Furthermore, the group that received 7 % CM showed the highest abundance of Desulfobacteria. Bacteroidetes participate in various metabolic activities, including the utilization of nitrogen compounds, carbohydrate digestion, and maintenance of intestinal microecological balance (Sun et al., 2019). Therefore, laying hens in the 7 % and 9 % CM groups exhibited lower feed conversion ratios, which may be correlated with a decreased abundance of Bacteroidetes. Increased levels of Firmicutes and decreased levels of Bacteroidetes have been associated with gut inflammation and barrier dysfunction (Las Heras et al., 2019; Rabot et al., 2016). Wang et al. (2019) indicated that the abundance of Firmicutes exhibited a strong positive correlation with the expression of IL-1β, TNF-α, and IFN-γ, and the abundance of Bacteroidetes showed a negative correlation with the expression of TNF-α. The increased abundance of Bacteroidetes coupled with the reduced abundance of Firmicutes in the cecum may explain the beneficial effects of CM supplementation on the anti-inflammatory responses of laying hens. *Desulfovibrio* and *Olsenella* levels increased in the 7 % and 9 % CM groups, and *Megamonas* levels decreased. *Desulfovibrio* is associated with the production of propionic acid (Liu et al., 2019). In the cecum, *Desulfovibrio* was positively correlated with serum IgM levels, suggesting its role in enhancing the anti-inflammatory capacity of laying hens supplemented with 7 % or 9 % CM. *Megamonas* inhibits Salmonella growth and produces short-chain fatty acids (Barnes and Impey, 1974; Mancabelli et al., 2016). Lower levels of *Megamonas* in chickens correlated with reduced serum and jejunal IL-6 levels, indicating that a reduction in pro-inflammatory factors can diminish inflammatory responses. These findings suggest that CM supplementation may enhance intestinal barrier integrity, morphology, and immune function by optimizing the gut microbiome composition and increasing the number of beneficial bacteria.

In laying hens, fatty liver represents a prevalent metabolic challenge, especially when they are on LP diets characterized by imbalanced energy- to-protein ratios. Studies have confirmed that chili pepper, specifically its active component, capsaicin, can alleviate lipid metabolism disorders in mice subjected to high-fat diets and reduce lipid

accumulation in mesenteric and epididymal adipose tissues (Azlan et al., 2022; Li et al., 2019). Puvača et al. (2019) reported that diets containing 0.5 % and 1.0 % chili pepper significantly reduced serum TC, TG, and LDL-C levels while increasing HDL-C levels in broilers. In this study, a diet comprising 5 %–9 % CM significantly decreased serum TG levels in laying hens fed LP diets, suggesting that CM may aid in decreasing lipid deposition. However, no significant changes were observed in serum TC, HDL-C, or LDL-C levels. The effects of chili pepper on lipid metabolism are primarily attributed to capsaicin, which is present at lower concentrations in CM than in whole chili pepper, potentially accounting for its reduced impact on lipid metabolism. The liver is a crucial metabolic organ that plays a significant role in lipid synthesis, degradation, and transport in laying hens. ACC and FAS are critical rate-limiting enzymes involved in de novo lipogenesis, and their activities are regulated by PPAR and SREBP-1C (Song et al., 2018). CPT1 and ACOX-1 are essential enzymes that regulate mitochondrial fatty acid oxidation (Lu et al., 2024; Schlaepfer and Joshi, 2020). We found that the mRNA levels of *FAS, ACC*, and *SREBP-1C/2* in the livers of laying hens fed LP diets were downregulated by dietary supplementation with CM. Conversely, the mRNA levels of *PPAR-α, CPT1*, and *ACOX-1* were upregulated by CM supplementation. These results suggest that CM reduces lipid deposition in laying hens fed LP diets by inhibiting the synthesis and enhancing the degradation of fatty acid-related genes.

Conclusion

CM, a by-product of chili pepper oil or pigment extraction, was incorporated as a feed ingredient in the diets of laying hens at levels of up to 5 %, without any adverse effects on productive performance. Additionally, CM significantly enhanced the color of egg yolk. Furthermore, dietary supplementation with CM improved the anti-inflammatory capacity, cecal microbiome composition, and lipid metabolism in laying hens. Therefore, CM has the potential to be developed as an unconventional feed resource for laying hens during the egg production period.

Disclosures

The authors declare no conflict of interest in this manuscript.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2025.105001.

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