

Effects of carrot leaves on digestibility of feed, and cholesterol and β -carotene content of egg yolks

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Abstract

The purpose of this study was to evaluate the effects of carrot leaf supplementation on feed digestibility and the cholesterol and β -carotene contents of the yolks of eggs produced by Lohmann Brown laying hens. A feeding trial was conducted using 240 healthy 30-week-old laying hens kept in colony cages to evaluate the effect of dietary supplementation with carrot leaves. Carrot leaf extract (CLE) was prepared by macerating carrot leaves in distilled water (1:1, w/w). The hens were fed diets i) without carrot leaves (C0), ii) supplemented with 2% carrot leaf flour (CLF)(C1); iii) supplemented with 2% CLE(C2); and iv) supplemented with 1% CLF and 1% CLE (C3). Supplementation of CLE, CLF and in combination increased dry matter, organic matter and protein digestibility significantly. Feed efficiency was improved, and eggshell thickness, yolk colour and β -carotene content in yolk increased. Supplementation with CFL or CLE produced significantly lower serum and yolk cholesterol contents. Dietary supplementation of laying hens with CLF and CLE also increased egg production and β -carotene contents in yolk.

Key words: *Daucus carota*, eggshell thickness, lipid profile

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Introduction

Eggs are one of the cheapest sources of quality animal protein. Egg quality and price are affected substantially by their internal and external characteristics (Hanusová *et al.*, 2015; Orhan *et al.*, 2016).

The use of antibiotic growth promoters was banned in Indonesia, because they could cause the development of antibiotic-resistant bacteria. Excessive use of antibiotics, even in low doses administered continually, could leave residue in poultry eggs. Efforts to reduce the use of antibiotics motivated the need to find alternatives.

The use of herbal leaves in feed or drinking water has been shown to increase feed digestibility, egg quality and yolk colour, and reduce cholesterol levels in yolk (Siti *et al.*, 2019; 2017; Bidura *et al.*, 2017; Duru & Sahin, 2015; Kaya & Yildirim, 2011; Puspani *et al.*, 2018; Wibawa *et al.*, 2017). These plants have been previously shown to improve the digestive system and performance of broiler chickens (Sahin & Duru, 2010; Ekayuni *et al.*, 2017) and the serum lipid profile of broilers (Duru & Sahin, 2012). The high phenolic content of herbal plants, supported by the presence of alkaloids, saponins, flavonoids, and triterpenoids in them also can be efficacious as an anti-bacterial (Yuniza & Yuherman, 2015). But secondary metabolites of herbal plants that have antimicrobial activity may interfere with the absorption of nutrients in the lumen of the gastrointestinal tract (Goel, 2013; Enemuor *et al.*, 2011). Terpenoids can be antibacterial in that they damage pathogenic bacterial cell membranes in the gastro-intestinal tract of chickens (Cowan, 1999). Sanchez *et al.* (2006), Ekayuni *et al.* (2017), and Hernandez *et al.* (2004) reported that herbal plants such as *Moringa oleifera* do not have toxic effects or contain factors that limit nutrient absorption and digestion. The results of studies on rats (Ramakrishna *et al.*, 2003) found that herbal extracts were able to increase the activity of pancreatic enzymes and microenvironmental conditions for better utilization of nutrients.

Carrot (*Daucus carota*) production in Indonesia reached 609,634 tons in 2018 and produced waste products amounting to 40% to 50% of the carrot harvest. These leaves could be used as a new feed supplement from this by-product of the agro-industry (Hammershøj *et al.*, 2010). They contain phytochemical compounds such as saponins, flavonoids, and tannins (Puspani *et al.*, 2019), have antimicrobial activity

(Çetingül *et al.*, 2020), are high in nutrients, and are good sources of α -carotene, β -carotene, and β -cryptoxanthin (Hammershoj *et al.*, 2010). The β -carotene content of carrot leaves is an active carcass colour agent (Ayssiwede *et al.*, 2011), and intensifies the yellow colour of egg yolk, making the eggs more desirable to consumers. Pale egg yolks have been a problem for the poultry industry for many years (Qiao *et al.*, 2001). Thus carrot leaves may be a viable option for dietary supplementation of laying hens to increase egg yolk colour and carotenoid concentration (Titcomb *et al.*, 2019). Therefore, this study aimed to study the effect of CLF and CLE aspects of egg production, feed digestibility, cholesterol levels and β -carotene in yolk of eggs produced by laying hens.

Material and Methods

This research was approved by the Ethics Commission of the Faculty of Veterinary Medicine, Udayana University. Two hundred and forty 30-week-old Lohmann Brown laying hens were obtained from commercial poultry farms with an average bodyweight of 1643.7 ± 29.4 grams. All chickens were kept in a controlled environment. Carrot leaf extract was prepared by suspending carrot leaves in distilled water (1:1, w/w). Carrot leaf flour was made by collecting the leaves at harvest, drying them in the sun for up to two days, and grinding them to a fine powder. The birds were randomly divided into four treatments, namely an unsupplemented control (C0), a diet that was supplemented with 2% CLF (C1); a diet that was supplemented with 2% CLE (C2); and a diet that was supplemented with 1% CLF and 1% CLE (C3). All diets are isocaloric and isonitrogenous. There were six replications of each treatment. Each replicate was housed in a cage (experimental unit) measuring $100 \times 75 \times 40$ cm³, which contained ten hens. The basal diet was based on nutritional requirements for laying hens (Scott *et al.*, 1982). The feeding trial lasted for 10 weeks. The ingredients and chemical composition of feed are shown in Table 1.

Table 1 Composition of feed ingredients and nutritional content in diets of laying hens aged 30 - 40 weeks

Ingredients	Composition, %	Nutrients	Calculated nutrient analysis
Yellow corn	60.20	Metabolizable energy, kcal/kg	2900
Rice bran	17.00	Crude protein, %	16.0
Soybean	9.20	Crude fibre, %	3.90
Fish meal	9.17	Ether extract, %	7.12
Premix ¹	4.43	Calcium, %	2.93
		Phosphorus, %	1.10
		Arginine, %	1.16
		Lysine, %	1.07
		Methionine + cysteine, %	0.69
		Tryptophan, %	0.20

¹ Calcium: 4.9 kg, phosphorus: 1.4 kg, iron: 40000 mg, manganese 27500 mg; magnesium: 27.500 mg; zinc 25 mg; vitamin B₁₂: 4.50 mg, vitamin D₃: 500000 IU per 10 kg of premix

All chickens had free access to feed and drinking water. The birds were weighed at the beginning and end of the experiment. Eggs were collected twice daily. An egg multitest machine (model EMT-7300, No. 096173, Touhoku Rhythm Co. Ltd, Japan) equipped with calibration gauge kit CGK-02 was used to measure egg quality. Feed consumption was determined weekly. Feed conversion ratio (FCR), calculated as kg feed/kg egg weight, was recorded for each cage during the trial. Yolk colour was scored (scale 1 - 15) with a Roche yolk colour fan. Measurements of yolk and albumen weight, eggshell, eggshell thickness, yolk colour, albumen height and yolk height were recorded each week.

Yolk cholesterol levels were evaluated from egg yolk samples. These samples were mixed until smooth and homogeneous, frozen, and stored prior to being analysed. Cholesterol content in egg yolk samples was determined with a standard method using a UV-visible spectrophotometer (AOAC, 2005). Measurement of β -carotene content in yolk was conducted according to Siti *et al.* (2019). The serum lipid profile was assessed with the Lieberman-Burchard acetic anhydride test.

Digestibility of dry matter (DM), organic matter (OM), crude protein (CP), and ether extract (EE) were determined using the force-feeding technique (Bidura *et al.*, 2019). The amount of diet used was 100 g,

which was based on a preliminary test in which the consumption of laying hens was measured. All hens were fasted for 24 hours to ensure that the digestive tract was empty. Then, the 100 g feed was divided into two parts, namely 50 g that was fed for the first hour, and 50 g that was fed three hours later. The feed was introduced into the crop slowly with a plastic funnel with a 30 cm rod. Water was available ad libitum during this time. The excreta were collected in plastic trays under individual cages. Samples were weighed, dried in the sun, then in the oven at 105 °C, and ground through a 1-mm filter. Triplicate samples of the diet and excreta were analysed using AOAC (2005) methods.

All data were analysed with one-way analysis of variance to determine the significance of treatment effects. If significant ($P \leq 0.05$) differences were found, the treatment means were compared with Duncan's multiple range test.

Results and Discussion

The outcomes from supplemental carrot leaf on egg production performance and nutrient digestibility are shown in Table 2. Hens that were supplemented with carrot leaf, regardless of form, consumed significantly more feed and produced significantly more eggs over the 70-day test period than their unsupplemented contemporaries. Average egg weight was unaffected by the treatments ($P > 0.05$). Thus, total egg weight was increased as a result of the greater hen-day production and the birds consumed less feed to produce a gram of egg. These responses were similar for C1, C2, and C3.

Table 2 Effects of carrot leaf supplementation on the performance of mature Lohmann Brown laying hens and on the digestibility of nutrients

Variables	Treatments				SE
	C0	C1	C2	C3	
Initial bodyweight, g	1642.85	1648.03	1640.72	1645.19	46.92
Final bodyweight, g	1758.36	1764.25	1772.73	1770.95	54.07
Daily feed consumption, g	108.37 ^b	112.85 ^a	113.29 ^a	113.78 ^a	1.35
Number of eggs produced in 70 days	43.67 ^b	47.96 ^a	50.69 ^a	50.28 ^a	1.21
Hen-day production, %	62.39 ^b	68.52 ^a	72.41 ^a	71.83 ^a	1.80
Egg weight, g	57.48	57.72	58.06	57.91	0.69
Total egg weight	2510.15 ^b	2768.25 ^a	2943.06 ^a	2911.71 ^a	75.39
Egg mass, g	251.02 ^b	276.83 ^a	294.31 ^a	291.17 ^a	6.93
Feed conversion ratio	3.02 ^a	2.85 ^b	2.69 ^b	2.74 ^b	0.05
Dry matter digestibility, %	71.29 ^b	75.51 ^a	76.34 ^a	76.19 ^a	1.19
Organic matter digestibility, %	73.51 ^b	77.39 ^a	78.25 ^a	78.03 ^a	1.21
Crude protein digestibility, %	74.02 ^b	78.62 ^a	79.85 ^a	79.53 ^a	1.47
Ether extract digestibility, %	80.71	79.95	79.72	78.96	1.59

C0: without carrot leaves, C1: supplemented with 2% carrot leaf flour (CLF); C2: supplemented with 2% carrot leaf aqueous extract (CLE); and C3: supplemented with 1% CLF and 1% CLE

^{a,b} Means with a common superscript were not different at probability $P = 0.05$

Dietary supplementation with carrot leaf, regardless of form, increased the digestibility of dry matter, organic matter, and CP ($P < 0.05$). However, the digestibility of ether extract (EE) did not show any significant difference ($P > 0.05$) between treatments (Table 2). These responses were similar for C1, C2, and C3.

Table 3 presents the characteristics of eggs produced by the Lohmann Brown laying hens during the 70-day test. The eggs in C0 and C1 had a lower percentage of shell and a higher percentage of yolk than C2 and C3 ($P < 0.05$). The shells in C0 were thinner than those from C1, C2, and C3. However, the percentages of albumen and Haugh unit for these eggs were similar across treatments ($P > 0.05$). The yolks from eggs produced by C1, C2, and C3 were more yellow and contained more β -carotene than the yolks of C0 ($P < 0.05$).

Table 3 Effects of carrot leaf supplementation on characteristics of eggs produced by mature Lohmann Brown laying hens

Variables	Treatments				SE
	C0	C1	C2	C3	
Eggshell, %	10.75 ^b	11.39 ^b	11.82 ^a	11.64 ^a	0.19
Egg yolk, %	29.64 ^b	31.27 ^b	31.46 ^a	31.39 ^a	0.48
Egg albumen, %	59.61	57.34	56.72	56.97	1.53
Haugh unit	76.07	75.84	75.92	76.17	0.87
Egg shape (egg width/egg length) x 100	74.85	74.17	75.08	75.26	1.05
Relative density, g/cm ³	1.05	1.04	1.05	1.05	0.07
Eggshell thickness, mm	0.40 ^b	0.46 ^a	0.48 ^a	0.47 ^a	0.01
Yolk colour (1–15)	7.02 ^b	8.45 ^a	8.59 ^a	8.63 ^a	0.31
β-carotene, µg/kg	1.489 ^b	1.597 ^a	1.631 ^a	1.624 ^a	0.02
Egg yolk cholesterol, mg/100 g yolk	12.95 ^a	12.71 ^a	11.04 ^b	11.19 ^b	0.44

C0: without carrot leaves, C1: supplemented with 2% carrot leaf flour (CLF); C2: supplemented with 2% carrot leaf aqueous extract (CLE); and C3: supplemented with 1% CLF and 1% CLE

^{a,b} Means with a common superscript were not different at probability $P=0.05$

Serum cholesterol, triglycerides, and low density lipoproteins were lower in the Lohmann Brown laying hens that were provided with C1, C2, and C3 compared with those fed C0 (Table 4).

Table 4 Effects of carrot leaf supplementation on serum lipid profile of mature Lohmann Brown laying hens

Variables	Treatments				SE
	C0	C1	C2	C3	
Total cholesterol	135.82 ^a	124.05 ^b	119.83 ^b	123.16 ^b	3.06
Triglyceride	26.69 ^a	21.72 ^b	19.81 ^b	20.81 ^b	1.17
High density lipoprotein	99.74	98.59	97.04	100.03	2.38
Low density lipoprotein	38.61 ^a	32.17 ^b	30.05 ^b	31.27 ^b	1.71

C0: without carrot leaves, C1: supplemented with 2% carrot leaf flour (CLF); C2: supplemented with 2% carrot leaf aqueous extract (CLE); and C3: supplemented with 1% CLF and 1% CLE

^{a,b} Means with a common superscript were not different at probability $P=0.05$

Supplementation of laying hens with carrot leaf increased the consumption of feed significantly. This showed that carrot leaf (flour or water extract) could increase chicken appetite, which would have a positive impact on the quantity and quality of egg production. This result was supported by the findings that feeding with *Moringa* leaf powder improved live weight, bodyweight gain, dressing percentage, and FCR in broilers (David *et al.*, 2012). Increased feed consumption implies increased nutrient consumption, which causes better egg production and increased egg weight. Relative to C0, the use of CLF or CLE improved feed conversion by birds in C1, C2, and C3 significantly.

Herbal leaves could be used as an effective feed supplement in poultry to improve feed efficiency (Akhouri *et al.*, 2013; Sanchez *et al.*, 2006), and could improve the absorption of protein, energy, and minerals (Ossebi, 2010). Bidura *et al.* (2020) found that laying hens supplemented with *Moringa* leaf flour showed greater differences in egg production, egg weight, feed efficiency (feed consumption/egg mass), and feed digestibility than control chickens. Administration of herbal extracts was shown to increase egg production and egg mass in laying hens significantly (Bidura *et al.*, 2017; Khan *et al.*, 2012).

Digestibility of DM, OM, and CP in laying hens was increased by supplementation with carrot leaf because of the absence in carrot leaves of antinutrient compounds that could affect feed digestibility. Ürüsan

et al. (2018) reported that supplementing carrot seed oil in the ration could increase the number of lactic acid bacteria in the digestive tract of broiler chickens significantly. Lactic acid bacteria act as probiotics that could provide beneficial effects for the host. However, Teteh *et al.* (2013) found that increased levels of saponin sourced from herbal leaves as antinutrients could reduce absorption of nutrients, especially lipids, in poultry.

The laying hens in C1, C2, and C3 showed significantly higher egg production, total egg weight, and eggshell thickness compared with C0. This may have been caused by the herbal extract significantly increasing in the digestibility of protein, energy, and minerals (Ossebi, 2010). Azizah *et al.* (2017) reported that the use of carrot flour in poultry rations could activate protease enzymes, so that calcium could be absorbed more readily and used for eggshell synthesis. In addition, carrot leaves contain high concentrations of calcium and phosphorus (Olalude *et al.*, 2015). Increased protein consumption and protein digestibility also affect the mass of calcium in the eggshell (Syafitri *et al.*, 2015). On the other hand, Duru *et al.* (2017) observed that supplementation of laying hens with 0.1% to 0.8% CLF did not affect egg quality variables significantly, including albumen index, egg index, egg weight, Haugh unit, egg yolk index, and egg yolk colour.

The yolk colour values in eggs from chickens in C1, C2, and C3 increased significantly compared with C0. This increase may be because carrot leaves have a high β -carotene content (Puspani *et al.*, 2019). Carotenoids that are suitable for this purpose include β -carotene (Stahl & Sies, 2012) and provitamin A (Olson, 1999). The types and amounts of carotenoid pigment consumed by chickens are the main factors in pigmentation of the yolk (Tourniaire *et al.*, 2009). Pigment carotenoids from carrots would be absorbed by blood, circulate through the body, then accumulate in the tissues, which would affect the colour of the egg yolk ultimately (Hammershoj *et al.*, 2010). The content of β -carotene in poultry eggs varies greatly, and depends on the feed consumed. The content of β -carotene in chicken egg was between 1.07 and 2.12 $\mu\text{g}/\text{kg}$ (Kotrbaček *et al.*, 2013), whereas according to Islam *et al.* (2017) it ranged from 0.16 to 1.62 $\mu\text{g}/\text{kg}$. Calislar (2019) reported that the β -carotene content in egg yolk from wild chickens was 25 - 30% higher than in domestic chicken eggs. Hammershoj *et al.* (2010) and Souza *et al.* (2019) reported that providing carrot flour to laying chickens could increase the colour value of egg yolks effectively, especially their lutein, alpha-carotene, and β -carotene contents.

The decrease in cholesterol, low-density lipoprotein (LDL) and triglyceride in the serum of supplemented chickens may have been caused by the presence of saponin in carrot leaves. Teteh *et al.* (2013) reported that the decrease in fat and cholesterol content in egg yolk, caused by increased levels of saponin, could reduce absorption of lipids. Elamin *et al.* (2019) found that baobab flour (*Adansonia digitata*) could function as a natural antioxidant to decrease triglyceride, cholesterol, and LDL levels compared with the control. Lovati *et al.* (2015) also reported that supplementation of Noni extracts in drinking water could reduce total cholesterol, triglycerides, and LDL in the serum of chickens. The cholesterol content in the egg yolk could also have been reduced as a consequence of the high calcium mineral content in carrot leaves (Olalude *et al.*, 2015), and fat metabolism could be also influenced by the presence of Ca (Azizah *et al.*, 2017). High Ca content in the body would cause fat metabolism to increase owing to the saponification process, and fat would be wasted, and thus fat deposition in eggs would be lowered. Carrot consumption also reduced cholesterol and triglyceride levels in the liver significantly, and increased plasma vitamin E levels (Nicolle & Cardinault, 2003).

Conclusion

Supplementation of 30- to 40-week-old laying hens with CLM, CLE and in combination could be beneficial in terms of increased feed consumption, egg production, and feed efficiency. These benefits may result, at least in part, from the effects on the digestibility of DM, OM, and CP. Consumers may appreciate the improvements in egg yolk colour, eggshell thickness, and β -carotene and cholesterol contents of egg yolk because of this supplement.

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Authors' Contributions

IGNGB and NWS conceived the idea, conducted the trial, collected the samples, analysed the data, and wrote the article.

Conflict of Interest Declaration

Authors declare they have no conflict of interest regarding this research.

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