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Cherry laurel (*Laurocerasus officinalis* Roem.) unshelled kernel: A potential feed additive to improve laying performance, yolk fatty acid profile, and antioxidant capacity in laying hens housed with outdoor access

Caroço sem casca de louro-cereja (*Laurocerasus officinalis* Roemer): Aditivo alimentar potencial para melhorar o desempenho, a composição em ácidos gordos e a capacidade antioxidante dos ovos de galinhas poedeiras

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ABSTRACT

Phytogenic products are useful poultry feed additives owing to their beneficial effects on health, laying performance, and product quality. This study aimed to determine the effect of cherry laurel (*Laurocerasus officinalis* Roem.) unshelled kernel (CLUK) dietary supplementation on the laying performance, egg quality, and yolk fatty acid (FA) and antioxidant contents in laying hens. 400 Lohmann Brown hens, housed with outdoor access, were randomly allocated into four groups. Each group comprised four replicates of 25 hens. Hens in the control group were fed on a basal diet without CLUK (0CK), whereas those in the treatment groups were fed on a basal diet supplemented with CLUK at doses of 2.5 (2.5CK), 5 (5CK), or 10 (10CK) g per kg diet for 12 weeks. The egg weight in the 5CK group was higher than that in the 0CK and 2.5CK groups. The feed conversation rate (FCR) in the 2.5CK and 10CK groups was lower than that in the 0CK group. The yolk heptadecanoic acid content was higher in the 5CK group. The yolk docosahexaenoic acid content was upregulated in the 2.5CK and 10CK groups. The yolk n6/n3 ratio was higher in the 0CK. Principal component analysis plot revealed seven egg yolk FAs. The egg yolk 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity in the 10CK group was higher than that in the 0CK and 2.5CK groups. These findings suggest that CLUK is a potential laying hen feed additive and improves egg weight, FCR, and yolk FA profile, DPPH scavenging potential, and n6/n3 ratio.

Index terms: Fruit kernel; phytogenic feed additive; omega-6/omega-3 ratio; egg production; egg quality.

RESUMO

Produtos fitogênicos são aditivos promissores na alimentação de aves, com efeitos positivos na saúde, desempenho de postura e qualidade dos ovos. Este estudo avaliou os efeitos da suplementação dietética com grãos sem casca de louro-cereja (CLUK) sobre o desempenho produtivo, qualidade dos ovos, perfil de ácidos graxos (AG) e capacidade antioxidante da gema em galinhas poedeiras. Foram utilizadas 400 galinhas Lohmann Brown com acesso ao ar livre, distribuídas aleatoriamente em quatro grupos com quatro repetições de 25 aves. O grupo controle (0CK) recebeu dieta basal sem CLUK, enquanto os grupos experimentais receberam 2,5 (2,5CK), 5 (5CK) ou 10 (10CK) g/kg de CLUK por 12 semanas. O peso dos ovos foi maior no grupo 5CK em comparação aos grupos 0CK e 2,5CK. A conversão alimentar foi melhor nos grupos 2,5CK e 10CK. O conteúdo de ácido heptadecanóico foi mais elevado no grupo 5CK, enquanto o teor de ácido docosahexaenóico (DHA) aumentou nos grupos 2,5CK e 10CK. A razão n6/n3 foi maior no grupo controle. A análise de componentes principais destacou sete AGs na gema. O grupo 10CK apresentou maior capacidade antioxidante (DPPH) em comparação aos grupos 0CK e 2,5CK. Os resultados indicam que o CLUK pode ser um aditivo eficaz na dieta de galinhas poedeiras, melhorando o desempenho produtivo, a qualidade nutricional da gema e a atividade antioxidante.

Termos para indexação: Caroço do fruto; aditivo fitogênico para ração; relação ômega-6/ômega-3; produção de ovos; qualidade dos ovos.

Introduction

The demand for eggs produced under free-range conditions and functional foods has increased research interest in functional egg production with a focus on poultry nutrition using this rearing system. To improve poultry nutrition, several potential natural feed additives have been examined. In addition to being rich in environmentally friendly and safe bioactive compounds, phytogenic supplements are effective antioxidant, anti-microbial, anti-inflammatory, and immunostimulant alternatives to improve egg composition and animal performance (Baghban-Kanani et al., 2019; Shirzadi et al., 2020; Abdelli, Solà-Oriol & Pérez, 2021; Aikpitanyi & Imasuen, 2023; Liu et al., 2023). The beneficial effects of phytogenic feed additives on poultry production and health can be attributed to their rich contents of active compounds, such as essential oils, flavonoids, polyphenols,

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tannins, and saponins (Aminullah et al., 2025). Fruit kernels with high phenolic contents are potential feed additives (Tareen et al., 2017). Palm kernel, which is rich in bioactive compounds with anti-inflammatory and antibacterial properties, has been used to maintain health and maximize the productivity of laying hens (Al-Zuhairi, Mhyson & Mohammed, 2024). Meanwhile, the supplementation of grape kernels, which contain the antioxidant resveratrol, to quail diets improves feed efficiency (Silici, Güçlü & Kara, 2011). These findings indicate that the by-products of fruit processing are an effective and economical source of bioactive compounds with antioxidant and synbiotic properties (Ibrahim et al., 2017; Jideani et al., 2021). However, Vlaicu et al. (2023) revealed that the benefits of dietary supplementation of these by-products in poultry feeds are inconsistent, with some by-products exerting adverse effects. Therefore, there is a need to identify alternative feed additives with natural and bioactive properties to develop sustainable and healthy solutions.

Cherry laurel (*Laurocerasus officinalis* Roem.) fruit is a rich source of antioxidants, including phenolics (chlorogenic acid, phenolic acids, anthocyanins, and vanillic acid) and ascorbic acid (Yaylacı-Karahalil & Şahin, 2011). Additionally, cherry laurel unshelled kernel (CLUK) is a potential poultry feed additive as it contains various bioactive compounds with antioxidant properties (Yaylacı-Karahalil & Şahin, 2011; Bilenler & Karabulut, 2023). The transfer of dietary bioactive compounds to the yolk (Panaite et al., 2016; Grčević et al., 2019) increases the nutraceutical value of eggs and enhances consumer acceptance. Compared with other fruit kernels, CLUK is minimally processed and is locally available. Thus, CLUK can minimize environmental impacts, enabling sustainability and innovation. This study hypothesized that CLUK enhances the performance of laying hens, improves egg quality, and enables the production of functional foods by enriching eggs with antioxidants. To validate this hypothesis, this study aimed to determine the effects of dietary supplementation of different CLUK doses on the body weight (BW), egg production, feed intake (FI), and feed conversion ratio (FCR) of laying hens housed with outdoor access, the internal and external egg quality traits, and the egg yolk fatty acid (FA) and antioxidant contents.

Material and Methods

Ethics approval

This study was performed between May and July 2021 at the poultry facilities of the Faculty of Agriculture, Eskişehir Osmangazi University, Eskişehir, Türkiye (39°45′42″ N and 30°28′40″ E, 813 m above sea level). The Experimental Animals Ethics Committee of Eskişehir Osmangazi University (Eskişehir, Türkiye) reviewed and approved all the experimental procedures of the animal study. The committee also determined that this study was not an unnecessary repetition of previous studies (Approval number: 802/2020).

Diets, hens, and experimental design

Hens were provided with the experimental diets in a mash form. Water was provided ad libitum throughout the experimental period. The ingredients, chemical composition, FA composition, and antioxidant capacity of the basal diet are presented in Table 1.

The preparation of CLUK has been previously described by Barasoğlu and Kop-Bozbay (2024). Briefly, kernels of harvested cherry laurel fruits were separated and dried. The peel was removed and ground to a particle size of 1 mm. Next, the nutrient content, total phenolic content, antioxidant activity, ascorbic acid, and FA composition were determined (Table 2).

This study randomly allocated 400 Lohmann Brown laying hens aged 22 weeks into four groups with four replicates (25 hens per replicate). Hens in the control group were fed on a basal diet with no CLUK (0CK), whereas those in the treatment group were fed on the basal diet supplemented with CLUK at a dosage of 2.5 (2.5CK), 5 (5CK), or 10 (10CK) g per kg diet. CLUK doses were determined by considering the total polyphenol compound content (Barasoğlu & Kop-Bozbay, 2024). CLUK was supplemented during the preparation of the experimental diets, ensuring that the test diets contained total extractable polyphenols at the previously reported doses (Mahfuz, Shang & Piao, 2021). The treatment period was from week 22 to week 34 of the hen age. The initial two-week period constituted the pre-trial phase.

The laying hens were housed in an experimental poultry house with a floor-litter system and outdoor access. The poultry house was mechanically ventilated and illuminated with white LED lightbulbs (16 hours of light and 8 hours of darkness). Each replicate was allocated 2.5 × 3 m indoor pens and 2.5 × 10 m outdoor plots. The indoor pens were equipped with a perch, individual nests/five hens, and an equal number of automatic drinkers and feeders. The outdoor pens were equipped with automatic drinkers. Sixteen-floor pens were evenly distributed along the long axis of the poultry house. Each pen had one exit doorway (32 × 32 cm) leading from the indoor to the outdoor pen. Hens freely accessed a non-vegetated soil area during the experiment based on weather conditions. The temperature and relative humidity in the poultry house were maintained at 20 ± 1°C and 60%–70%, respectively.

Data collection, measurements, and analysis

At the beginning and end of the experiment, the BW of individual birds was measured and the FI and FCR were calculated. The FCR was expressed as the compound feed consumed per kilogram of egg mass. The number of eggs collected daily from all groups was determined, and the egg-laying rate (%) was calculated for two weeks. Mortality was determined daily during the experimental period. No deaths occurred in any group.

Table 1: The ingredients and nutrient levels of the basal diet (%, as-fed basis).

Ingredient	
Corn	44.24
Soybean meal (46% CP)	23.9
Wheat	21.7
Dicalcium phosphate	0.85
Limestone (38%)	8.5
DL-methionine	0.25
L-Lysine	0.05
Salt	0.26
Premix †	0.25
Nutrient levels	
Metabolizable energy (Kcal/kg)	2750
Crude protein‡	16.91
Ether extract	2.40
Crude fiber	2.99
Lysine	1.04
Methionine + cysteine	0.78
Ash	12.33
Calcium	4.03
Available phosphorus	0.31
Fatty acids‡	
Palmitic acid (16:0)	13.59
Palmitoleic acid (16:1)	-
Heptadecanoic acid (17:1)	-
Stearic acid (18:0)	3.29
Oleic acid (18:1)	31.63
Linoleic acid (C18:2)	47.04
Alpha-linolenic acid (18:3)	2.65
Arachidic acid (20:0)	0.43
Eicosenoic acid (20:1)	0.50
Eicosatrienoic acid (20:3)	0.34
Linolenic acid (20:3)	0.34
Lignoceric acid (24:0)	0.19
Σ SFA	17.50
Σ MUFA	32.13
Σ PUFA	50.37
DPPH‡	29.20

† Provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 2400 IU; vitamin K3, 2,5 mg; vitamin E, 1.75 IU; vitamin B12, 0,015 mg; vitamin B2, 7 mg; D-pantothenic acid, 8.02 mg; folic acid: 750 mg; niacin, 19.8 mg; choline, 382.9 mg; Co, 100 mg; Cu, 5.0 mg; I, 100 mg; Fe, 50.35 mg; Mn, 80 mg; Se, 0.3 mg; Zn, 40 mg. ‡ Analyzed values, whereas the others were calculated values. SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; DPPH: 2,2 diphenyl-1-picrylhydrazyl.

Table 2: Nutrient content, bioactive component, and fatty acid profile of cherry laurel unshelled kernel (Barasoğlu & Kop-Bozbay, 2024).

Item	Quantity
Nutrient, g/100 g	
Dry matter	95.22
Ash	3.60
Crude protein	28.94
Ether extract	34.55
Acid detergent fiber	26.25
Neutral detergent fiber	36.70
Bioactive component	
Total phenolic content, mg GAE/g	3.31
Antioxidant activity, µg TE/mg	
DPPH radical scavenging	11.79
ABTS radical scavenging	8.00
FRAP scores	139.84
Ascorbic acid, %	1.57
Fatty acid, g/100 g FA	
Palmitic acid (C16:0)	11.78
Palmitoleic acid (C16:1)	2.60
Heptadecenoic acid (C17:1)	0.09
Stearic acid (C18:0)	2.54
Oleic acid (C18:1)	66.61
Linoleic acid (C18:2)	15.61
Alpha-linolenic acid (C18:3)	nd
Arachidic acid (C20:0)	0.48
Eicosenoic acid (C20:1)	0.19
Eicosatrienoic acid (C20:3)	nd
Linolenic acid (C20:3)	0.06
Lignoceric acid (C24:0)	0.05
Σ SFA	14.85
Σ MUFA	69.49
Σ PUFA	15.67

GAE, Gallic acid equivalent, TE, Trolox equivalent; DPPH, 2,2-diphenyl −1-picrylhydrazyl; ABTS, 2,2'-casino-bis (3-ethylbenzothiazoline-6-sulfonic acid); FRAP, Ferric reducing antioxidant power; nd, not detected; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Internal and external egg qualities were measured with four eggs (64 eggs) in each pen at 14-day intervals. The egg weights were determined using a precision balance with an accuracy of 0.001 g. The length and width of the egg were measured with a digital caliper (Dasqua Ip54-0.01 mm). The egg shape index

was calculated as follows: egg shape index = (width/height) × 100. To obtain the eggshell, the eggs were cracked on a flat surface (a glass stand with a mirror), and any white residues adhering to the shell were removed. The eggshell was weighed. The shell thickness (mm) was calculated at three specific points (blunt, equator, and pointy ends) using a digital caliper (Dasqua 2110-8105, 150mm Ip54).

To determine internal quality characteristics, yolk, and albumen lengths were measured with a tripod micrometer (Mitutoyo-0.01 mm). The diameter of the yolk and the length and width of the albumen were measured using a digital caliper. Haugh unit (HU) was calculated as follows: HU = 100 Log (albumen height (mm) + 7.57 − 1.7 egg weight (g)$^{0.37}$)). The values of the yolk CIE Lab (L*: brightness, a*: redness, b*: yellowness) were determined using Minolta CR-400. The egg yolk and albumen were carefully placed in a sterile container and weighed using a precision balance. The percentage weight of yolk was calculated as follows: yolk percentage = (yolk weight × 100)/egg weight. Meanwhile, the percentage weight of albumen was calculated as follows: albumen percentage = (albumen weight × 100)/egg weight. The pH of the yolk and albumen was measured using a digital pH meter (Hanna HI-2002-02).

On the last day of the experiment, six eggs from each replicate (96 eggs in total) were collected to determine the FA profile and total antioxidant level in the yolk. The FA composition of the basal diet and egg yolk was determined following the methods of Folch et al. (1957). The sample (0.15−1.20 g) was collected in a glass tube, weighed, and boiled with 2.5 mL of 0.5 M NaOH for 10 min. After cooling, the sample was boiled with 2 mL of boron trifluoride for 5 min. The boiled sample was cooled and boiled with 2 mL of N-heptane for 1 min. Next, the sample was cooled, mixed with 5 mL of saturated sodium chloride, and centrifuged at 4,000 rpm and 4°C for 10 min. The supernatant was injected into a gas chromatography system (7890A, Agilent Tech., USA). The FA values were expressed as weight percentages relative to the total FAs. The antioxidant levels of the basal diet and egg yolk were analyzed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, following the modified methods of Sacchetti et al. (2005). Briefly, the sample (2 g) was subjected to ultrasonication with 25 mL pure methanol for 20 min. The filtered sample was transferred to 0.1 mL glass tubes and incubated with 2.9 mL of DPPH solution (0.0025 g DPPH in 100 mL methanol). The absorbance of the mixture at 517 nm was measured using a spectrophotometer (UV-160A, Shimadzu, Japan). The samples were read at the 60-minute mark, and the DPPH values were calculated as follows: [(control absorbance − sample absorbance)/control absorbance] × 100.

Statistical analysis

Performance data were analyzed using one-way analysis of variance (ANOVA) with the repeated measurement tab of the general linear model procedure in the SPSS package. The number of replications was determined based on sample size and power estimation analysis. The egg quality characteristics were evaluated using the based on the weekly mean values. The means were compared using the Duncan test. Normality, which is one of the basic assumptions of ANOVA, was evaluated using the Kolmogorov-Smirnov test. The homogeneity of variance was evaluated using the Levene test. The normality of the distribution was evaluated using the Kolmogorov-Smirnov test, which revealed that the data were suitable for analysis. The homogeneity of variance between groups was examined using Levene's test. Principal component analysis (PCA) was used to examine the effect of the CLUK on the egg yolk FA profile, simultaneously considering all variables. The Kaiser-Meyer Olkin and Bartlett's tests confirmed that the data were suitable for factor analysis. Thus, a new set of 18 orthogonal variables (loading vectors) was generated via PCA. PCA was performed using the Kaiser criterion to determine the principal components (PCs) and the factor procedure of the relevant package program (Jolliffe, 2002).

Results and Discussion

To the best of our knowledge, this is the first study to report the effects of dietary CLUK supplementation on the performance, internal egg quality (yolk DPPH scavenging activity and yolk FA contents), and external egg quality in laying hens reared in a free-range system and/or conventional systems. The egg weight in the 5CK group was higher than that in the 0CK and 2.5CK groups (Table 3), ($P < 0.05$). Additionally, hens in the 2.5CK and 10CK groups exhibited decreased FCR. These findings suggest that CLUK is a potential feed additive to improve overall health and performance in poultry (Dhama et al., 2014). Feed additives improve the FCR by regulating the intestinal microbiota, increasing nutrient digestibility and absorption, and improving ovarian characteristics (Saki et al., 2014; Bai & Li, 2022; Liu et al., 2023).

Feed supplementation with phenolic-enriched extracts is reported to promote intestinal health by modulating the gut microbiota dynamics and adaptive immune responses (Das et al., 2020), improving villus development, intestinal barrier function, immunity, and beneficial intestinal microflora (Zhao et al., 2025), and regulating metabolism. Furthermore, feed additives with antioxidant compounds enhance the endogenous antioxidant defense in laying hens through various mechanisms, such as scavenging free radicals and regulating enzymatic activity. Thus, these feed additives effectively increase egg weight and improve egg quality (Wen et al., 2019; Shahid et al., 2022). This study did not examine the effect of CLUK on these parameters. Based on previous findings, the beneficial effect of CLUK with high antioxidant activity and phenolic compounds on FCR and egg weight can be attributed to its regulatory effect

on villus development, intestinal barrier function, immunity, and beneficial intestinal microflora (Das et al., 2020; Shahid et al., 2022; Zhao et al., 2025) of hens. Limited studies have examined the effect of fruit kernels on egg production. However, various studies have investigated the effect of herbal preparations on egg production. Consistent with the findings of this study, the supplementation of various phytogenic feed additives rich in bioactive compounds, such as *Artemisia annua* leaf (Baghban-Kanani et al., 2019), fenugreek powder and extract (Samani et al., 2020), black pepper and red pepper (Aikpitanyi & Imasuen, 2023), and date kernel (Al-Zuhairi, Mhyson & Mohammed, 2024) to laying hen diet is reported to improve FCR, increase egg weight, and enhance egg quality. Previous studies have demonstrated that secondary metabolites of plants can support animal growth, enhance immunity, and improve the quality of animal products (Mahfuz, Shang & Piao, 2021). Thus, the bioactive compounds of CLUK may also improve FCR and increase egg weight. The improvement of FCR (from 2.36 to 2.28) can significantly affect commercial poultry production. In large-scale operations, minor improvements can lead to significant economic savings by decreasing feed costs and increasing productivity, which results in cost savings. The maximum external supplementation of CLUK to the basal diet in this study was 1%. However, the supplementation at this dose does not decrease the nutritional composition of the diet to adversely affect laying performance. Further studies are needed to determine if the positive effect of CLUK on egg weight and consequently FCR in laying hens is due to the active nutrient content or the feed raw material properties.

In free-range poultry production, several factors, including individual animal differences, stress tolerance, and social status, can influence the welfare of chickens. Housing and feeding practices can adversely affect animal welfare and the profitability of the enterprise (Kop-Bozbay et al., 2021). In this study, mortality was not observed in any treatment groups and the performance was satisfactory for the genotype, indicating that all treatments can enhance profitability. The increased metabolic activity resulting from the intensified poultry breeding enhances the production of intracellular free radicals. These free radicals can cause oxidative stress, trigger inflammation, and disrupt various cellular processes (Hosseinzadeh et al., 2015). Phytochemicals and plants play an essential role in the health and productivity of animals (Shirzadi et al., 2020; Abdelli, Solà-Oriol & Pérez, 2021; Abd El-Hack et al., 2023). CLUK supplementation effectively increased the antioxidant system function and immunity of animals, which can be attributed to its bioactive constituents.

The quality of eggs can be enhanced by regulating the diet of laying hens (Kop-Bozbay et al., 2021). As shown in Table 4, the internal and external quality characteristics of the eggs were not significantly different ($P > 0.05$). CLUK supplementation to laying hen diets in a free-range system did not exert adverse effects on yolk weight, albumen weight, shell weight, egg yolk/albumen ratio, shell thickness, albumen index, yolk index, shape index, HU, egg yolk and albumen pH values, and egg yolk color. The efficacy of phytochemical supplementation in dietary regimens is dependent on the specific active compounds at a well-defined dose. The differential efficacy can be attributed to the doses utilized, which can explain the observed lack of response. This suggests that CLUK did not adversely affect the investigated parameters or was not administered at levels that would exert these adverse effects. Furthermore, the absence of any discernible variation in egg quality could not be addressed due to the absence of studies evaluating egg quality in a uniform diet supplemented with CLUK. Future studies must investigate the effects of CLUK on the relevant parameters.

Table 3: Effects of dietary supplementation of cherry laurel unshelled kernel (CLUK) on the laying performance of laying hens housed with outdoor access.

Item†	Treatment groups				SEM	P value
	0CK	2.5CK	5CK	10CK		
Initial body weight (g/hen)	1691.92	1691.68	1691.81	1691.61	2.197	1.000
Final body weight (g/hen)	1827.22	1811.53	1864.18	1806.00	12.946	0.413
Egg-laying rate (%)	93.28	95.97	93.30	93.21	0.716	0.485
Feed intake (g/hen/day)	125.54	125.19	125.70	122.14	0.924	0.521
Egg weight (g)	56.82^{b}	56.82^{b}	57.81^{a}	57.26^{ab}	0.147	0.033
Egg mass (g/hen/day)	53.00	54.54	53.94	53.36	0.408	0.609
FCR (g feed: g egg mass)	2.36^{a}	2.29^{b}	2.32^{ab}	2.28^{b}	0.012	0.041
Cracked egg ratio (%)	0.92	0.72	0.79	0.82	0.143	0.973

Means with different lowercase letters in the same row are statistically different at $P<0.05$. 0CK, fed a basal diet with no CLUK; 2.5CK, 5CK, and 10CK fed the basal diet supplemented with CLUK at the level of 2.5, 5, and 10 g per kg diet, respectively; SEM: Standard error of the mean. † Means represent four pens of 25 layers per treatment at 14-day intervals.

Table 4: Effects of dietary supplementation of cherry laurel unshelled kernel (CLUK) on egg quality traits in laying hens housed with outdoor access.

Item †	Treatment groups				SEM	P value
	0CK	2.5CK	5CK	10CK		
Haugh units	84.86	83.97	81.44	82.61	0.512	0.089
Yolk ratio (g/100 g egg)	24.16	24.39	23.73	23.92	0.130	0.366
Albumen ratio (g/100 g egg)	62.51	62.33	63.18	62.85	0.173	0.387
Eggshell ratio (g/100 g egg)	13.25	13.31	13.11	13.19	0.056	0.671
Yolk/albumen ratio	0.38	0.39	0.37	0.38	0.003	0.352
Eggshell thickness (μm)	0.39	0.39	0.40	0.39	0.001	0.421
Albumen index	9.47	9.28	8.69	9.16	0.135	0.222
Yolk index	44.32	44.07	44.97	44.28	0.186	0.389
Shape index	79.99	78.31	78.99	79.66	0.285	0.169
pH						
Yolk	6.32	6.36	6.34	6.37	0.008	0.173
Albumen	8.63	8.61	8.69	8.65	0.013	0.243
Yolk color						
L*	51.56	51.66	52.03	52.11	0.124	0.317
a*	4.26	4.55	4.09	4.16	0.080	0.214
b*	39.67	39.45	39.36	39.32	0.172	0.899

Means with different lowercase letters in the same row are statistically different at P<0.05. 0CK, fed a basal diet with no CLUK; 2.5CK, 5CK, and 10CK fed the basal diet supplemented with CLUK at the level of 2.5, 5, and 10 g per kg diet, respectively; SEM: Standard error of the mean. † Means represent 16 eggs per treatment at 14-day intervals.

The FA composition of egg yolk is an indicator of egg quality. Eggs are an important dietary source of FAs, which are critical for human health. Docosahexaenoic acid (DHA), a long-chain n3 FA, is metabolically active and exerts higher beneficial effects than the short-chain FA alpha-linolenic acid (Neijat, Eck & House, 2017). Previous studies reported that n3 polyunsaturated FAs (PUFAs), including DHA, have critical roles in human nutrition as they mitigate diseases, such as hypertension, diabetes, and coronary arteries (Shahidi & Ambigaipalan, 2018). The yolk contents of heptadecanoic acid, a monounsaturated FA (MUFA), in the 5CK group, were higher than those in the other groups (Table 5, $P < 0.01$). Furthermore, the yolk DHA contents in the 2.5CK and 10CK groups were higher than those in the 0CK group ($P < 0.05$). The upregulation of unsaturated FAs, especially in foods of animal origin, benefits consumers and producers. Several studies have demonstrated that the levels of n3 PUFAs in eggs can be increased through dietary manipulation and that the FA composition of the egg yolk is dependent on that of the feed (Neijat, Eck & House, 2017; Panaite et al., 2019; Li et al., 2023). This study could not determine the alpha-linolenic acid content of the basal diet and CLUK. Thus, the transfer of dietary DHA to egg yolk could not be determined (Neijat, Eck & House, 2017). As all test groups were fed with the same basal diet in this study, the FAs in CLUK can be assumed to have determined the FA profile of egg yolk. The differential FA levels can be attributed to the rearing system or the doses of CLUK (Popova et al., 2020; Kop-Bozbay et al., 2021). Consuming eggs containing high DHA can meet the daily DHA needs of humans. To the best of our knowledge, the effect of CLUK on the FA composition of egg yolk has not been previously reported.

The most significant PCs in the yolk FA data and their statistical analysis are shown in Figure 1. The PC1 and PC2 explained 28.5% and 17.1% of the total variance, respectively.

Although FAs were distributed in all quadrants of the PCA, the loadings (or scores) corresponding to the PCs indicated high contributions from three groups. Therefore, seven main FA profiles were identified based on natural groupings in the PC2 versus PC1 plot. Group 1 comprised FAs with positive loadings for PC1 and PC2 (eicosadienoic (0.741 and 0.457), linolenic (0.188 and 0.206), alpha-linolenic (0.619 and 0.096), arachidic (0.530 and 0.375), eicosenoic (0.084 and 0.377), linoleic (0.922 and 0.289), and pentadecanoic (0.752 and 0.100) acids). Group 2 comprised FAs with positive loadings for PC1 and negative loadings for PC2 (heptadecenoic (0.653 and −0.031) and margaric (0.767 and −0.367) acids). Group 3 comprised FAs with negative loadings for PC1 (palmitoleic acid (−0.586 and 0.618) and palmitic acid (−0.447 and 0.655), myristic acid (−0.616 and 0.584), nervonic acid (−0.445 and 0.384), lignoceric acid (−0.283 and 0.059), eicosatrienoic acid (−0.132 and −0.211), stearic acid (−0.276 and −0.522), oleic acid (−0.401 and −0.790), and DHA (−0.022 and −0.265)). Based on the correlation matrix

loadings of the variables, eicosadienoic, linoleic, pentadecanoic, and margaric acids were deemed to strongly contribute to PC1, while eicosenoic, linolenic, alfa-linolenic, arachidic, and heptadecenoic acids contributed less strongly to PC1. Seven FAs (eicosadienoic, linolenic, alpha-linolenic, arachidic, eicosenoic, linoleic, and pentadecanoic acids) can be used to identify the dose response of dietary CLUK supplementation and do not exert adverse effects on egg performance or egg quality.

The diet of chickens is prepared using a corn-soybean meal. These feed raw materials are rich in nutrients and exhibit excellent amino acid profiles, promoting the growth of chickens and egg production. Corn and soybeans contain a high n6/n3 FA ratio (Simopoulos, 2002). The n6/n3 ratio in the CLUK groups was lower than that in the 0CK group (Table 5, $P < 0.01$). The differential levels of MUFAs and PUFAs between groups may have resulted from the interconversion of FAs. Furthermore, this can be explained by the high levels of DHA in the egg yolk of hens fed on CLUK-supplemented diets. The diets of humans are low in n3 PUFA and relatively high in n6 FAs (Grčević et al., 2019). The current prevalence of non-communicable diseases, including cardiovascular disease, cancer, and diabetes, is attributed to this imbalanced FA ratio, which has led to a growing interest in producing animal products enriched with n3 (Jeong et al., 2014). CLUK may improve egg quality through FA profile alteration, contributing to consumer health. This is a beneficial effect considering that table eggs exhibit high contents of n6 FA and low contents of n3 FA (Attia et al., 2015).

Table 5: Effects of dietary supplementation of cherry laurel unshelled kernel (CLUK) on the yolk fatty acid profile and DPPH content in laying hens housed with outdoor access.

Item †	Treatment groups				SEM	P value
	0CK	2.5CK	5CK	10CK		
Myristic acid (14:0)	0.29	0.31	0.29	0.29	0.008	0.890
Palmitic acid (16:0)	24.57	25.38	24.61	24.80	0.200	0.453
Margaric acid (17:0)	0.12	0.10	0.11	0.12	0.006	0.575
Stearic acid (18:0)	6.07	6.13	5.96	6.50	0.093	0.217
Arachidic acid (20:0)	0.10	0.09	0.09	0.09	0.003	0.276
Lignoceric acid (24:0)	0.08	0.10	0.07	0.09	0.008	0.375
Pentadecanoic acid (15:0)	0.03	0.02	0.04	0.02	0.004	0.456
∑ SFA	31.31	32.35	31.21	31.97	0.201	0.138
Nervonic acid (24:1)	0.25	0.30	0.27	0.32	0.017	0.561
Oleic acid (18:1)	45.43	45.81	45.43	45.07	0.291	0.851
Palmitoleic acid (16:1)	4.09	4.40	4.16	3.97	0.096	0.452
Heptadecenoic acid (17:1)	0.13^{b}	0.13^{b}	0.16^{a}	0.12^{b}	0.003	0.001
Eicosenoic acid (20:1)	0.34	0.34	0.33	0.33	0.005	0.871
∑ MUFA	50.26	50.96	50.46	49.84	0.286	0.595
Linolenic acid (20:3)	0.08	0.08	0.08	0.08	0.004	0.965
Docosahexaenoic acid (22:6)	0.31^{b}	0.38^{a}	0.34^{ab}	0.37^{a}	0.010	0.041
Linoleic acid (18:2)	16.31	15.12	16.22	15.90	0.293	0.460
Alpha-Linolenic acid (18:3)	0.52	0.52	0.61	0.59	0.023	0.406
Eicosadienoic acid (20:2)	0.13	0.11	0.13	0.13	0.005	0.377
Eicosatrienoic acid (20:3)	0.96	1.04	0.95	1.05	0.020	0.231
∑ PUFA	18.32	17.19	18.30	18.15	0.312	0.548
∑ n3	0.88	0.90	0.94	0.96	0.018	0.420
∑ n6	16.31	15.03	16.18	15.90	0.293	0.427
n6/n3	18.49^{a}	16.70^{b}	17.23^{b}	16.45^{b}	0.242	0.009
DPPH (%)	7.59^{b}	8.29^{b}	9.37^{ab}	10.66^{a}	0.372	0.020

Means with different lowercase letters in the same row are statistically different at $P<0.05$. 0CK, fed a basal diet with no CLUK; 2.5CK, 5CK, and 10CK fed the basal diet supplemented with CLUK at the level of 2.5, 5, and 10 g per kg diet, respectively; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; n3: omega-3 fatty acids; n6: omega-6 fatty acids; DPPH: 2,2 diphenyl-1-picrylhydrazyl; SEM: Standard error of the mean. † Means represent 24 eggs per treatment.

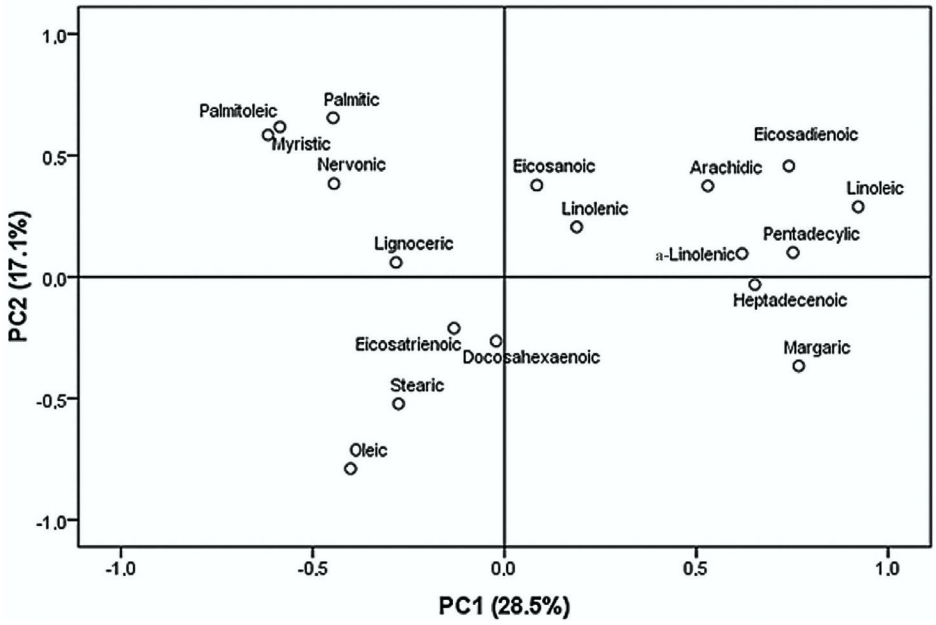

Figure 1: Loading principal component (PC1 and PC2) plots to identify the effects of dietary supplementation of cherry laurel unshelled kernel on the yolk fatty acid profile of laying hens housed with outdoor access.

Phytogenic feed additives are a cost-effective source of antioxidant components (Li et al., 2023). The consumption of animal products rich in antioxidants, such as meat and eggs, improves human health and immunity (Barbosa et al., 2021). The egg yolk DPPH content in the 10CK group was higher than that in the 0CK and 2.5CK groups (Table 5), ($P < 0.05$). The antioxidant profile of egg yolk accurately reflects the diets consumed by the hens. Consistently, the antioxidants in the egg yolk were upregulated in hens fed on a 10CK diet (Baghban-Kanani et al., 2019; Untea et al., 2020). This is attributed to the significant role of bioactive compounds in CLUK, a natural source of antioxidants (Barasoğlu & Kop-Bozbay, 2024). FAs (especially DHA) and antioxidants in eggs are essential components for human nutrition (Burdge, Tan & Henry, 2017; Grčević et al., 2019). Consequently, CLUK supplementation to the diet of laying hens can contribute to the functional enrichment of eggs.

The presence of FAs (especially n3 PUFAs) and antioxidants in eggs improves human health (Burdge, Tan & Henry, 2017). The oxidation of n3 FAs is easier than that of n6 FAs. Thus, feed additives that improve egg yolk DHA and antioxidant contents should be preferred. The usage of rich n3 sources in the diet significantly enhances lipid peroxidation, which adversely affects the antioxidant and immune systems in laying hens (Ebeid et al., 2006). Thus, the antioxidant content of egg yolk can be simultaneously increased. The findings of this study indicated that CLUK dietary supplementation delays the lipid oxidation of unsaturated FAs in eggs by simultaneously increasing the antioxidant content of egg yolk (Zeb, 2004). Furthermore, the enhanced PUFA content in the egg yolks of CLUK-fed laying hens can be attributed to the protective effect of CLUK bioactive compounds against PUFA oxidation. Based on the FA profile of CLUK (Barasoğlu & Kop-Bozbay, 2024), dietary CLUK supplementation is a practical approach for producing desired functional eggs (Grčević et al., 2019; Kop-Bozbay et al., 2021).

Conclusions

The supplementation of CLUK to the diets of free-range laying hens decreased FCR, increased egg weight, upregulated the yolk DHA and antioxidant contents, and enhanced the n6/n3 ratio. Thus, CLUK may be a promising feed ingredient for functional egg production and sustainable nutrition. However, further studies are needed to determine the long-term effects, cost, toxicity, intestinal function, oxidative stress biomarkers, transfer of bioactive compounds to the egg, ovarian characteristics, yolk bioavailability, and oxidative stability of CLUK as feed additives.

Author Contributions

Conceptual idea: Kop-Bozbay, C.; Barasoğlu, E.; Methodology design: Kop-Bozbay, C.; Data collection: Kop-Bozbay, C.; Barasoğlu, E.; Data analysis and interpretation: Kop-Bozbay, C.; and Writing and editing: Kop-Bozbay, C.

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