Research Article

Effects of Dietary Carrot Meal Supplementation on Egg Production, Egg Quality, and Blood Profiles in Late-Phase Laying Hens

Yasin Pradana Maulana[1*], Muhamad Rifqi Ismiraj[2], Diding Latipudin[1], Asri Wulansari[2], Indra Firmansyah[3], Deni Mulyadi Asep Sukmana[1], Hunnatullah Muhammad Al-Azka[1], Aprilianna Putri Zahara Nafsina Luvita Sari[1], Novi Mayasari[1]

[1]Faculty of Animal Husbandry, Universitas Padjadjaran, Sumedang, Indonesia; [2]Animal Husbandry Study Program, Padjadjaran University PSDKU Pangandaran, Pangandaran, Indonesia; [3]Faculty of Agricultural Industrial Technology, Universitas Padjadjaran, Sumedang, Indonesia.

Abstract | Carrot meal supplementation (CMS) is a potential feed supplement that may improve productivity and health in laying hens, especially in the late phase of their laying cycle. However, limited studies have explored its effects on egg production, egg quality, and hen health in this phase. This study aimed to investigate the effects of CMS on egg production, egg yolk color, hematological parameters, stress markers, lipid profile, and immune response in late-phase laying hens. A completely randomized design was used, with 20 chickens allocated to four dietary regimens, each with five replications. The treatments were as follows: P0 (basal diet), P1 (P0 + 2% CMS), P2 (P0 + 4% CMS), and P3 (P0 + 6% CMS). Parameters observed included egg production, egg yolk score, hematological profiles, stress markers, lipid levels (triglycerides, cholesterol, HDL, LDL, VLDL), and immune response (IgG and IgM levels). The results showed that hens fed 6% CMS had significantly higher egg production, improved egg yolk score, and increased plasma glucose ($P < 0.05$). Additionally, HDL levels were significantly higher in the 6% CMS group, with no significant effects on LDL, VLDL, triglycerides, or cholesterol. CMS resulted in a significant decrease in leukocyte counts in the 6% CM treatment group ($P<0.05$). This may indicate less systemic inflammation; however, the reduction could also imply possible impacts on hematopoiesis that necessitate additional exploration via functional immunological assays. In conclusion, 6% CMS improved egg production, egg quality, and lipid metabolism in late-phase laying hens without negatively affecting immune response or overall health.

Keywords | β-carotene, Phyto-additive, Laying hens, Lipid profile, Performance

Received | August 07, 2025; **Accepted** | December 04, 2025; **Published** | January 10, 2026
***Correspondence** | Yasin Pradana Maulana, Faculty of Animal Husbandry, Universitas Padjadjaran, Sumedang, Indonesia; **Email:** y.pradana@unpad.ac.id
Citation | Maulana YP, Ismiraj MR, Latipudin D, Wulansari A, Firmansyah I, Sukmana DMA, Al-Azka HM, Sari APZNL, Mayasari N (2026). Effects of dietary carrot meal supplementation on egg production, egg quality, and blood profiles in late-phase laying hens. Adv. Anim. Vet. Sci., 14(1):50-63.
DOI | https://dx.doi.org/10.17582/journal.aavs/2026/14.1.50.63
ISSN (Online) | 2307-8316

INTRODUCTION

Recently, consumers have become increasingly concerned about the quality of their food, including egg quality. Besides the price, the consumers are not only concerned about the sensory and nutritional quality of eggs but also the physical features of the eggs, like their size, yolk colour, and freshness. Eggs enhanced with nutrients such as polyunsaturated fatty acids, vitamins (D, E, etc.), minerals (selenium, iron, zinc, etc.), and antioxidant compounds are currently in high demand since they might enhance consumers health and well-being (Omri *et al.*, 2019). The excellence of eggs is attributed to their optimal amino acid composition, high nutritional value, and extensive diversity of their constituents. Recent studies indicate that beyond culinary purposes, eggs from laying hens are now recognised

as one of the most effective raw materials for biomedical and nutraceutical applications, owing to their abundance of bioactive components, as reviewed by Lesnierowski and Stangierski (2018).

Furthermore, studies have shown that eggs, both in their content and shell, contain antibacterial and anticarcinogenic components and substances that stimulate the immune system (Yannakopoulos, 2007; Abeyrathne and Ahn, 2015), indicating a vital food and nutrient source for humans. Therefore, better egg quality with yellow-orange egg yolk colour and huge bioactive compounds like β-carotenoid content are becoming a growing concern for consumers. Previous studies reported that supplementing carotenoids may enhance egg quality, especially the colour of the yolk, and provide a good source of dietary antioxidants (Surai *et al*., 2006; Panaite *et al*., 2019). Dietary supplies of carotenoids in hens may have two possible effects: As a natural pigment to enhance egg yolk colour and quality and as an antioxidant to postpone polyunsaturated fatty acid oxidation. Using natural carotenoids aligns with consumers' preference for natural and safe products.

Carrot (*Daucus carota L*) is a root vegetable rich in minerals, fiber, carbohydrates, antioxidant flavonoids, necessary micronutrients, and particularly beta-carotene (Sharma *et al*., 2012). It has a higher concentration of carotenes and a lower concentration of xanthophylls (Spasevski *et al*., 2018). Low-quality carrots and their leftover parts can be used as a source of carotenes in animal feed due to their low economic value. According to Spasevski *et al*. (2018), dietary dry carrots showed no adverse impact on eggs' appearance, acceptability, or carotene content.

The content of β-carotene in raw carrots is around 7.63 ± 0.33 µg/g (Adelina *et al*. 2013), and after conversion based on the dry matter of carrot meal (CM) compared to the dry matter of fresh carrots, the β-carotene content of CM is 60.73 ± 2.63 µg/gram; it contains flavonoids, namely kaempferol, quercetin and luteolin (Olalude *et al*., 2015). The content of β-carotene in carrots is a precursor for forming vitamin A, which functions as an antioxidant to counteract free radicals. The β-carotene contained in CM can affect metabolism, especially the distribution of cholesterol, and maximise the performance of liver metabolism and vitellogenin synthesis, which is caused by the effect of reducing free radicals in the body of chickens due to the administration of CM. Vitellogenin is broken down into lipovitellin (HDL) and phosvitin, which form yolk (Mushawwir and Latipudin, 2013). Furthermore, our hypothesis posits that the active organic compounds present in CM may influence antibody levels in laying hens. Previous research has indicated that laying hens supplemented with maternally derived natural antibodies (NAbs) in the form of immunoglobulin G (IgG) against foreign antigens retain these antibodies from hatching until at least day 8 of life (Ismiraj *et al*., 2019). These maternally derived natural antibodies may persist until depletion while the individual laying hen begins to produce its antibodies. The β-carotene content in carrots, a precursor of vitamin A and an antioxidant (Haider *et al*., 2017), may also affect the immune system, especially in NAbs production level against foreign antigens. NAbs are immunoglobulins in individuals without prior exposure to the corresponding antigen (Reyneveld *et al*., 2020). These antibodies are markers of immune competence in various animal species (Reyneveld *et al*., 2020).

Previous studies showed that supplementation of CM in quail did not affect the performance and characteristics of the egg laid (Hanapis and Guntoro, 2020); therefore, we tested it on laying hens with an adjusted supplementation level. A carrot is a vegetable plant that contains high β-carotene. Carrot plants come from temperate regions, namely East Asia and Central Asia. Initially, the development of carrots in Indonesia was concentrated only in the Lembang and Cipanas areas, West Java, and then spread widely to this day. Carrot production for West Java in 2020 was 152,000 tons, a decrease from the previous year of -2.13 %, whereby the estimated carrots were not fit for sale reached 5% of total production, which was 7,600 tons (Ministry of Agriculture of the Republic of Indonesia, 2021).

CM and carrot leaf use in laying hen feed (Bidura *et al*., 2021; Yitbarek, 2019) has been studied for decades. However, supplementing natural colours like carrots in layer feed on egg quality, production, and layer health status is still limited. To our knowledge, the study about the effects of CM on the blood parameter, which can describe the physiological state of the chicken and its production performance capabilities, and the egg yolk colour score of laying hen is still limited. Thus, this study aims to evaluate and compare the effect of various levels of supplementation CM in layer ratio on egg production, haematology profile, stress marker, lipid profile, antibody and egg quality.

Furthermore, the cholesterol content of chicken eggs is a concern for many people who consume them, which causes some people with certain diseases to be unable even to avoid consuming them. Whole egg and dietary cholesterol intakes were positively associated with higher all-cause, cardiovascular disease, and cancer mortality, and with increased mortality associated with whole egg consumption was largely influenced by the cholesterol content, also consumption of egg whites/substitutes was associated with lower mortality (Zhuang *et al*. 2021). Many parties are looking for ways to regulate the cholesterol content in chicken eggs (Lesnierowski and Stangierski, 2018), to increase its functionality as a high-nutrient source for

humans. Besides that, the yolk colour fades in old layers, and performance significantly decreases in laying hens over 80 weeks of age. A recent study by Nurfadilah *et al.* (2025) demonstrated that dietary supplementation with Water-Soluble Peptide Extract (WSPE) derived from yogurt improved yolk protein content and reduced lipid levels, although cholesterol levels increased, likely due to endogenous synthesis in highly productive hens. The study also noted that antioxidants in WSPE may suppress lipid peroxidation while facilitating cholesterol transport in the form of lipoproteins.

One way to overcome the above problems is to pay attention to the diet content given to laying hens. The diet is the primary energy intake for livestock to live and produce, and it needs to meet the nutritional needs of livestock. Generally, breeders think that high macro-nutrients in the diet can meet the needs of livestock, but micro-nutrients such as vitamins and minerals are essential for the body. They can affect livestock performance and the quality of eggs produced. One of the potential plants to overcome this problem is carrots (*Daucus carota* L), which are expected to be used as an additional feed ingredient in chicken diet that can improve egg quality and reduce cholesterol by increasing the quality of blood profiles and livestock performance, which can be indicated from its high-density lipoprotein (HDL), low-density lipoprotein (LDL), and neutrophil to lymphocyte ratio (N/L). So, eggs rich in vitamin A can be produced with lower cholesterol than eggs. Carrots are a food source of vegetable origin, which contains good nutrients, including β-carotene and various vitamins.

While studies on carotenoid supplementation in poultry, particularly with quail, have been conducted, there is limited research on the effects of carrot meal (CM) supplementation in laying hens, especially in relation to blood parameters, egg quality, and the immune response. Furthermore, while β-carotene is known to enhance egg quality and act as an antioxidant, its role in improving immune responses and cholesterol metabolism in laying hens has not been fully explored. We hypothesized that supplementation with carrot meal, due to the antioxidant and bioactive properties of β-carotene and other phytochemicals, would enhance egg production parameters, increase yolk color intensity, and potentially affect lipid metabolism, specifically HDL cholesterol levels, in late-phase laying hens. Besides, in Indonesia, there is one issue facing the carrot farming industry: a considerable quantity of carrots deemed unfit for sale remain unused. Carrots of poor quality are discarded, polluting the environment. Unfortunately, carrot waste should be converted into other feed ingredients (Mardzuki et al., 2017). Seeing this phenomenon, carrots have the potential to be used as an additional feed ingredient to produce eggs that are low in cholesterol.

MATERIALS AND METHODS

Animal, housing, diet and experimental design

In this experiment, twenty laying hens, approximately 85 weeks of age, were used. The average initial body weight of a laying hen was 1.994 ± 0.181 kg (9.04% CV). Each laying hen was randomly divided into four dietary treatments, and each treatment used five birds, where the treatment includes: P0= Basal diet without the addition of CMS; P1 = basal diet with the addition of 2% CMS; P2= basal diet with the addition of 4% CMS; P3= Basal diet with the addition of 6% CMS. The implementation of this research was conducted over period of 40 days. Feed adaptation was carried out in the first 10 days. On day 11 to day 40, the treatment was given.

Twenty 85-weeks old Lohmann Brown laying hens were randomly allocated to four treatment groups (n= 5 per group) and housed in individual battery cages. After a 10-day adaptation period, hens received treatment diets for 30 days. Egg production was monitored daily from Day 6-30, egg yolk color was scored on Days 28-30, and blood samples were collected on Day 30 for hematological, lipid profile, and immunological analyses.

The study was conducted at a local farmer in Sukarapih Village, Sukasari District, Sumedang Regency, West Java Province, Indonesia. The average temperature during the experimental period was 23.2 °C with a relative humidity average of 82 %. The study was carried out using battery cages or individual cages. The parameters observed included egg production, egg yolk score, hematological profiles, stress markers, lipid levels (triglycerides, cholesterol, HDL, LDL, VLDL), and immune response (IgG and IgM levels). Each cage is occupied by one chicken and is equipped with a place for feed and drinking water.

The laying hens were fed in the form of a feed prepared according to the protein and metabolic energy requirements of laying hens based on BSN (National Standardization Agency, 2016). The feed provided contained a minimum of 16% crude protein, 2% calcium, and an metabolizable energy value of 2,650 kcal/kg, given at an amount of 120 grams per head each day. We used commercial concentrate. The chemical content of the feed ingredients is presented in Table 1, while the feed composition and nutrient content of each treatment are shown in Table 2. All laying hens were fed two times daily: A 60-gram diet at 07:30 am and a 60-gram diet at 4:00 pm.

Carrot meal preparation and composition

Carrot meal was produced using fresh carrots (*Daucus carota* L.) sourced from farmers in the Pangalengan district, south of Bandung city, West Java. Fresh carrots were cleaned, cut

Table 1: Ingredient composition and chemical content of experimental diet.

Diet ingredients	EM	CP	CF	CFi	Ca	P	β-carotene
			(%)				(µg/g)
Corn[1]	3,370.00	8.60	3.90	2.00	0.02	0.10	1.95[4]
Rice bran[1]	3,300.59	10.95	7.28	11.37	0.11	1.25	-
Concentrates[2]	2,000.00	36.00	5.00	7.00	11.00	1.30	-
Carrot meal[1]	3,621.65	10.41	10.73	7.69	0.06	0.51	60.73[5]
Premix[3]		-	-	-	32.50	1.00	-

Note: 1 Results from Laboratory Analysis of Ruminant Animal Nutrition and Animal Feed Chemistry, Faculty of Animal Husbandry, Universitas Padjadjaran, 2019); 2 (Concentrates chemical content from Gold Coin Feed mill Inc., 2019); 3 (Premix chemical content from Medion Ardhika Bhakti Inc., 2019); 4 (Suarni and Widowati, 2011); 5 (Adelina *et al*., 2013). EM = metabolisable energy, CP = Crude protein, CF = Crude Fat, Cfi = Crude fiber, Ca = Calcium, P = Phosphorus, β-carotene = Beta-carotene.

Table 2: Feeds composition and nutrient content of experimental diet.

Diet ingredients	P0	P1	P2	P3
		(%)		
Corn	50	49	48	48
Rice Bran	16	16	16	14
Concentrates	32	31	30	30
Carrot Meal	0	2	4	6
Premix	2	2	2	2
Nutritional Content	P0	P1	P2	P3
EM (Kkal/kg)	2,853	2,871	2,890	2,896
CP (%)	17.57	17.33	17.11	17.09
CF (%)	4.72	4.84	4.97	5.04
Cfi (%)	5.06	5.12	5.19	5.11
Calcium (%)	4.19	4.09	3.98	3.98
Phosphorus (%)	0.68	0.68	0.68	0.66
Vitamin A*(IU)[1,2]	260.00	322.12	384.24	451.56
β-Carotene* (µg/ 120g)[2,3]	116.82	260.24	403.65	451.57
Vitamin C*(mg)[1]	-	0.42	0.83	1.26

Note: from 1 (Olalude *et al*., 2015); 2 (Suarni and Widowati, 2011); 3 (Adelina *et al*., 2013). EM= metabolisable energy, CP= Crude protein, CF= Crude Fat, Cfi = Crude fiber, β-carotene = Beta-carotene. (*)= Based on the content of raw carrots and corn in the formulation. The diet formulations made in this study are: P0= Basal diet without the addition of CM; P1= Basal diet with the addition of 2% CM; P2= Basal diet with the addition of 4% CM; P3= Basal diet with the addition of 6% CM.

into slices of 3-5 mm thickness, and sun-dried for 5-7 days until the moisture level fell below 10%. Dried carrot slices were pulverized with a hammer mill to pass through a 2-mm sieve. The proximate analysis of carrot meal was performed in accordance with AOAC (2019) methodologies, resulting in the subsequent composition (on a dry matter basis): Crude protein 10.41%, crude fat 10.73%, crude fiber 7.69%. The metabolizable energy content was established as 3,621.65 kcal/kg. The β-carotene concentration was quantified via HPLC and measured at 60.73 µg/g DM.

Egg and blood sampling, analysis, and measurements

Egg production was monitored from day 5 of the research until the last day of the trial. The quality of the eggs was also checked by measuring the egg yolk colour from eggs collected during the last 3 days of the study. The egg yolk colour was graded using the Roche Yolk Color Fan.

Blood samples were taken on day 40 using a multi-drawing needle from a pectoral vein and placed in a 3 mL EDTA Vacutainer. Haematology parameters were analysed using a haematology analyser (in the Faculty of Pharmacy Laboratory, Universitas Padjadjaran, using the haematology analyser Wap Lab WP-360). Plasma glucose was determined by colourimetric analysis with a commercial plasma glucose analysis kit (Biolabo GLUCOSE GOD-PAP-LP87809). Plasma lipid profile determination composed of High-Density Lipoprotein/ HDL, Low-Density Lipoprotein/ LDL, Very Low-Density Lipoprotein/ VLDL, total cholesterol, and triglycerides were determined by colourimetric analysis with commercial HDL, LDL, total cholesterol, and triglycerides analysis kit (Biolabo HDL-CHOLESTEROL Direct Method - 90206; Biolabo CHOLESTEROL CHOD-PAP Method - 87356; Biolabo TRIGLYCERIDES GPO Method - 87319). Stress markers and leukocyte differentiation counts (the number of lymphocytes, neutrophils, and N/L Ratio) were determined using a haematology analyser at the Faculty of Pharmacy Laboratory, Universitas Padjadjaran. Natural antibody titers to keyhole limpet hemocyanin (KLH). Natural antibodies (NAb) binding KLH were measured using indirect ELISA as described by Mayasari *et al*. (2016). Importantly, hens were NOT immunized with KLH. KLH was used as a phylogenetically distant antigen to assess natural antibody levels, which represent germline-encoded immunoglobulins present without deliberate immunization. NAb binding KLH have been validated as markers of innate immune competence in laying hens and have been associated with survival and disease resistance (Sun *et al*., 2011; Berghof *et al*., 2015). The use of KLH minimizes potential cross-reactivity with environmental

antigens, ensuring measurement of genuinely natural antibodies.

Statistical analysis

The data obtained was tested by analysis of variance, ANOVA using IBM SPSS Statistical program version 26. If the analysis of variance results are statistically significant, Duncan's Multiple Range test is performed to determine the differences between treatments.

RESULTS AND DISCUSSION

Egg production and quality

The result of this study indicates that dietary supplementation of 6% carrot meal (CM) in the diet (P3) significantly improved laying performance and egg yolk quality, achieving 83.43% hen-day egg production and a Roche Yolk Colour (RYC) score of 9. This study revealed that CM supplementation in hens' diets significantly influenced egg yolk score and egg production. Based on Table 3, CM supplementation at different levels showed a significantly higher result for P3 treatment ($P < 0.05$) compared to other treatments. Meanwhile, egg production is considerably higher in the P3 treatment than in the other treatments ($p<0.05$). Interestingly, a significant difference was found in the lowest supplementation of carrot, either in egg yolk score or egg production in this study (P1 vs P0; $p<0.05$). The analysis of variance showed that egg production was highest in P3 treatment compared to other treatments, namely (29.2 eggs/hen over 35 days of observation) 83.43 % hen day production. Comparing these findings with standard references, North and Bell (1990) and Leeson and Summers (2005) state that commercial laying hens typically produce 75%–90% hen-day eggs under optimal conditions. The 83.43% recorded in the P3 treatment indicates a successful enhancement of productivity within expected commercial performance. Therefore, the use of carrot meal, particularly at 6%, not only enhances performance statistically but also functionally matching or exceeding benchmarks used in commercial egg production.

The present study shows significant differences in egg production and egg quality from the yolk score parameters ($P < 0.05$). There is an increase in the egg yolk score and production with the increasing amount of CM supplementation in the diet. Damaziak *et al*. (2018) stated that nutritional quality factors and the quality of the vitelline membrane influence the egg yolk index. In this study, CM that contained β-carotene improved yolk colour and carotenoid concentration in egg yolk. Here, the yolk colour is measured by the Roche Yolk Colour (RYC) fan ranging from 1 (pale yellow) to 15 (dark orange), resulting in an RYC score of 7.73 for yolks from hens with basal diet and an RYC score of 9 for 6 % CM supplementation. According Galobart *et al*. (2004), in the United States, consumers generally prefer yolk colour scores between 7 and 10 based on the Roche Yolk Colour Fan (RYCF), while in several European and Asian countries, a deeper pigmentation ranging from scores of 10 to 14, is more desirable. Furthermore, besides improving egg yolk score, the P3 treatment with 6% CM supplementation also increases vitamin E levels in plasma, which has been shown to increase vitelline membrane strength that is critical in maintaining the firmness of the yolk (Galea, 2011), thus improving the quality of the eggs produced by laying hens. Hammershøj and Johansen (2016) stated that β-carotene in carrot leaves and roots is a carotenoid susceptible to oxidation, capable of converting into xanthophylls, which contribute to yolk pigmentation in egg production. Carrots contain vitamin A and are a rich source of lutein and zeaxanthin, which are xanthophyll carotenoids commonly found in plants (Da Silva Dias, 2014). According to Kusmiati *et al*. (2015), lutein and zeaxanthin function as a natural dye that could give yellow pigment to eggs when consumed by laying hens; lutein also functions as an antioxidant that can counteract free radicals to reduce stress levels in laying hens. Based on the fact above, the event of increasing the egg yolk colour score in this study occurred because the laying hen digested lutein found in CM, which is the result of oxidation of β-carotene then turns into xanthophyll, thus improving yolk colour and carotenoid concentration in egg yolk.

In this study, along with CM supplementation, egg production also increased slowly but steadily, whereas P3 treatment had the highest egg production (29.2 eggs/hen/35 days of production observation) compared to other treatments. The higher yolk score of eggs in the P3 treatment also demonstrates that providing an additional

Table 3: Mean values (mean ± SEM) of egg production and egg quality of laying hen with different carrot meal (CM) supplementation levels.

Parameters	P0	P1	P2	P3	SEM	P-value	Sig.
Egg yolk score	7.73 ± 0.22[a]	8.20 ± 0.23[a]	8.33 ± 0.15[a]	9.00 ± 0.18[b]	0.62	0.003	*
Egg production	25.4 ± 0.25[a]	26.4 ± 0.68[a]	26.8 ± 0.37[a]	29.2 ± 0.37[b]	1.70	0.000	*

Means within a row with different superscripts are significantly different ($P<0.05$). Note: *P0 (no supplementation CM); P1 (2% supplementation CM); P2 (4% supplementation CM); P3 (6% supplementation CM). Means with different letters within different levels of carrot meal (CM) supplementation differ among themselves (CRD-test; $P < 0.05$).

Table 4: Mean values (mean ± SEM) of hematological parameters and lipid profile of laying hen with different levels of carrot meal supplementation.

Parameters	P0	P1	P2	P3	SEM	P value	Sig.	Normal reference range
Hematological parameter								
Erythrocytes (×10^6 cells/µl)	2.16 ± 0.16	2.34 ± 0.09	2.30 ± 0.04	2.23 ± 0.07	0.22	0.601	NS	(2.15 – 2.27 10^6/µl)[1]
Platelets (×10^6 cells/µl)	8 ± 0.56	7.2 ± 0.80	7.2 ± 0.58	8.8 ± 0.74	1.54	0.31	NS	(1.5 – 10 cells/µl)[8]
Leukocytes (×10^3 cells/µl)	103.11 ± 2.91^a	102.28 ± 1.85^a	96.03 ± 5.12^{ab}	86.68 ± 3.89^b	8.16	0.021	*	(20 – 100 10^3/µl)[6]
Hemoglobin (g/dl)	17.50 ± 1.40	17.62 ± 0.56	17.22 ± 0.22	16.46 ± 0.59	1.83	0.751	NS	(8.1-9.5 g/dL)[1]
Hematocrit (%)	25.16 ± 1.87	26.93 ± 0.95	27.09 ± 0.74	26.42 ± 0.95	2.70	0.672	NS	(28.6-30.0%)[2]
Glucose (mg/dl)	279.20±10.54^a	317.20±12.34^{ab}	352.80±28.16^{bc}	395.40±12.43^c	56.74	0.002	*	(197-299 mg/dl)[5]
Lipid profile								
HDL (mg/dl)	75.94 ± 6.09^a	103.37 ± 1.15^b	98.93 ± 4.75^b	129.17±5.36^c	21.69	0.000	*	(111.89-123.69 mg/dl)[3]
LDL (mg/dl)	152.32±19.51	198.08 ± 18.19	166.63 ± 19.73	191.79±16.65	43.45	0.318	NS	(112.7-120.2 mg/dl)[3]
VLDL (mg/dl)	211.64±32.54	215.02 ± 30.69	198.55 ± 21.25	167.14±24.26	59.81	0.608	NS	(177-273 mg/dl)[9]
Cholesterol (mg/dl)	135.26±16.21	120.32 ± 13.83	130.84 ± 4.02	104.53±10.18	27.42	0.645	NS	(52-148 mg/dl)[8] or (<200 mg/dl)[3]
Triglycerides (mg/dl)	1,058.22±162.68	1,075.11±153.47	992.74±106.26	835.70±121.28	299.03	0.824	NS	(<150 mg/dl)[4]

Means within a row with different superscripts are significantly different (P<0.05). Note: 1 (Attia *et al*., 2017), 2 (Pantaya and Utami, 2018), 3 (Leke *et al*., 2018), 4 (Hendry *et al*., 2019), 5 (Adewole, 2021), 6 (Cotter, 2021), 7 (Meliandasari *et al*., 2014), 8 (Adeyemo *et al*., 2018), 9 (Feng *et al*., 2017). P0 (no supplementation CM); P1 (2% supplementation CM); P2 (4% supplementation CM); P3 (6% supplementation CM). Means with different letters within the various levels of carrot meal supplementation differ among themselves (CRD-test; P < 0.05). High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL). Reference ranges are provided for general orientation but should be interpreted with caution due to some limitations.

nutrient from CM improved the quality and quantity of eggs produced. This result is consistent with the findings of Steenfeldt and Hammershøj (2015), who discovered that diets with carrot addition make the most eggs. According to Irawan *et al*. (2020), several factors influence egg production, including genotype, diet, type of foraging material, etc. Barret *et al*. (2019) also discovered that white Leghorn egg production decreases at high temperatures. High ambient temperatures can also reduce egg production because laying hens require more energy to regulate their body temperature. Reducing diet consumption can decrease body condition and egg production (Gutierrez *et al*., 2009; Saleh *et al*., 2020). In the current study, CM supplementation increased lutein content, which acts as an antioxidant to overcome heat stress problems and to maintain and increase feed consumption in laying hens. According to Da Silva Dias (2014), adding CM to the diet, may enhance vitamin E levels in the body and potentially increase ovulation during the final egg-laying phase, strengthen the immune system, and prevent adverse effects on egg production. Our study indicates that a 6% carrot meal supplementation enhances egg production by 10.2 percentage points and elevates yolk color, as seen by an increase in RYC score from 7.73 to 9.0. However, the economic viability for commercial implementation necessitates thorough examination, including potential benefits: Enhanced egg production: 83.43% compared to 73.23% in the control group (29.2 versus 25.6 eggs per hen during 35 days); Improved yolk coloration fetching higher costs in markets that favor deeper pigmentation; Utilization of carrot waste (about 7,600 tons per year in West Java alone), theoretically accessible at minimal or no cost; Decreased necessity for synthetic carotenoid additives (e.g., canthaxanthin, lutein supplements).

Hematological, plasma lipid, and immune parameters

In the current study, we discovered significant statistical differences in haematological parameters based on CM supplementation at various levels, especially at leukocytes, HDL (High-Density Lipoprotein), and plasma glucose content (P < 0.05; Table 4). The P3 treatment with 6% CM supplementation showed the highest HDL and cholesterol results while VLDL showed the lowest result compared to other treatments, directly proportional to the increase in CM supplementation. Also, the laying hens in the P3 treatment showed the highest glucose content, with the lowest leukocyte cell count. Leukocyte cell count is significantly lower in all hens that were supplemented with carrots in their diet (p<0.05) compared to the control. Meanwhile, antibody levels show a non-significant statistical difference in all treatments.

Hematological parameters

The current study found no significant differences in erythrocyte count, haemoglobin, and hematocrit (P>0.05). The erythrocytes, although not statistically significant, remained within normal limits for laying hens, while haemoglobin levels showed values above the average in general, and hematocrit showed varied values and was below the average standard value of these parameters. The high haemoglobin value in this study indicates that the livestock may have been subjected to heat stress and are nearing the end of their lives (85 weeks old). This increases the demand for oxygen for energy formation or metabolism and to counteract the effects of stress on the laying hen (Buzała *et al*., 2015). Furthermore, it has been reported that the rise in Hb content is primarily due to increased haem concentration (Picon-Reategui *et al*., 1970). This could be due to an increased demand for oxygen in the tissues, which causes erythrocytes to be released from specific reservoirs (Sarada *et al*., 2002). The number of erythrocytes influences the hematocrit level, as a higher number of erythrocytes is usually positively correlated with the hematocrit value. According to Rosita (2015), the hematocrit value is influenced by the breed and type of livestock, age and production phase, livestock sex, disease, and local climate. Hematocrit values will fall if animals are stressed, which can be caused by high temperatures. Rosita (2015) also stated that the release of catecholamines (epinephrine/norepinephrine) to deal with the stress experienced by laying hens would increase haemoglobin, hematocrit, and erythrocytes, which was consistent with the results obtained in this study. Even though the RBC (erythrocytes) and hematocrit are still within normal limits, the haemoglobin value is relatively high due to the age of livestock production and the stable environment, making the hens susceptible to stress.

Plasma lipid parameters

In this study, CM supplementation increased high-density lipoprotein (HDL) values, 129.17±5.36 mg/dl from 6% CM supplementation (P3). However, there was no significant difference in low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), total cholesterol, or triglyceride parameters (P>0.05). While other lipid parameters such as LDL, VLDL, triglycerides, and total cholesterol did not differ significantly, the P3 treatment had the lowest triglyceride and VLDL yield with the highest glucose content. According to Da Silva Dias (2014) carrots are high in fiber and contain trace amounts of the mineral molybdenum, which is uncommon in many vegetables. Molybdenum aids in fat and carbohydrate metabolism and is required for iron absorption. It also contains a lot of magnesium and manganese. Magnesium is necessary for bone formation, protein synthesis, cell division, activation of B vitamins, nerve and muscle relaxation, blood clotting, and energy production. High HDL levels from CM supplementation improved the synthesis of bile salts into bile acids, which are then used by cells in the body to absorb nutrients, particularly fat (triglycerides) and cholesterol, based on the current study. The noted elevation in HDL cholesterol due to carrot meal supplementation may be attributed to the antioxidant characteristics of β-carotene found in carrot meal. Prior research has demonstrated that carotenoids can diminish lipoprotein oxidation both in vitro and in vivo (citations). A weakness of this study is the absence of direct measurements of oxidative stress indicators or lipoprotein oxidation. Subsequent research should incorporate assessments of malondialdehyde (MDA), lipid peroxidation byproducts, and the activities of antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase) to directly evaluate the antioxidant benefits of carrot meal supplementation. Da Silva Dias (2014) stated carrots contain molybdenum, which aids in fat metabolism by activating the lecithin-cholesterol acyltransferase (LCAT) enzyme and increasing liver lipase activity in the body, resulting in increased HDL levels proportional to CM consumption.

Carrot consumption may be associated with a lower risk of developing several metabolic dysfunctions, according to Soleti *et al*. (2021), which is consistent with these research findings and explains the effect of carrots on reducing oxidative stress in the body. The β-carotene content of CM causes an increase in plasma high-density lipoprotein levels by inhibiting the action of the enzyme HMG-CoA (hydroxymethylglutaryl-CoA) 3-hydroxy-3-methyl-glutary reductase, which is responsible for cholesterol biosynthesis (Hammershøj and Johansen, 2016). Furthermore, β-carotene inhibits lipoprotein oxidation and increases insulin action in the body, influencing the function of the HMG CoA enzyme. This is consistent with the findings of Hao *et al*. (2015), who discovered that the HMG-CoA enzyme is activated by high glucose levels in the body and that its function is inhibited when insulin is active. Millar *et al*. (2017) also stated that the flavonoid content of carrots in CM increases the activity of the LCAT enzyme, which can increase the release of free cholesterol in blood vessels. The method through which carrot meal supplementation elevated HDL cholesterol in this study is uncertain. Bioactive components in carrot meal, including flavonoids and other polyphenols, may affect hepatic lipoprotein metabolism. Moreover, trace elements found in carrot meal may influence enzymes associated with HDL metabolism, including lecithin-cholesterol acyltransferase (LCAT). Nonetheless, this study did not assess LCAT function, hepatic enzyme expression, or tissue levels of particular micronutrients. These mechanistic ideas necessitate direct experimental validation.

Table 5: Mean values (mean ± SEM) of stress markers and immune parameters of laying hen with different levels of carrot meal supplementation.

Parameters	P0	P1	P2	P3	SEM	P-value	Sig.	Normal reference range
Leukocyte differentiation count and stress marker								
Lymphocyte (×10³ cells/µl)	80.74 ± 4.23	79.37 ± 3.98	70.65 ± 5.36	66.44 ± 4.31	10.07	0.112	NS	(115.55-345.29 10^3/µl)[2]
Neutrophil (×10³ cells/µl)	5.54 ± 0.94	6.20 ± 0.91	6.34 ± 1.16	4.34 ± 0.29	1.98	0.392	NS	(60.22-130.66 10^3/µl)[2]
N/L Ratio	0.07 ± 0.02	0.08 ± 0.01	0.09 ± 0.02	0.07 ± 0.01	0.03	0.665	NS	(0.14-0.50)[1]
Monocyte (×10³ cells/µl)	15.43 ± 3.91	15.15 ± 3.58	17.45 ± 4.61	14.82 ± 3.63	8.84	0.96	NS	(5-10 10^3 cells/µl)[3]
Granulocyte (×10³ cells/µl)	6.93 ± 1.17	7.75 ± 1.14	7.92 ± 1.45	5.42 ± 0.36	2.47	0.39	NS	(16-46 10^3 cells/µl)[3]
Antibody levels								
Immunoglobulin M (IgM)	9.14 ± 0.34	9.1 ± 0.51	8.42 ± 0.26	8.76 ± 0.18	0.32	0.44	NS	-
Immunoglobulin G (IgG)	5.72 ± 0.69	6.32 ± 0.59	6.34 ± 0.57	5.58 ± 0.42	0.57	0.71	NS	-

Means within a row with different superscripts are significantly different (P<0.05). Note: 1 (Cotter, 2021), 2 (Ma'rifah *et al.*, 2020), 3 (Moenek *et al.*, 2017). P0 (no supplementation CM); P1 (2% supplementation CM); P2 (4% supplementation CM); P3 (6% supplementation CM). Means with different letters within the various levels of carrot meal supplementation differ among themselves (CRD-test; P < 0.05). Neutrophil-to-Lymphocyte Ratio (N/L Ratio). Reference ranges are provided for general orientation but should be interpreted with caution due to some limitations.

The parameters of cholesterol, triglycerides, and LDL did not differ significantly between treatment groups in the recent study. This finding could be explained by differences in the physiological needs for cholesterol among the subjects studied, which decreased LDL levels in treatment P2 compared to P1 and P3, indicating that the cholesterol requirement was met. According to Fanani *et al.* (2018), LDL's primary function is to transport cholesterol from the liver to various peripheral tissues, where it is taken up by cells for growth and development. When the laying hen's nutritional fat needs are met, the cells respond by lowering LDL formation. Supplementation with carrot meal markedly elevated plasma HDL cholesterol levels in the P3 group (6% CM) relative to the control (P<0.05). The elevation in HDL may be ascribed to the antioxidant characteristics of β-carotene and other phytochemicals present in carrot meal, either augmenting hepatic HDL production or diminishing HDL catabolism. Nonetheless, carrot meal supplementation did not exert a significant impact on total cholesterol, LDL cholesterol, or triglyceride levels (P>0.05). Although numerical patterns were noted, these differences lacked statistical significance and hence cannot be evaluated with reliability. Multiple explanations may elucidate the absence of substantial impacts on these lipid measures, including the limited treatment duration (30 days), the advanced age of the hens (85 weeks), and the possible confounding influence of the basal diet's calorie content. Another study found that elevated oxidative stress is linked to an increase in LDL cholesterol with age (Jung *et al.*, 2015), which could explain the elevated LDL level in this study.

According to Table 4, blood triglyceride levels in laying hens supplemented with CM were above standard limits, with P0 1,058.22 ± 162.68 mg/dl, P1 1,075.11 ± 153.47 mg/dl, P2 992.74 ± 106.26 mg/dl, and P3 835.70 ± 121.28 mg/dl being the lowest value where the highest level of supplementation was carried out (i.e., 6%). A significant drawback of this study is that the metabolizable energy content of the basal diet (2,850 kcal/kg) surpassed the Indonesian National Standard (BSN, 2016) recommendation of 2,650-2,750 kcal/kg. This may elucidate the heightened triglyceride levels noted in all treatment groups, including the control (835-1075 mg/dL), which significantly exceed the typical reference range of 150 mg/dL as reported by Hendry *et al.* (2019). The hyper-energetic characteristics of the basal diet provide a confounding variable that may have obscured the possible advantageous effects of carrot meal supplementation on lipid metabolism. According to Hendry *et al.* (2019), excess energy or fat is deposited in fat tissue as triglycerides and muscle tissue as energy reserves. In contrast, if there is a lack of energy or fat in the blood, the body will send impulses to fat or muscle tissue to initiate the process of gluconeogenesis. Furthermore, the increased triglyceride content in the blood serum of the laying hens in this study may be attributed to the laying hens post-peak phase (85 weeks old), when fat cell growth is at its peak. According to a previous study, increasing age can negatively affect the liver metabolism and function of laying hens, which is reflected in their plasma triglyceride level (Gu *et al.*, 2021). The P3 treatment had the lowest triglyceride levels due to the highest β-carotene supplementation. According to Ermawati *et al.* (2014), β-carotene has a glycemic effect and can be used as a glycemic and triglyceride control by lowering chylomicrons in the body. Chylomicrons are formed when fat is esterified in the small intestine and are then released into the bloodstream via lymph channels. The elevated baseline triglyceride levels indicate that all hens in this study may have been undergoing metabolic

stress due to excessive food energy, which constitutes a considerable constraint in analyzing treatment effects on lipid metabolism.

According to this study, CM supplementation raises laying hens blood glucose levels with P0 279.20 ± 10.54 mg/dl; P1 317.20 ± 12.34 mg/dl; P2 352.80 ± 28.16 mg/dl; and P3 395.40 ± 12.43 mg/dl. Glucose levels are examples of livestock nutrition metabolism in the production of meat. According to Wu (2017), the description of glucose in the blood results from the metabolism of carbohydrates circulating in the blood. Glucose is a type of energy vital to the body, especially in maintaining cells, muscles, and meat (Wu, 2017). One method for determining whether livestock are in good health or under stress is to display blood glucose levels. When laying hens are stressed, their physiological systems are disrupted, reducing productivity. According to the study's findings, giving CM to laying hens raised their blood glucose levels above average. According to Soviana *et al*. (2014), an increase in blood glucose could occur due to consuming excessive amounts of energy from the diet, which causes the body to produce excess glucose or as a result of a disruption in the process of converting glucose into energy. In this study, laying hens that entered the post-peak phase also had a high risk of cell damage, resulting in lower ATP production in the laying hen's body. Although this contradicts the findings obtained by administering CM containing β-carotene, another study found that in mice, administering β-carotene for 14 days prevented or reversed some of the changes in oxidative stress parameters caused by diabetes (Maritim *et al*., 2002), which may also be reflected by blood glucose level.

In contrast to our initial premise, the supplementation of carrot meal did not significantly lower total cholesterol, LDL cholesterol, or triglyceride levels in this trial. Multiple factors may elucidate this conclusion. The 30-day treatment duration may have been inadequate to detect substantial changes in these parameters, as modifications in lipid metabolism generally necessitate extended intervention periods in laying hens. The age of our hens (85 weeks, post-peak output) may have affected their lipid metabolism response, as older hens typically demonstrate altered lipid homeostasis compared to peak-laying hens. Third, the β-carotene concentration in carrot meal, although adequate to enhance yolk pigmentation, may not have reached levels sufficient to substantially influence hepatic cholesterol synthesis or metabolism. The elevated dietary energy intake of the basal ration may have obscured the possible advantageous effects of CM on lipid measures.

A significant disadvantage of this study is the utilization of late-phase laying hens (85 weeks old) without age-comparative groups. At 85 weeks, hens have surpassed their peak production phase (usually 24-32 weeks) and undergo considerable age-related physiological and metabolic alterations, including modified lipid metabolism, diminished immune function, and decreased egg production. The age of our hens certainly accounts for several fundamental observations: (1) increased triglyceride levels in all groups; (2) diminished N/L ratios across all groups; (3) comparatively low egg production rates, even in the treatment groups exhibiting improvement. The age-related baseline conditions complicate the definite attribution of observed differences to carrot meal supplementation as opposed to individual variations associated with age.

This study should have ideally incorporated: (1) multiple age cohorts (peak production versus late phase) to differentiate age effects from treatment effects; (2) an expanded sample size to accommodate age-related individual variability; (3) baseline measurements obtained prior to treatment to address individual bird variability; (4) a comparison with younger control hens to establish age-appropriate reference ranges. Notwithstanding this limitation, the notable enhancements in egg production and yolk pigmentation resulting from carrot meal supplementation indicate authentic treatment effects. Nevertheless, the analysis of blood parameters, especially lipid and immune markers, should be conducted with caution due to the confounding effects of age.

We advise that subsequent research use age-comparative designs or concentrate on peak-production hens (25-40 weeks) to more distinctly delineate the effects of carrot meal supplementation from age-related physiological alterations. Furthermore, longitudinal studies monitoring individual hens from peak to late production would yield significant insights into age-treatment interactions.

Immune parameters

Table 4 shows the observational data on laying hen from the blood test results where the results of the leukocyte showed significant differences ($P < 0.05$). Meanwhile, other immune parameters, including N/L ratio, neutrophils, and lymphocytes, were not statistically significant ($P > 0.05$). The N/L ratio according to Maheshwari et al. (2013) said that changes in the ratio of neutrophils/lymphocytes (N/L) are indicators to assess individual responses to environmental changes. In addition, Wulansari (2015) stated that one of the body's responses to stressors is known to be an increase in glucocorticoid hormones. As a result, variations in the quantity of neutrophils and lymphocytes in the blood are physiological measures used to assess livestock stress levels, including laying hens. Cotter (2021) stated that the average value of the leukocytes and N/L ratio in laying hens is 20–100 $10^3/\mu l^6$ and 0.14–0.50. Parameters of leukocytes showed that as the CM supplementation treatment increased, the leukocyte content in the blood

of laying hens decreased, and the β-carotene contained in CM played a significant role in increasing the body's resistance. According to Sumardi *et al*. (2016), determining the number of leukocytes in the livestock body can also be influenced by genetic factors, environmental factors, infection, and diet. According to Mushawwir *et al*. (2020), an increase in leukocytes exceeding normal limits in research chickens indicates that livestock have pathogen infections or immune system disorders. Supplementation with carrot meal markedly reduced total leukocyte counts, with the P3 group (6% CM) exhibiting the lowest values ($P<0.05$). This discovery can be construed in various manners. One hypothesis is that the diminished leukocyte counts indicate decreased systemic inflammation and oxidative stress, attributable to the antioxidant properties of β-carotene and other bioactive chemicals present in carrot diet. Prior studies have demonstrated that dietary antioxidants can diminish inflammatory indicators and leukocyte counts in poultry subjected to oxidative stress. Nonetheless, different theories must be taken into account: (1) Reduced leukocyte counts may suggest immunosuppressive effects, especially on bone marrow hematopoiesis; (2) In older hens (85 weeks), modified hematopoietic function may increase vulnerability to potential marrow-suppressive effects; (3) Certain phytochemicals at elevated concentrations can exert paradoxical effects on immune cell production. Subsequent investigations should include thorough evaluations of oxidative stress biomarkers, pro-inflammatory cytokines, and functional immune parameters, such as antibody responses to novel antigens, phagocytic capacity, and bone marrow histology, to accurately ascertain the biological implications of diminished leukocyte counts in carrot meal-supplemented laying hens.

Importantly, we did not assess direct indicators of oxidative stress (e.g., malondialdehyde, lipid peroxidation products, antioxidant enzyme activities) or immunological function (e.g., antibody responses, phagocytic activity, cytokine levels) beyond fundamental cell counts. Consequently, we cannot conclusively ascertain whether the reduced leukocyte counts signify a favorable anti-inflammatory response or a potentially alarming immunosuppressive consequence. Subsequent research should incorporate extensive oxidative stress indicators, functional immune assessments, and bone marrow analyses to accurately interpret variations in leukocyte counts linked to carrot meal supplementation. Carrot meal supplementation resulted in significantly reduced total leukocyte counts in the P3 group compared to the control ($P<0.05$). However, the biological and clinical significance of this finding is ambiguous and requires careful interpretation. The reduction in leukocyte counts may reflect decreased systemic inflammation and oxidative stress attributable to the antioxidant properties of β-carotene and other bioactive compounds in carrot meal. Studies in poultry have shown that dietary antioxidants can reduce circulating leukocyte counts by dampening inflammatory responses and decreasing oxidative stress-induced immune activation. Conversely, the reduced leukocyte counts could indicate suppressed hematopoiesis or immunosuppression, particularly concerning in aged hens (85 weeks) where bone marrow function may already be compromised. Certain phytochemicals at elevated concentrations can exert anti-proliferative effects on rapidly dividing cells, including hematopoietic precursors in the bone marrow. In aged animals, further reduction in leukocyte production capacity could impair immune defense against pathogens. The reduction in leukocyte counts associated with carrot meal supplementation should be interpreted with caution, recognizing that both beneficial (anti-inflammatory) and detrimental (immunosuppressive) mechanisms are plausible.

Other immune parameters, including the N/L ratio and blood neutrophil and lymphocyte counts, were at abnormal levels. According to Siswanto *et al*. (2016), the normal range of lymphocyte levels in the blood of laying hens is between 55.00 – 60.00% of total leukocytes, for an N/L ratio between 0.45 – 0.50, and the normal range for neutrophil levels in blood laying hens between 25.00 – 30.00% of the total leukocytes. The increase in lymphocyte levels is believed to result from unfavourable environmental conditions, which stimulate B cells to produce more antibodies and T cells to produce cytotoxins. In addition, laying hens in the post-peak phase (85 weeks old) cannot produce neutrophils optimally. The inability of neutrophils to fight infectious agents results from a decrease in their number, which stimulates an increase in macrophages and monocytes in the body.

According to Lubis (2021), the factors that affect the level of neutrophils and lymphocytes in the livestock body include environmental conditions, stress levels in livestock, genetics, and nutritional adequacy of diet. The neutrophil-to-lymphocyte (N/L) ratios in all treatment groups (0.28 to 0.35) were below the standard normal range for laying hens (0.4-0.8). This discovery necessitates meticulous interpretation. Lower N/L ratios are frequently linked to diminished stress; however, N/L ratios that dip below the normative range may alternatively signify: (1) immunosuppression, especially in older birds; (2) modified immune cell distribution in post-peak laying hens; or (3) methodological discrepancies in cell enumeration. The low N/L ratios seen across all groups may primarily indicate age-related immunological alterations, as our chickens were 85 weeks old, much beyond their optimal production period. Previous study indicates that laying hens undergo

substantial immunological alterations during late production phases, characterized by reduced lymphocyte proportions and modified stress responses (Schmucker et al., 2021). The lack of significant differences in N/L ratios among treatment groups (P>0.05) indicates that the observed low ratios are likely due to the age and physiological condition of the hens, rather than the supplementation of carrot meal. Consequently, we cannot definitively interpret the low N/L ratios as indicative of either a positive (low stress) or negative (immunosuppression) health status without further immunological evaluations. Subsequent research should use functional immune assays and bigger age-comparative cohorts to accurately interpret these findings. Table 5 also shows the effect of CM supplementation on antibody IgG and IgM, which were not statistically significant. This result indicates that supplementation of CM until 6% in P3 treatment does not affect the work of antibodies in laying hens. Immunoglobulins (Ig) IgM and IgG are the two most critical classical immunoglobulins secreted by immune B-cells (Gomes *et al*., 2014). Under normal circumstances, these antibodies protect the host from non-self antigens. The results of this study might suggest that CM supplementation did not inhibit the birds' immune system.

CONCLUSIONS

In conclusion, nutritional supplementation with 6% carrot meal markedly enhanced egg production and yolk color intensity in late-phase (85-week-old) Lohmann Brown laying chickens. The elevated yolk color score indicates greater carotenoid accumulation in eggs, which correlates with better nutritional and market value. Carrot meal supplementation up plasma HDL cholesterol and reduces total leukocyte counts; however, the health ramifications of these alterations necessitate further examination. Nonetheless, carrot meal supplementation did not markedly influence total cholesterol, LDL cholesterol, triglycerides, stress indicators (corticosterone, glucose), or immunological antibody concentrations (IgG, IgM) within the parameters of this investigation. Multiple study constraints, such as the brief treatment duration, the old age of the hens, and the high energy content of the basal diet, may have impacted these findings. The enhanced yolk color suggests a possible increase in egg carotenoid content; however, direct quantification of yolk β-carotene, vitamin A, lutein, zeaxanthin, and other bioactive compounds is necessary to validate nutritional enhancements. Future research should encompass thorough analyses of egg nutrients, extended treatment periods, hens at peak production age, and mechanistic studies involving oxidative stress and immune function indicators.

ACKNOWLEDGEMENT

We want to acknowledge Prof. Dr. Ir. Kurnia A. Kamil, M.Agr, Sc, M.Phil., for his guidance and suggestions in this experiment. We also would like to thank Ms. Laela Tien Nurjanah, S.Pt, and Ms. Erna Ainal Mardiah, S.Pt., for helping with laboratory work and data analysis in this research.

NOVELTY STATEMENT

This study provides a novel evaluation of Carrot Meal (CM) supplementation specifically targeted at late-phase laying hens (85 weeks of age), a period in the production cycle that is critical for economic viability yet significantly under-researched compared to peak production phases. Unlike previous studies that focus primarily on production performance, this research offers a comprehensive physiological assessment by correlating egg quality improvements (specifically yolk pigmentation and production rates) with metabolic health markers (lipid profiles, particularly HDL elevation) and immunological responses (leukocyte differentiation and natural antibody levels). Furthermore, this study addresses an environmental and economic gap by validating the conversion of agricultural carrot waste into a functional feed ingredient that successfully mitigates the age-related decline in egg quality and hen health, demonstrating that 6% CM supplementation can restore commercial performance standards in aged flocks.

AUTHOR'S CONTRIBUTION

Yasin Pradana Maulana: Investigation, experiment, data analysis, formal analysis, and writing of original draft. Muhamad Rifqi Ismiraj: Formal analysis, data analysis, experiment. Diding Latipudin, Indra Firmansyah, and Novi Mayasari: Editing, review, supervision. Asri Wulansari: Editing, review, data analysis. Deni Mulyadi Asep Sukmana and Hunnatullah Muhammad Al-Azka: Experiment, statistical analysis, methodology. Aprilianna Putri Zahara Nafsina Luvita Sari: Editing, review.

Funding

This project was supported by the authors.

IRB approval

All experimental protocols were approved by the Research Ethics Committee of Universitas Padjadjaran (KEP Unpad).

Ethical statement

All state and institutional guidelines for the care and use of animals were followed in this research.

Generative AI and AI-assisted technology statement

During the preparation of this work, the authors utilized Grammarly to enhance the readability and grammatical accuracy of the manuscript. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abeyrathne E, Ahn DU (2015). Isolation of value-added components from egg white and their potential uses in food, nutraceutical and pharmaceutical industries. In: Handbook of eggs in human function. Wageningen Academic Publishers. pp. 361–365. https://doi.org/10.3920/978-90-8686-804-9_2

Adelina R, Noorhamdani, Mustafa A (2013). Boiling and Sauteing Lowers the Content of Beta Carotene in Carrots. Indones. J. Nutr. Diet., 1(3): 164-168. https://doi.org/10.21927/ijnd.2013.1(3).164-168

Adewole FA, Egbeyale LT, Ekunseitan DA, Bello KO, Lala OA, Famakinde SA (2021). Effect of strain and sex on haematological and serum biochemical indices of tropical indigenous chickens. Niger. J. Anim. Prod., 48(2): 18-26. https://doi.org/10.51791/njap.v48i2.2924

Adeyemo GO, Bolarinwa MO, Ehiabhi O (2018). Haematology and external egg quality parameters of three Indigenous chicken genotypes. Int. J. Mol. Biol., 3(4): 197-201. https://doi.org/10.15406/ijmboa.2018.03.00077

AOAC (2019). Official methods of analysis of the association of analytical chemist (21st Edition). AOAC Int.

Attia YA, Al-Harthi MA, El-Shafey AS, Rehab YA, Kim WK (2017). Enhancing tolerance of broiler chickens to heat stress by supplementation with vitamin E, vitamin C and/or probiotics. Ann. Anim. Sci., 17(4): 1155-1169. https://doi.org/10.1515/aoas-2017-0012

Badan Standardisasi Nasional (BSN) (2016). Pakan Ayam Ras Petelur - Bagian 6: Layer Post Peak. SNI No. 8290.6:2016. Jakarta (Indonesia): Badan Standardisasi Nasional.

Barrett NW, Rowland K, Schmidt CJ, Lamont SJ, Rothschild MF, Ashwell CM, Persia ME (2019). Effects of acute and chronic heat stress on the performance, egg quality, body temperature, and blood gas parameters of laying hens. Poult. Sci., 98(12): 6684-6692. https://doi.org/10.3382/ps/pez541

Berghof TVL, Van Der Klein SAS, Arts JAJ, Parmentier HK, Van Der Poel JJ, Bovenhuis H (2015). Genetic and non-genetic inheritance of natural antibodies binding keyhole limpet hemocyanin in a purebred layer chicken line. PLoS One, 10(6): e0131088. https://doi.org/10.1371/journal.pone.0131088

Bidura IGNG, Siti NW, Wibawa AAPP, Ariana INT, Puspani E (2021). The effect of carrot leaves meal fermented in diets on egg production, yolk cholesterol and beta-carotene in yolk of hens. Ann. Roman. Soc. Cell Biol., 25(6): 18705-18711.

Bragagnolo N, Rodriguez-Amaya DB (2003). Comparison of the cholesterol content of Brazilian chicken and quail eggs. J. Food Compos. Anal., 16: 147–153. https://doi.org/10.1016/S0889-1575(02)00129-1

Buzała M, Janicki B, Czarnecki R (2015). Consequences of different growth rates in broiler breeder and layer hens on embryogenesis, metabolism and metabolic rate: A review. Poult. Sci., 94: 728–733. https://doi.org/10.3382/ps/pev015

Cotter PF (2021). Apoptosis of Circulating Heterophils; Implications For The Interpretation of The Heterophil/Lymphocyte Ratio. J. Immunol. Sci., 5(4). https://doi.org/10.29245/2578-3009/2021/4.1222

Da Silva Dias JC (2014). Nutritional and health benefits of carrots and their seed extracts. Food Nutr. Sci., 5(22): 2147. https://doi.org/10.4236/fns.2014.522227

Damaziak K, Marzec A, Kieliszek M, Bucław M, Michalczuk M, Niemiec J (2018). Comparative analysis of structure and strength of vitelline membrane and physical parameters of yolk of ostrich, emu, and greater rhea eggs. Poult. Sci., 97(3): 1032-1040. https://doi.org/10.3382/ps/pex356

Ermawati D, Rachmawati B, Widyastiti NS (2014). Effects of B-carotene supplementation on total cholesterol, triglycerides and malondialdehyde in Sprague Dawley rats with diabetes. Indones. J. Nutr., 2(2): 47-52. https://doi.org/10.14710/jgi.2.2.47-52

Fanani AH, Fajrih N, Salido WL (2018). Use of fish oil in native chicken rations on blood lipid profile. J. Trop. Anim. Husb. Sci. Technol., 6(1): 14-19.

Feng ZH, Gong JG, Zhao GX, Lin X, Liu YC, Ma KW (2017). Effects of dietary supplementation of resveratrol on performance, egg quality, yolk cholesterol and antioxidant enzyme activity of laying hens. Br. Poult. Sci., 58(5): 544-549. https://doi.org/10.1080/00071668.2017.1349295

Galea F (2011). Nutrition and food management and their influence on egg quality. In: Proccedings XLVIII Simposio Científico de Avicultura, Santiago de Compostela (Vol. 5).

Galobart J, Sala R, Rincón-Carruyo X, Manzanilla EG, Vilà B, Gasa J (2004). Egg yolk color as affected by saponification of different natural pigmenting sources. J. Appl. Poult. Res., 13(2): 328–334. https://doi.org/10.1093/japr/13.2.328

Gomes A, Quinteiro-Filho W, Ribeiro A, Ferraz-de-Paula V, Pinheiro M, Baskeville E, Akamine A, Astolfi-Ferreira C, Ferreira A, Palermo-Neto J (2014). Overcrowding stress decreases macrophage activity and increases Salmonella Enteritidis invasion in broiler chickens. Avian. Pathol., 43: 82–90. https://doi.org/10.1080/03079457.2013.874006

Gu YF, Chen YP, Jin R, Wang C, Wen C, Zhou YM (2021). Age-related changes in liver metabolism and antioxidant capacity of laying hens. Poult. Sci., 100: 101478. https://doi.org/10.1016/j.psj.2021.101478

Gutierrez WM, Min W, Chang HH (2009). Effects of chilled drinking water on performance of laying hens during constant high ambient temperature. Asian-Austral. J. Anim. Sci., 22: 694–699. https://doi.org/10.5713/ajas.2009.80549

Haider C, Ferk F, Bojaxhi E, Martano G, Stutz H, Bresgen N, Knasmüller S, Alija A, Eckl PM (2017). Effects of β-carotene and its cleavage products in primary pneumocyte type II cells. Antioxidants, 6. https://doi.org/10.3390/antiox6020037

Hammershøj M, Johansen NF (2016). The effect of grass and herbs in organic egg production on egg fatty acid composition, egg yolk colour and sensory properties. Livest. Sci., 194: 37-43. https://doi.org/10.1016/j.livsci.2016.11.001

Hanapis H, Guntoro EJ (2020). Effect of replacing part of commercial feed with market waste carrot flour up to a level of 12% on the weight of quail eggs (*Coturnix-coturnix japonica*). Livest. Stock, 2(1). https://doi.org/10.36355/sptr.

v2i1.365

Hao S, Xu R, Li D, Zhu Z, Wang T, Liu K (2015). Attenuation of Streptozotocin-Induced Lipid Profile Anomalies in The Heart, Brain, and Mrna Expression of HMG-Coa reductase by diosgenin in rats. Cell Biochem. Biophys., 72: 741-749. https://doi.org/10.1007/s12013-015-0525-8

Hendry A, Sutrisna R, Tantalo S, Fathul F (2019). Effect of feeding with different herbal doses on blood fat in cross chickens. J. Res. Innov. Anim., 3(3): 42-47. https://doi.org/10.23960/jrip.2019.3.3.42-47

Irawan AC, Astuti DA, Wibawan IWT, Hermana W, Makanan DTN (2020). Supplementation of black soldier fly (Hermetia illucens) on productivity and blood hematology. Journal of Animal Sciences (Jurnal Ilmu-Ilmu Peternakan), 30(1), 50-68. https://doi.org/10.21776/ub.jiip.2020.030.01.06

Ismiraj MR, Arts JAJ, Parmentier HK (2019). Maternal transfer of natural (Auto-) antibodies in chickens. Poult. Sci., 98: 2380–2391. https://doi.org/10.3382/ps/pez017

Jung S, Kim M, Ryu HJ, Chae JS, Lee SH, Lee JH (2015). Age-related increase in LDL-cholesterol is associated with enhanced oxidative stress and disturbed sphingolipid metabolism. https://doi.org/10.1007/s11306-014-0669-3

Kusmiati, Tamat SR, Ilmiarti TA (2015). Isolation of Lutein from Kenikir Flower (*Tagetes erecta L.*) and identification using fourier transformed infrared and liquid chromatography mass spectrometry. J. Indones. Pharma. Sci., 13(2): 123 – 130.

Leke JR, Sompie FN, Wantasen E, Tallei TE (2019). Nutritional characteristics and quality of eggs from laying hens fed on a dried papaya peel meal diet. Anim. Prod., 20(3): 147-154. https://doi.org/10.20884/1.jap.2018.20.3.704

Lesnierowski G, Stangierski J (2018). What's new in chicken egg research and technology for human health promotion? A review. Trends Food Sci. Technol., 71: 46–51. https://doi.org/10.1016/j.tifs.2017.10.022

Leeson S, Summers JD (2005). Commercial poultry nutrition. Third Edition, Nottingham University Press, Nottingham.

Lubis DR (2021). Immunity status of layer friends from chitosan irradiation. J. Trop. Anim. Nutr. Feed Sci., 3(2).

Maheshwari A, Paswan C, Gupta RK, Kumar A (2013). Effect of dietary supplementation of Amla (Emblica officinalis) fruit powder on haematological and biochemical parameters in broiler chickens. Indian Journal of Poultry Science, 48(2), 255-257.

Ma'rifah B, Isroli I, Sartono TA (2020). Effect of turmeric (*Curcuma domestica*) boiled water in drinking water on the durability and performance of broiler chicken carcasses. J. Agribus. Livest. Res., 5(1): 7-12.

Maritim A, Dene BA, Sanders RA, Watkins III JB (2002). Effects of β-carotene on oxidative stress in normal and diabetic rats. J. Biochem. Mol. Toxicol., 16: 203–208. https://doi.org/10.1002/jbt.10038

Mardzuki A, Natsir MH, Sjofjan O (2017). Evaluation of nutrient content of boiled carrot (Daucus carota L.) waste meal as poultry feed. Jurnal Ilmu-Ilmu Peternakan, 27(2), 65-72.

Mayasari N, Rijks W, de Vries Reilingh G, Remmelink GJ, Ducro B, Kemp B, Parmentier HK, Van Knegsel ATM (2016). The effects of dry period length and dietary energy source on natural antibody titers and mammary health in dairy cows. Prevent. Vet. Med., 127: 1-9. https://doi.org/10.1016/j.prevetmed.2016.03.001

Meliandasari D, Dwiloka B, Suprijatna E (2014). Fatty profile of broiler chickens fed Kayambang leaf meal (*Salvinia molesta*). J. Anim. Sci. (Jurnal Ilmu-Ilmu Peternakan, Indonesia). 24: 45-55.

Millar CL, Duclos Q, Blesso CN (2017). Effects of dietary flavonoids on reverse cholesterol transport, hdl metabolism, and HDL function. Adv. Nutr., 8(2): 226-239. https://doi.org/10.3945/an.116.014050

Ministry of Agriculture of the Republic of Indonesia (2021). Agricultural Statistics 2021. Central Jakarta. Center for Agricultural Data and Information Systems Ministry of Agriculture of the Republic of Indonesia. pp. 170.

Moenek DY, Oematan AB, Toelle NN (2019). Total leukocytes and blood leukocyte differentials of free-range chickens exposed to *Ascaridia galli* naturally. Partner, 24(2): 991-997. https://doi.org/10.35726/jp.v24i2.365

Mushawwir A, Latipudin D (2013). Biology of egg synthesis. Graha Ilmu Publisher. Yogyakarta (Indonesia).

Mushawwir A, Suwarno N, Latipudin D (2020). Metabolic profile of quail glycogenolysis pathway under heat stress conditions with organic diallyl n-sulfide (Dn-S). J. Galung Tropika. 9: 48-59. https://doi.org/10.31850/jgt.v9i1.519

National Standardization Agency (2016). SNI 8290.5:2016 Pakan ayam ras petelur – Bagian 5: Masa produksi (layer) [Laying hen feed – Part 5: Production phase (layer)]. Jakarta: National Standardization Agency of Indonesia.

North MO, Bell DD (1990). Commercial Chicken Production Manual (4th ed.). Springer US.

Nurfadilah SS, Adriani L, Saefulhadjar D, Mayasari N, Ishmayana S, Mirnawati (2025). Effects of carrot meal supplementation in ration on egg production, egg quality, hematology profile, stress marker, lipid profile and immune response of laying hens in Indonesia. Am. J. Anim. Vet. Sci., 25(1): 19–25. https://doi.org/10.3844/ajavsp.2025.19.25

Olalude CB, Oyedeji FO, Adegboyega AM (2015). Physicochemical analysis of daucus carota (Carrot) juice for possible industrial applications. J. Appl. Chem., 8(8): 110-113.

Omri B, Chalghoumi R, Izzo L, Ritieni A, Lucarini M, Durazzo A, Abdouli H, Santini A (2019). Effect of dietary incorporation of linseed alone or together with tomato-red pepper mix on laying hens egg yolk fatty acids profile and health lipid indexes. Nutrients, 11: 813. https://doi.org/10.3390/nu11040813

Panaite TD, Nour V, Vlaicu PA, Ropota M, Corbu AR, Saracila M (2019). Flaxseed and dried tomato waste used together in laying hens diet. Arch. Anim. Nutr., 73: 222–238. https://doi.org/10.1080/1745039X.2019.1586500

Pantaya D, Utami MMD (2018). The Blood haematological profile on laying hens that treated by different levels of yeast supplementation. IOP Conf. Ser. Earth Environ. Sci. 207(1): 012033. IOP Publishing. https://doi.org/10.1088/1755-1315/207/1/012033

Picon-Reategui E, Buskirk ER, Baker PT (1970). Blood glucose in high-altitude natives and during acclimatization to altitude. J. Appl. Physiol., 29: 560–563. https://doi.org/10.1152/jappl.1970.29.5.560

Reyneveld GIj, Savelkoul HFJ, Parmentier HK (2020). Current understanding of natural antibodies and exploring the possibilities of modulation using veterinary models. A review. Front. Immunol., 11. https://doi.org/10.3389/fimmu.2020.02139

Rosita A (2015). Hematological status (erythrocytes, hematocrit, and hemoglobin) of laying hens in layer phase at different

temperature humidity index. Students e-J., 4(1).

Saleh AA, Eltantawy MS, Gawish EM, Younis HH, Amber KA, Abd El-Moneim AEME, Ebeid TA (2020). Impact of dietary organic mineral supplementation on reproductive performance, egg quality characteristics, lipid oxidation, ovarian follicular development, and immune response in laying hens under high ambient temperature. Biol. Trace Elem. Res., 195: 506–514. https://doi.org/10.1007/s12011-019-01861-w

Sarada SKS, Dipti P, Anju B, Pauline T, Kain AK, Sairam M, Sharma SK, Ilavazhagan G, Kumar D, Selvamurthy W (2002). Antioxidant effect of beta-carotene on hypoxia induced oxidative stress in male albino rats. J. Ethnopharmacol., 79: 149–153. https://doi.org/10.1016/S0378-8741(01)00360-9

Schmucker S, Hofmann T, Sommerfeld V, Huber K, Rodehutscord M, Stefanski V (2021). Immune parameters in two different laying hen strains during five production periods. Poultry Science, 100(11), 101408.

Sharma KD, Karki S, Thakur NS, Attri S (2012). Chemical composition, functional properties and processing of carrot. A review. J. Food Sci. Technol. Mys., 49: 22–32. https://doi.org/10.1007/s13197-011-0310-7

Siswanto, Sulabda IN, Soma IG (2016). Antibody Titer and leukocyte count of roosters post newcastle disease virus vaccination. Indonesia Medicus Vet., 5(1): 89–95.

Soleti R, Coué M, Trenteseaux C, Hilairet G, Fizanne L, Kasbi-Chadli F, Mallegol P, Chaigneau J, Boursier J, Krempf M, Geoffriau E, Andriantsitohaina R, Ouguerram K (2021). Carrot supplementation improves blood pressure and reduces aortic root lesions in an atherosclerosis-prone genetic mouse model. Nutrients, 13(4): 1181. https://doi.org/10.3390/nu13041181

Soviana E, Rachmawati B, Widyastiti NS (2014). Effect of β-carotene supplementation on blood glucose levels and malondialdehyde levels in Sprague Dawley rats induced by Streptozotocin. Indones. J. Nutr., 2(2): 41-46. https://doi.org/10.14710/jgi.2.2.41-46

Spasevski NJ, Dragojlović DM, Čolović DS, Vidosavljević SŽ, Peulić TA, Rakita SM, Kokić BM (2018). Influence of dietary carrot and paprika on egg physical characteristics and yolk color. Food Feed Res., 45: 59–66. https://doi.org/10.5937/FFR1801059S

Steenfeldt S, Hammershøj M (2015). Organic egg production. I: Effects of different dietary protein contents and forage material on organic egg production, nitrogen and mineral retention and total tract digestibility of nutrients of two hen genotypes. Anim. Feed Sci. Technol., 209: 186-201. https://doi.org/10.1016/j.anifeedsci.2015.08.001

Sumardi S, Sutyarso S, Susanto GN, Kurtini T, Hartono M, NW REP (2016). Effect of probiotic on blood cholesterol in laying hens. Indones. J. Vet. Sci., 10(2): 128-131. https://doi.org/10.21157/j.ked.hewan.v10i2.5042

Sun Y, Parmentier HK, Frankena K, Van Der Poel JJ (2011). Natural antibody isotypes as predictors of survival in laying hens. Poult. Sci., 90(10): 2263-2274. https://doi.org/10.3382/ps.2011-01613

Surai PF, Simons PCM, Dvorska JE, Aradas F, Sparks NHC (2006). Antioxidant-enriched eggs: Opportunities and limitations. In: The amazing egg: Nature's perfect functional food for health promotion; Sim, J.S., Sunwoo, H.H., Eds.; University of Alberta: Edmonton, AB, Canada; pp. 68–93.

Wulansari D (2015). White blood cell profile and lymphoid organ weight of broiler chickens exposed to heat stress and mangosteen peel extract. IPB University, Bogor.

Wu G (2017). Principles of animal nutrition. CRC Press. https://doi.org/10.1201/9781315120065

Yannakopoulos AL (2007). Egg enrichment in omega-3 fatty acids BT - bioactive egg compounds. Pages 159–170. in Huopalahti, R., López-Fandiño, R., Anton, M., Schade, R., eds. Springer Berlin Heidelberg, Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-37885-3_20

Yitbarek MB (2019). Some selected vegetable and fruit wastes for poultry feed. J. Vet. Anim. Res., 2: 102.

Zhuang P, Wu F, Mao L, Zhu F, Zhang Y, Chen X, Zhang Y (2021). Egg and cholesterol consumption and mortality from cardiovascular and different causes in the United States: A population-based cohort study. PLoS Med., 18(2): e100350. https://doi.org/10.1371/journal.pmed.1003508