



Effect of Palm Oil on Laying Performance, Egg Quality and Yolk Fatty Acid Composition of White Leghorn Layers

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■Keywords

Palm oil, laying hen performance, egg
quality, egg fatty acid profile.



Section Editor: Tatiana Carlesso dos Santos

Submitted: 06/March/2025

Approved: 25/August/2025

ABSTRACT

This study examined the effects of palm oil supplementation on the performance of laying hens, egg quality, and yolk fatty acid composition. A total of 120 Lohmann LSL-Lite layers aged 25 weeks were assigned to four dietary treatment groups (T_1 – T_4), each receiving varying levels of palm oil supplementation (0%, 1.5%, 3.0%, and 4.5%). Each treatment had three replicates with 10 hens per replicate, following a completely randomized design. The T_3 group exhibited the highest feed intake, while the final body weight was best in the T_1 and T_4 groups. Weekly egg production improved in T_2 , while T_3 had the highest average egg weight, egg mass, and improved feed conversion ratio. Hen-day egg production was enhanced in T_2 , T_3 , and T_4 , whereas hen-house egg production was higher in T_2 and T_4 . In terms of egg quality, T_3 had the highest egg weight, while T_2 and T_3 had superior eggshell thickness. Additionally, T_2 showed greater eggshell weight, albumen height, and yolk index, whereas yolk height was highest in T_4 . The T_3 group also had the highest Haugh unit, yolk diameter, and yolk color, while yolk and albumen weight were greater in T_1 and T_3 . Saturated fatty acids increased with 4.5% palm oil, while unsaturated fatty acids were significantly higher in T_3 . Among the groups, omega-3 fatty acids were highest in T_2 , whereas omega-6 fatty acids were highest in T_4 . In conclusion, 3.0% palm oil supplementation effectively improved egg production, feed efficiency, egg quality, and yolk fatty acid profile.

INTRODUCTION

Poultry production plays a crucial role in meeting the global demand for high-quality protein, with eggs serving as a major source of essential nutrients (Gül *et al.*, 2020; Korver, 2023). Over the years, various dietary strategies have been explored to optimize the productivity of laying hens, improve egg quality, and enhance the nutritional profile of eggs. Among these strategies, lipid supplementation has gained considerable attention for its potential impact on energy efficiency, production performance, and the fatty acid composition of eggs (Gao *et al.*, 2024). The inclusion of fats in poultry diet not only meets the energy requirements of birds (Maliwan *et al.*, 2022) but also improves the physical characteristics of the feed, reduces dustiness, and enhances feed efficiency (Winkel *et al.*, 2016). Additionally, dietary fats play a crucial role in the absorption of fat-soluble vitamins (Diarran, 2020) and provide essential fatty acids that are vital for various physiological functions (Ehr *et al.*, 2017). Previous studies have indicated that dietary fat sources, such as vegetable oils, can alter the deposition of fatty acids in egg yolks, thereby influencing their health benefits for humans (Yang *et al.*, 2021). Among these vegetable oils, palm oil is a widely available option that serves as a rich source of both saturated and unsaturated



fatty acids. It has been studied as a dietary energy source for poultry (Izuddin *et al.*, 2022).

Palm oil, derived from the fruit of the oil palm (*Elaeis guineensis*), is one of the most widely produced and consumed vegetable oils globally. Its composition features a balanced ratio of saturated and unsaturated fatty acids, predominantly palmitic acid (C16:0) and oleic acid (C18:1) (Izuddin *et al.*, 2022). This unique fatty acid profile contributes to its oxidative stability (Kamal-Eldin & Budilarto, 2015), making it a viable option for inclusion in animal feeds. Studies have demonstrated its potential to enhance growth performance and egg production in layers when included at optimal levels (Gao *et al.*, 2021). Therefore, the inclusion of palm oil in layer diets can influence various production parameters, including feed intake, body weight, and feed efficiency. The fatty acid composition of palm oil may also affect the fatty acid profile of egg yolks (Huang, 2016; Batkowski *et al.*, 2021; Panaite *et al.*, 2021), potentially increasing the levels of beneficial unsaturated fatty acids (Wang *et al.*, 2024). Although palm oil has been investigated as a dietary energy source in poultry nutrition (Izuddin *et al.*, 2022), its specific effects on laying performance, egg quality, and yolk fatty acid composition require further investigation. Therefore, the present study was undertaken to evaluate the effects of dietary palm oil supplementation on production performance, egg quality traits, and the yolk fatty acid profile in laying hens.

MATERIALS AND METHODS

Experimental site, design, and husbandry

This experiment was conducted in a well-ventilated, semi-controlled poultry house at the Poultry Research Center, University of Agriculture, Faisalabad. A total of 120 Lohmann LSL-Lite layers aged 25 weeks were assigned to four dietary treatment groups (T_1 – T_4), each receiving different levels of palm oil supplementation (0%, 1.5%, 3.0%, or 4.5%) (Table 1). Each treatment had three replicates with 10 hens per replicate, following a completely randomized design (CRD). The experimental diets were provided to the hens from 26 to 45 weeks of age for a total duration of 20 weeks. Each hen was housed individually in a cage measuring 41 × 39 × 37 cm, equipped with separate feeding and drinking systems to ensure uniform access to feed and water. The birds were provided mash feed every morning between 07:00 and 08:00, while water

was continuously available through nipple drinkers. A uniform lighting schedule of 16 hours per day was maintained throughout the experimental period. During the feeding trial, the temperature ranged from 24°C to 32°C, with an average humidity of 80% ± 5%. Before the experiment began, all experimental diets were analyzed for their proximate composition in the Poultry Nutrition Laboratory at the Institute of Animal and Dairy Sciences, Faculty of Animal Husbandry, University of Agriculture, Faisalabad.

Table 1 – Composition of the experimental diets.

Ingredients	T_1	T_2	T_3	T_4
Maize	52.200	60.700	54.300	45.500
Soybean meal 44%	23.200	19.400	18.500	15.700
Limestone	8.550	8.400	8.560	8.750
Rice polishing	0.000	0.120	4.000	10.000
Rice tips	10.400	0.000	0.000	0.000
Canola meal 36 %	0.000	4.470	6.000	10.000
Guar meal	3.000	3.000	3.000	3.000
Dicalcium phosphate	1.540	1.520	1.620	1.430
Palm oil	0.000	1.500	3.000	4.500
Sodium bicarbonate	0.330	0.470	0.470	0.470
NaCl	0.270	0.300	0.240	0.240
Premix*	0.220	0.220	0.220	0.220
DL- Methionine	0.740	0.150	0.150	0.150
Lysine sulphate 55%	0.050	0.060	0.060	0.060
Phytase 10000C	0.005	0.005	0.005	0.005
Nutrient composition (calculated)				
Metabolizable energy (kcal/kg)	2750	2750	2750	2750
Crude Protein (%)	16.00	16.00	16.00	16.00
Calcium (%)	3.75	3.75	3.75	3.75
Avail. Phosphorus (%)	0.39	0.39	0.39	0.39
Lysine (%)	0.82	0.82	0.82	0.82
Methionine (%)	0.40	0.40	0.40	0.40
Methionine + Cystine (%)	0.68	0.68	0.68	0.68
Arginine (%)	1.08	1.08	1.08	1.08
Tryptophan (%)	0.18	0.18	0.18	0.18
Threonine (%)	0.60	0.60	0.60	0.60

*Each kg of premix will provide per kg of diet: vitamin A 3000IU, Vitamin D3 300 IU, Vitamin E 5 IU, Vitamin K 0.5 IU, Riboflavin 2.5 mg, Pantothenic acid 2 mg, Choline 1050 mg, Niacin 10 mg, Vitamin B12 0.004 mg, Folic acid 0.95 mg, Biotin 0.1 mg, Thiamine 0.7 mg, Chlorine 0.13%, Pyridoxin 2.5 mg, Potassium 0.15%, Sodium 0.15%, Magnesium 500 mg, Manganese 20 mg, Iodine 0.035 mg, Zinc 35 mg, Iron 45 mg.

Data collection

Production performance and egg quality

Upon arrival, all laying hens were individually weighed in each experimental unit. A premeasured quantity of feed was provided, and residual feed was collected and weighed to calculate weekly feed intake for each group. Weekly egg production was also



monitored (Mangnale *et al.*, 2019). Additionally, the feed conversion ratio per dozen eggs was calculated.

Each week, three eggs were collected per replicate from each treatment group to evaluate multiple quality parameters, including egg weight, specific gravity, eggshell weight and thickness, yolk color, yolk and albumen height, Haugh unit, yolk diameter, yolk weight, albumen weight, and the yolk index. A digital electronic weighing balance (Model JJ3000B) was used to measure total egg weight, eggshell weight, albumen weight, and yolk weight. Yolk color was assessed using a yolk color fan. Eggshell thickness was measured using an egg thickness meter (Model P-1, Meg Co Ltd., Ozaki, Japan). Readings were taken from the broad end, narrow end, and middle of the shell. After carefully removing the eggshell membrane, the final thickness value was calculated as the average of these three measurements (Dilawar *et al.*, 2021). The specific gravity of the eggs was assessed by immersing them in different salt solutions. Specific gravity is defined as the ratio of an object's weight to the weight of an equal volume of water. A digital vernier caliper (0–150 mm) was used to measure the yolk diameter. The caliper jaws were carefully positioned around the yolk and adjusted until they lightly touched its edges. At this point, the yolk diameter was recorded (Borille *et al.*, 2013). The yolk index was then calculated using the following formula:

$$\text{Yolk Index} = \frac{\text{Yolk Height (mm)}}{\text{Yolk Diameter (mm)}}$$

The specific gravity of an egg reflects the ratio of its shell to its internal components. In this study, nine 3-liter beakers were prepared, each containing salt solutions of varying concentrations with known specific gravities. The required amount of salt was measured using a digital balance and added to a specific volume of water in each beaker. The solution was then stirred manually until the salt was fully dissolved. To begin the process, each egg was gently placed in a beaker of pure water (pre-dip solution) to allow it to acclimate. Afterward, a spoon was used to carefully lower the egg into the first beaker containing a salt solution of known concentration, ensuring slow immersion along the side of the beaker. The egg remained in the solution for 15 to 20 seconds. If it floated, it was immediately removed. If the egg did not float, it was briefly dipped again in the pre-dip solution before being transferred to the next beaker with a higher salt concentration. This process was repeated until the egg floated in one of the beakers, or until the final (ninth)

beaker was reached. The specific gravity of the egg was considered to be equivalent to that of the solution in which it remained buoyant.

Albumen height and yolk height were measured using an egg quality stand (OSK 13471 Model, Ogawa Seiki, Co. Ltd, Japan). The probe of the meter was carefully positioned at the center of the albumen and yolk, then gradually lowered until it made contact with their surfaces. The reading on the needle was recorded at the point of contact with both the albumen and yolk (Dilawar *et al.*, 2021). The following formula was used to determine the Haugh unit score:

$$HU = 100 \log H + 7.37 - 1.71W^{0.37}$$

Where:

HU = Haugh Unit

W = Egg weight in grams

H = Height of albumin in mm

Determination of yolk fatty acid composition

At the end of the experiment, three eggs from each replicate were randomly selected for analysis of their yolk fatty acid profiles. Gas chromatography was used to determine the fatty acid composition of the yolks. Fatty acid methyl esters were prepared using 2N NaOH in ethanol, extracted with n-hexane, and subsequently analyzed through gas chromatography (Alty, 2009). The separation of fatty acid methyl esters was performed using a GC-9A gas chromatograph equipped with a flame ionization detector and a DB WEX 30M 0.25 mm column. Nitrogen was used as the carrier gas at a flow rate of 30 mL/min, while hydrogen and air pressures were maintained at 0.5 kg/cm². The injector temperature was initially set at 140°C for 5 minutes, and then gradually increased to 240°C at a rate of 4°C per minute over the course of 30 minutes. The fatty acid composition was expressed as a percentage of the total fatty acids (Ding *et al.*, 2017).

Statistical analysis

Data were analyzed using one-way ANOVA under a completely randomized design with the General Linear Model procedure in SAS (SAS Institute Inc., Cary, NC, 2002–2003). Mean comparisons were conducted using Duncan's Multiple Range Test, with significance set at a 5% probability level. The following mathematical model was applied:

$$Y_{ij} = \mu + \tau_i + \epsilon_{ij}$$

Where:



Y_{ij} = observation of dependent variable recorded on i th treatment

μ = Population mean; τ_i = Effect of i th treatment ($i = 1, 2, 3, 4$)

ϵ_{ij} = Residual effect of j th observation in i th treatment $NID \sim 0, \sigma^2$.

RESULTS

Laying performance

Table 2 summarizes the performance parameters, highlighting significant differences among the treatment groups. These parameters include average daily feed intake, final body weight, body weight gain, weekly egg production, average egg weight, average egg mass, feed conversion ratio, hen-day egg production, and hen-house egg production. Among the groups, layers in the T_3 group (supplemented with 3% palm oil) demonstrated the highest feed intake. The highest final body weight was recorded in hens from the T_1 and T_4 groups ($p < 0.05$). Furthermore, the results showed that weekly egg production improved in T_2 , while average egg weight was highest in T_3 . Both average egg mass and feed conversion ratio improved in T_3 and T_4 . Additionally, hen-day egg production was enhanced in T_2 , T_3 , and T_4 , whereas hen-house egg production was highest in T_2 and T_4 .

Table 2 – Effect of dietary inclusion of palm oil on the production performance of laying hens¹.

Parameters ²	Treatments ³				SEM	p-value
	T_1	T_2	T_3	T_4		
FI (g/bird)	826 ^{ab}	825 ^{ab}	827 ^a	825 ^c	0.892	0.041
FBW (g/bird)	1500 ^a	1493 ^b	1487 ^c	1500 ^a	1.549	0.043
WG (g/bird)	122 ^{ab}	123 ^a	109 ^d	112 ^c	1.959	0.02
WEP (%)	5.6 ^d	5.9 ^a	5.8 ^c	5.9 ^{ab}	1.691	0.0187
AEW (g)	56 ^{cd}	57 ^c	59 ^a	58 ^{ab}	1.691	0.0397
AEM (g/week)	64 ^c	68 ^b	70 ^a	70 ^a	4.769	0.0267
FCR (kg/dz)	1.72 ^b	1.73 ^a	1.60 ^c	1.59 ^c	1.891	0.0217
HDEP (%)	80 ^b	84 ^a	83 ^a	84 ^a	2.419	0.0377
HHEP (%)	80 ^c	84 ^a	83 ^b	84 ^a	3.769	0.0023

^{a-d}The treatment means in a row containing different superscripts differ significantly ($p < 0.05$).

¹Data represent the means of three replicates ($n=3$), each with 10 birds.

²FI: feed intake, FBW: final body weight, WG: weight gain, WEP: weekly egg production, AEW: average egg weight, AEM: average egg mass, FCR: feed conversion ratio, HDEP: hen day egg production, HHEP: hen house egg production.

³ T_1 : diet containing 0% palm oil, T_2 : diet containing 1.5% palm oil, T_3 : diet containing 3% palm oil, T_4 : diet containing 4.5% palm oil.

Egg quality characteristics

Table 3 summarizes egg quality parameters, including egg weight, eggshell weight, eggshell

thickness, albumen weight and height, yolk weight and height, egg specific gravity, yolk color, yolk diameter, and yolk index. The supplementation of palm oil in the layer diets significantly affected all these parameters ($p < 0.05$). The T_3 group exhibited the highest egg weight, while both T_3 and T_4 showed greater egg specific gravity. Both T_2 and T_3 showed increased eggshell thickness, while T_2 had the highest eggshell weight. Yolk height was greatest in T_4 , whereas albumen height was highest in T_2 . The Haugh unit and yolk diameter were superior in T_3 . Furthermore, yolk and albumen weights were greater in both T_1 and T_3 . Yolk color was most pronounced in T_3 , and the yolk index was highest in T_2 .

Table 3 – Effect of dietary inclusion of palm oil on the egg quality characteristics of laying hens¹.

Parameters ²	Treatments ³				SEM	p-value
	T_1	T_2	T_3	T_4		
EW (g)	55 ^c	57 ^b	57 ^a	56 ^{ab}	0.899	0.025
ESG	1.09 ^{ab}	1.08 ^c	1.10 ^a	1.09 ^a	1.359	0.036
EST (mm)	0.41 ^b	0.45 ^a	0.42 ^a	0.31 ^b	0.699	0.022
ESW (g)	8.9 ^b	9.0 ^a	8.5 ^b	8.5 ^b	1.699	0.043
YH (mm)	18.5 ^{ab}	19.0 ^{ab}	17.5 ^c	19.4 ^a	1.459	0.029
AH (mm)	8.4 ^{ab}	8.9 ^a	8.0 ^c	8.5 ^{ab}	1.649	0.021
HU	87 ^d	89 ^b	90 ^a	89 ^b	1.529	0.011
YD (mm)	38.4 ^d	39.5 ^b	41.5 ^a	38.2 ^c	1.099	0.054
YW (g)	17.4 ^a	16.5 ^b	16.9 ^b	17.3 ^a	1.789	0.039
AW (g)	33.4 ^a	32.8 ^b	31.8 ^c	33.9 ^a	1.499	0.019
YI	0.42 ^c	0.65 ^a	0.55 ^b	0.41 ^c	2.589	0.046

^{a-d}The treatment means in a row containing different superscripts differ significantly ($p < 0.05$).

¹Data represent the means of three replicates ($n=3$), each with 10 birds.

²EW: egg weight, ESG: egg specific gravity, EST: eggshell thickness, ESW: eggshell weight, YH: yolk height, AH: albumen height, HU: Haugh unit, YD: yolk diameter, YW: yolk weight, AW: albumen weight, YI: yolk index.

³ T_1 : diet containing 0% palm oil, T_2 : diet containing 1.5% palm oil, T_3 : diet containing 3% palm oil, T_4 : diet containing 4.5% palm oil.

Yolk fatty acid profile

The concentration of saturated fatty acids (C4:0, C8:0, C10:0, C11:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0) in the egg yolk significantly increased in hens fed a diet containing 4.5% palm oil. Similarly, the levels of unsaturated fatty acids (C18:1, C18:2, C18:3, C20:4) also rose with the inclusion of 4.5% palm oil in the diet. However, omega-3 fatty acids were notably lower across all treatment groups. Among the treatments, the T_2 group, which received 1.5% palm oil, had the highest concentration of omega-3 fatty acids compared to the other groups. In contrast, omega-6 fatty acids increased proportionally with the rising levels of palm oil in the diet (Table 4).



Table 4 – Effect of dietary inclusion of palm oil on the fatty acid profile of egg yolk¹.

Parameters ²	Treatments ³				SEM	p-value
	T ₁	T ₂	T ₃	T ₄		
Butyric acid	0.26	0.80	0.01	0.30	0.057	0.063
Undecanoic acid	0.20	3.30	11.00	13.50	0.081	0.057
Octanoic Acid	0.10	0.64	0.90	0.15	0.046	0.059
Myristic acid	7	11	6	7	0.053	0.059
Pentadecanoic acid	0.05	0.50	0.30	13.10	0.058	0.07
Margaric acid	0.03	0.66	0.66	0.80	0.074	0.047
Palmitic acid	0.05	2.40	0.05	0.43	0.134	0.14
Stearic acid	0.07	3.45	0.45	0.01	0.052	0.058
Behenic acid	17	8	9	10	0.066	0.062
Arachidic acid	4.50	2.75	0.04	4.85	0.07	0.076
Heptadecenoic acid	0.03	0.02	0.04	18.10	0.062	0.048
Henicosanoic acid	0.66	3.70	0.43	0.22	0.048	0.062
Oleic acid	0.06	0.75	0.04	0.01	0.063	0.069
Linolenic acid	0.07	1.15	0.03	0.03	0.049	0.055
Gamma linolenic acid	7	16	15	7	0.056	0.041
Linoleic acid	0.03	2.60	6.50	0.00	0.071	0.07
Alpha linolenic acid	0.11	0.55	3.90	5.90	0.074	0.058
Arachidonic acid	9	1	15	18	0.051	0.067
Total SFA	29	37	29	68		
Total UFA	17	22	40	31		
Total omega 3 FA	4	6	2	4		
Total omega 6 FA	16	20	29	31		

¹Data represent the means of three replicates (n=3), each with 10 birds.

²FA: fatty acid, SFA: saturated fatty acid, UFA: unsaturated fatty acid.

³T₁: diet containing 0% palm oil, T₂: diet containing 1.5% palm oil, T₃: diet containing 3% palm oil, T₄: diet containing 4.5% palm oil.

DISCUSSION

Laying performance

The study results indicated that the T₃ group, which received 3% palm oil, exhibited the highest feed intake. This can be attributed to enhanced palatability, improved digestibility, and increased energy density of the diet. Dietary fats contribute to better texture and flavor, making the feed more appealing to hens (Gao *et al.*, 2021). Research suggests that moderate fat inclusion stimulates feed consumption by enhancing the diet's sensory characteristics (Zulkifli *et al.*, 2003). Poultry regulate their feed intake based on dietary energy levels. Lower-energy diets lead to increased consumption to meet energy needs, while excessively high-energy diets result in reduced intake due to satiety effects (Richards & Proszkowiec-Weglarz, 2007). Similar results have been reported in previous studies, where palm oil supplementation positively influenced feed intake in laying hens (Kolani *et al.*, 2018; Areerob *et al.*, 2019).

The highest final body weight was observed in the T₁ (0%) and T₄ (4.5%) groups. In the T₁, the carbohydrate-based diet likely led to increased feed intake as the birds compensated for the lower energy density. This higher consumption may have contributed to greater gut fill and overall weight gain (Ravindran & Abdollahi, 2021). Conversely, in the T₄ group, despite lower feed intake, the higher dietary energy content facilitated greater body fat deposition, resulting in increased body weight (Noetzold *et al.*, 2025). Dietary fats are known to enhance energy efficiency and promote fat accumulation in poultry (Sanz *et al.*, 2000). However, excessive fat levels can reduce protein accretion due to increased oxidation of amino acids for energy (Simonson *et al.*, 2020). This may explain why the intermediate groups (T₂ and T₃) did not achieve the highest body weights. These findings align with the results of Hosseini-Vashan & Afzali (2008), who observed that increasing levels of palm oil in the diet of laying hens led to higher body weight gain.

The highest weekly egg production was observed in the T₂ group (1.5% palm oil). This moderate level of fat inclusion may have improved energy utilization and reproductive hormone activity, resulting in enhanced reproductive performance. Dietary fat increases lipid availability, which plays a critical role in yolk precursor synthesis and ovulation (Gao *et al.*, 2021). However, excessive fat levels can redirect energy toward body weight gain rather than egg production, thereby reducing reproductive efficiency (Wang *et al.*, 2024). The observed improvement in hen-day egg production suggests that dietary fat positively influences ovarian function and yolk formation. Furthermore, the increase in hen-house egg production may be attributed to enhanced energy efficiency and consistent egg production throughout the laying period. These findings align with those of Kolani *et al.* (2018) and Areerob *et al.* (2019), who reported that palm oil supplementation in the diets of laying hens improve egg production rates. However, Hosseini-Vashan *et al.* (2011) found no significant effect of dietary palm olein oil on egg production, which may be attributed to differences in the type of fat used or the experimental conditions.

The T₃ group (3% palm oil) produced the heaviest eggs, likely due to the role of dietary fat in enhancing yolk lipid deposition, which contributes to increased egg weight (Grobas *et al.*, 1999). Additionally, the inclusion of 3% palm oil might have provided an optimal energy balance, supporting egg development without causing excessive body weight gain. Whitehead *et al.* (1995)



suggested that lipid supplementation can increase egg weight by stimulating the synthesis of oviduct proteins, which are regulated by estrogen. These findings align with El-Husseiny *et al.* (2008), who reported higher egg weights in hens fed palm oil. However, other studies have shown inconsistent results. Cachaldora *et al.* (2008) and Hosseini-Vashan & Afzali (2008) found that the source of dietary fat, including palm oil, had no significant effect on egg weight. These variations may be due to differences in fat composition, inclusion levels, basal diet formulation, husbandry practices, or the health status of the birds. The observed increase in egg mass in the T₃ group can be attributed to the higher egg weight, as egg mass is determined by both egg weight and the rate of egg production.

The feed conversion ratio is a key indicator of production efficiency (Li *et al.*, 2024). An increase in feed conversion ratio can lead to higher production costs (Adaszyńska-Skwirzyńska *et al.*, 2025). In this study, the T₃ (3% palm oil) and T₄ (4.5% palm oil) groups demonstrated a superior feed conversion ratio, indicating improved feed efficiency. The inclusion of dietary fat likely enhanced metabolic efficiency, reducing the amount of feed required per unit of egg mass (Han *et al.*, 2023). Lipids are known to improve digestibility and nutrient absorption, leading to a more efficient feed conversion ratio (Han *et al.*, 2023). These findings align with those of Kolani *et al.* (2018) and Areerob *et al.* (2019), who reported improved feed conversion ratios in laying hens fed palm oil-supplemented diets. However, Hosseini-Vashan & Afzali (2008) and Hosseini-Vashan *et al.* (2011) found no significant impact of palm oil inclusion on feed conversion ratios, which may be attributed to differences in the type of fat used or the experimental conditions.

Egg quality characteristics

Egg weight is primarily determined by yolk lipid deposition, energy intake, and protein metabolism. The T₃ group (3% palm oil) produced the heaviest eggs, likely due to the contribution of dietary lipids to yolk formation and overall egg development (Grobas *et al.*, 1999). Research suggests that moderate inclusion of fats helps maintain an optimal energy balance, promoting efficient nutrient utilization for egg formation without causing excessive body fat accumulation (Grobas *et al.*, 1999). Furthermore, fat supplementation has been shown to enhance the synthesis of oviduct proteins, which is a crucial factor

in determining egg weight (Whitehead *et al.*, 1995). These findings align with Areerob *et al.* (2019), who reported increased egg weight in layers supplemented with palm oil. However, Hosseini-Vashan & Afzali (2008) reported that fat sources had no significant effect on egg weight, suggesting that egg weight variation depends on fat type, inclusion level, and overall dietary composition.

Eggshell thickness and weight are key indicators of shell quality, influenced by calcium metabolism, dietary fat, and vitamin D absorption (Jiang *et al.*, 2013). The increased eggshell thickness observed in T₂ and T₃ may be linked to enhanced calcium absorption and utilization facilitated by dietary fat. Palm oil improves the absorption of fat-soluble vitamins, such as vitamin D (Diarran, 2020), which is essential for calcium metabolism and eggshell formation (Plaimast *et al.*, 2015). The highest eggshell weight in T₂ suggests that a moderate inclusion of palm oil (1.5%) provided sufficient energy for calcium retention and shell formation without redirecting nutrients toward excessive fat accumulation (Richards & Proszkowiec-Weglarz, 2007). These findings are in line with those of Li *et al.* (2024), who reported that moderate lipid inclusion benefits eggshell quality, while higher fat levels can reduce calcium retention.

Yolk and albumen heights are key indicators of egg freshness and protein quality (Silversides & Budgell, 2004). The increased yolk height observed in T₄ suggests that higher dietary fat levels promoted greater yolk lipid deposition, resulting in a more structured and dense yolk (Abeyrathne *et al.*, 2022). However, excessive fat intake may shift nutrient allocation toward body fat accumulation, potentially affecting other egg quality traits (Wang *et al.*, 2024). Similar results were observed by Agboola *et al.* (2016), who examined the impact of palm oil on production performance, egg quality, and yolk fatty acid composition in laying hens. The greater albumen height observed in T₂ may be attributed to the balanced utilization of energy and protein at moderate palm oil levels. Increased albumen height is linked to efficient protein synthesis and improved water retention within the egg, both of which are influenced by dietary energy balance and amino acid availability (Obianwuna *et al.*, 2022). These results align with Agboola *et al.* (2016), Gao *et al.* (2020), Gao *et al.* (2021), and Gao *et al.* (2022), who found that moderate dietary fat improves albumen height and egg protein quality. However, excessive fat levels



can lead to water loss in albumen, reducing albumen height (Kim *et al.*, 2007).

The Haugh unit is a widely recognized indicator of egg albumen quality, with higher values reflecting greater protein integrity and egg freshness (Eisen *et al.*, 1962). The elevated Haugh unit in T_3 suggests that a 3% palm oil inclusion provided sufficient energy for efficient protein synthesis while maintaining albumen structure (Wang *et al.*, 2024). Additionally, the increased yolk diameter in T_3 may be attributed to greater lipid availability, as dietary fat plays a crucial role in the synthesis and deposition yolk precursors (Gao *et al.*, 2021). These findings align with Areerob *et al.* (2019), who reported enhanced albumen and yolk quality with palm oil supplementation.

The increased yolk and albumen weights observed in T_1 and T_3 indicate different nutrient utilization mechanisms. In T_1 (control, 0% palm oil), hens may have consumed more feed to compensate for the lower energy density, resulting in higher protein intake and, consequently, greater yolk and albumen weights (Ravindran & Abdollahi, 2021). In contrast, the optimized lipid metabolism in T_3 , facilitated by dietary fat, likely promoted efficient nutrient partitioning toward egg components (Sanz *et al.*, 2000). However, an excessive dietary fat intake may reduce protein deposition due to increased amino acid oxidation, which could explain why higher fat levels in T_4 did not further enhance yolk and albumen weights (Simonson *et al.*, 2020). These findings align with Areerob *et al.* (2019), who reported that increasing the inclusion of crude palm oil in the diets of laying hens can improve specific egg quality traits.

Yolk color is primarily determined by carotenoid absorption (Kojima *et al.*, 2022), which is enhanced by dietary fats (Papadopoulos *et al.*, 2019). The deeper yolk pigmentation observed in T_3 suggests optimal carotenoid uptake and deposition (Kolani *et al.*, 2018). Similar results have been reported by Kolani *et al.* (2018), who found that palm oil supplementation improved yolk pigmentation. The yolk index, which indicates yolk shape and integrity (Kumar *et al.*, 2022) is higher when the yolk is more spherical and intact. The highest yolk index observed in T_2 suggests that moderate fat inclusion effectively supported lipid metabolism, resulting in improved yolk structural integrity. These findings align with Areerob *et al.* (2019), who demonstrated that palm oil supplementation enhances yolk quality.

Yolk fatty acid profile

This study investigated the effects of dietary palm oil supplementation (0%, 1.5%, 3.0%, and 4.5%) on the fatty acid composition of egg yolk. The results indicated a significant increase in both saturated and unsaturated fatty acids in hens fed 4.5% palm oil (T_4). The significant rise in saturated fatty acids (C4:0 to C22:0) in egg yolk at 4.5% palm oil inclusion (T_4) can be attributed to the fact that palm oil is naturally high in saturated fatty acids (40–50%). It contains palmitic acid (C16:0) and stearic acid (C18:0) as major saturated fatty acids. These are efficiently deposited into egg yolk due to their high digestibility and absorption rates (Raza *et al.*, 2016). Unlike polyunsaturated fatty acids, saturated fatty acids are less prone to oxidation, allowing higher retention in yolk lipids (Grobbs *et al.*, 1999). These results are in line with Hosseini-Vashan *et al.* (2011), who reported increased yolk saturated fatty acid content in hens supplemented with palm oil. Similarly, EL-Husseiny *et al.* (2008) found a significant increase in egg yolk saturated fatty acids with the addition of 4.8% vegetable oil. Additionally, other studies (Kang *et al.*, 2001; Muangkeow, 2011) found that different concentrations of dietary palm oil did not affect the saturated fatty acid content of the egg yolk.

The significant increase in monounsaturated (C18:1) and polyunsaturated fatty acids (C18:2, C18:3, C20:4) in the T_4 group can be explained by the fact that palm oil contains a high proportion (37–40%) of oleic acid (C18:1), a major monounsaturated fatty acid that is readily absorbed in the egg yolk (Kolani *et al.*, 2018). Polyunsaturated fatty acids (C18:2, C18:3, C20:4) are essential for egg development (Gao *et al.*, 2021). Higher dietary fat levels stimulate bile secretion and lipase activity, enhancing unsaturated fatty acid bioavailability and incorporation into yolk lipids (Wang *et al.*, 2024). These findings align with Hosseini-Vashan *et al.* (2011) and Punita & Chaturvedi (2000), who observed increased yolk monounsaturated fatty acids and polyunsaturated fatty acids in hens fed a high-level palm oil diet.

The low omega-3 fatty acid content in all treatments, but with the highest levels in T_2 (1.5% palm oil), can be attributed to the fact that palm oil is naturally low in omega-3 fatty acids. It contains minimal α -linolenic acid (ALA, C18:3), the precursor for omega-3 synthesis. The delta-6 desaturase enzyme is responsible for converting ALA (C18:3) into long-chain omega-3s (EPA, DHA). However, high omega-6 levels (from palm



oil) inhibit this conversion, leading to lower omega-3 deposition (Simopoulos, 2002). These findings align with those of Muangkeow (2011), who found that eggs from hens fed diets containing palm olein oil had a high percentage of n-3 PUFA. The proportional increase in omega-6 fatty acids (C18:2, C20:4) with rising palm oil levels could be explained by the fact that palm oil is naturally high in linoleic acid (C18:2), a key omega-6 fatty acid. Higher dietary palm oil levels directly increase linoleic acid deposition in egg yolks (Gao *et al.*, 2021). These findings are consistent with Areerob *et al.* (2019), who reported higher omega-6 fatty acid accumulation in yolks of hens fed palm oil-enriched diets.

CONCLUSION

Based on the present findings, it can be concluded that dietary inclusion of palm oil up to 3% effectively enhances production performance and egg quality attributes in laying hens. Moreover, moderate inclusion levels (1.5–3%) were found to optimize yolk lipid profile, particularly by increasing beneficial unsaturated fatty acids. In contrast, higher inclusion levels (4.5%) may negatively affect the omega-6 to omega-3 ratio, indicating the importance of careful formulation to maintain nutritional balance.

ACKNOWLEDGEMENTS

The authors extend their appreciation to Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2025R101), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. The authors also extend their appreciation to the administration of the Poultry Research Center, University of Agriculture Faisalabad for supporting the trial.

AUTHOR CONTRIBUTIONS

Conceptualization, MY and SL; methodology, MY, SL and MTK; software, FA, EB and GA; validation, KP, MSR, and HAB; formal analysis, MY and MTK; investigation, MY and SL; resources, MY, BS and FA; data curation, SL; writing—original draft preparation, MY, SL and MTK; writing—review and editing, FA, MSR, MMJ, and GA; visualization, EB and HD; supervision, MY; project administration, SL and MY; funding acquisition, AN and MOA. All authors have read and agreed to the published version of the manuscript.

FUNDING

None.

DATA AVAILABILITY STATEMENT

Data will be available upon request.

CONFLICT OF INTEREST

All authors of the present study declare that there is no conflict of interest, financial or otherwise.

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