

Effect of different levels of lutein on laying performance and egg quality in laying hens

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ABSTRACT

Seventy Brown-Nick laying hens (39-wk old) were divided into 5 treatment groups of similar mean body weight and egg production, comprising 14 birds in each. The birds were housed in individual cages in a completely randomized design and 16:8 h light:dark photoperiod was employed. The birds were fed standard layer diets containing different levels of lutein for 6 weeks. Treatment groups included control (without lutein), 100, 200, 400 and 800 ppm lutein respectively. The results attained from this study indicated that, different levels of lutein did not have a significant effect on final body weight, feed intake and feed conversion ratio but egg production was different between treatment groups. Also, it was observed that egg width, albumen width, shell weight, egg shape index, yolk index, yolk height and shell thickness were affected by lutein. Feeding diet containing different levels of lutein had a significant effect on egg yolk colour. It can be concluded that, adding of lutein in layer hens' diet can improve the laying performance and egg quality.

Keywords: Egg quality, Laying hens, Lutein

Egg colour is an important factor in the evaluation of egg quality, selection, and consumption by the consumers. Chickens cannot synthesize the colouring matter and therefore layer chickens use carotenoids containing colour substances (lutein, zeaxanthin, cantaxanthin, cryptoxanthin, echinone, violaxanthin, neoxanthin) that they take from feed and transfer to egg yolk (Nelson and Babbitt 1966, Marusich and Bauernfeind 1970). In the poultry industry, carotenoids have been used as means of pigmenting eggs and meat (Lesson and Summers 1997) and carotenoids content in egg and meat can be manipulated by adding different levels of carotenoid in layer hens' diet (Lesson and Caston 2004). The results of some experiments showed that, adding of coloured carrots or lutein and alga in laying hens' diet have a significant effect on egg yolk colour and egg yolk concentration of total carotenoids (Hammershoj *et al.* 2010, Englamaierova *et al.* 2013, Kotrbacek *et al.* 2013). Lutein is a natural pigment group known as xanthophyll carotenoids without vitamin A activity, and it is found in vegetables, fruits, grains, and eggs (Johnson 2004). Due to the lack of information about biological mechanisms on physiological functions and prevention of some diseases, lutein is not currently evaluated as an essential nutrient for humans and animals (Pitargue *et al.* 2019). However, adequate lutein intake is currently recommended to improve human health, for example, consuming eggs containing lutein

(oxygenated carotenoid) and zeaxanthin has been shown to reduce age related macular degeneration and cataracts (Olmedilla *et al.* 2003, Granado *et al.* 2003), incident of cancer and cardiovascular disease due to strong antioxidant capacity of lutein (Park *et al.* 1998, Sindhu *et al.* 2010). The present study was designed to determine the effect of adding different levels of lutein in layer hen's diet on laying performance and egg quality.

MATERIALS AND METHODS

The objective of present study was to investigate the effect of different levels of dietary lutein on performance and egg quality in brown-neck layer hens. For this experiment, 39 weeks-old brown-neck layer hens (70) were selected. The birds were kept in individual battery cages (35 cm × 45 cm × 40 cm) which contained nipple drinker and feeder. Room temperature (18-22°C) and humidity (55-60%) were maintained within the animal comfort zone using air conditioning and 16:8 h light:dark photoperiod was employed. All birds were fed *ad lib.* with a standard layer diet (Table 1) obtained from a commercial feed industry for two weeks (39-40 week-adaptation), during which body weight, daily egg production and egg weight were recorded daily. At the beginning of the experiment, when the birds were 41 weeks old, all the birds were divided into 5 experimental groups with similar mean body weight and egg production levels, comprising 14 birds in each group. Lutein source used in this experiment was Marigold flower extract. Experimental diets included: control (without adding lutein), 100, 200, 400, and 800 ppm

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Table 1. Ingredient and nutrient compositions of the experimental diets given to laying hens.

Ingredient (%)		Nutrient	
Yellow corn	54.49	Dry matter	88.52
Soybean meal-46	10.00	Crude protein	19.00
Full fat soybean	9.64	Crude cellulose	3.58
Limestone powder (GRN)	7.71	Crude fat	3.71
Sunflower meal-36	7.46	Crude ash	13.35
Corn gluten meal-60	3.86	Starch	34.99
Meat-bone-35	2.48	Metabolizable energy (kcal/kg)	2680
DCP-18	1.57	Ca	3.65
Soybean oil	2	Tot-P	0.78
Salt	0.24	Ave-P	0.50
Vitamin premix	0.20	Na	0.16
Mineral premix	0.10	Lysine	0.87
Sodium bicarbonate	0.10	Methionine	0.37
L-Lysine	0.06	Methionine + cystine	0.70
Choline-60	0.05	Tryptophan	0.20
DL-Methionine	0.04	Threonine	0.70
Total	100.00	Arginine	1.20
		Isoleucine	0.76
		Valine	0.89

For each kg of the diet: 15,000,000 IU, Vitamin A; 5,000,000 IU, Vitamin D₃; 100,000 mg, vitamin E; 3,000 mg, Vitamin K₃; 3,000 mg, Vitamin B₁; 8,000 mg, Vitamin B₂; 60,000 mg, Niacin; 15,000 mg, Ca-D-Pantothenate; 5,000 mg, Vitamin B₆; 20 mg, Vitamin B₁₂; 2,000 mg, Folik Asit; 200 mg, D-Biotin ve; 100,000 mg, Vitamin C; 120,000 mg, Mn; 80,000 mg, Fe; 80,000 mg, Zn; 16,000 mg, Cu; 1,250 mg, I; 200 mg, Co ve; 300 mg, Se.

lutein respectively. This experiment lasted 6 weeks (41 to 46 weeks-old).

During the experiment, eggs were collected every day and measured for exterior quality (size and weight). Feed intake, egg production and feed conversion ratio were also recorded. In the first week of the experiment and thereafter (41, 42, 43, 44, 45 and 46 weeks of age), eggs obtained on the third and fifth days of every week were analyzed for external and internal quality. Egg weight, shell weight, albumen weight and yolk weight were measured with a 0.01 precision scales. Also, egg width-length, albumen width-length-height and yolk width-height were measured by callipers. Egg shell thickness, egg shell strength, and yolk colour (l=lightness, a=redness and b=yellowness) were measured using micrometer, TA. XT. Plus Texture Analyser, and HunterLab, ColorFlex EZ, respectively. Shape index, albumen index, and Haugh unit were calculated according to following formula:

$$\text{Egg shape index} = \text{Egg width} / \text{Egg length} \times 100$$

$$\text{Yolk index} = \text{Yolk height} / \text{Yolk width} \times 100$$

$$\text{Albumen index} = \text{Albumen height (mm)} / [\text{Albumen length (mm)} + \text{Albumen width (mm)}] \times 100$$

$$\text{Haugh Unit} = 100 \log (\text{Albumen height} + 7.57 - 1.7 \times \text{Egg weight}^{0.37})$$

Statistical analyses: The data obtained from this experiment were analyzed using general linear model procedure of the Statistical Analysis System software (SAS 2005) to obtain the effect of lutein levels. Duncan's New Multiple Range Test in SAS was used to identify significant differences among treatments means. Results obtained from this experiment are presented as means per bird with

standard error of difference between means (SED) with P values.

RESULTS AND DISCUSSION

Laying performance: The results of this study indicated that adding different levels of lutein in laying hens' diets did not have a significant effect on final body weight, average and total feed intake and average egg weight ($p > 0.05$) but total and average egg weights were numerically higher in lutein groups than the control group (Table 2). Egg production was higher in the groups fed with lutein containing diet than the control group (Table 2), especially in the group fed a diet containing 400 ppm lutein ($p < 0.05$). Based on feed intake, it can be observed that there were no significant differences between experimental groups and the feed conversion ratio was lower in groups fed with diet containing 100 and 400 ppm lutein (1.78).

Results of this study are in accordance with finding of Shuhao *et al.* (2017) who reported that supplementation of 0.075%, 0.15%, 0.30% and 0.60% marigold extract does not have a significant effect on feed intake, body weight gain, and feed conversion ratio. Also, Alay and Karadas (2016), demonstrated that supplementation of 10 mg/kg apoester, canthaaxanrhin, 3% clover extract, paprika oleoresin and aztec marigold extract pigments in quail breeders' diet does not have a significant effect on body weight at the beginning, and at the end of the study also feed intake and feed conversion ratio of quail were not affected ($p > 0.05$). Byoung-Ki *et al.* (2014) showed that, adding of 1.34 mg/g and 5.36 mg/kg chlorella powder have a significant effect on egg production and daily egg mass but there was no

Table 2. Effect of using different levels of dietary lutein on laying performance

Parameter	Treatment					SED	p
	Control	100 ppm	200 ppm	400 ppm	800 ppm		
Initial weight (g)	1882	1887	1886	1885	1883	18.283	1.000
Final weight (g)	1822	1924	1890	1886	1906	19.389	0.534
Average feed intake (g/hen/7 day)	766.73	810.49	789.02	785.19	808.27	6.937	0.263
Total feed intake (g/hen/ 42 day)	4600	4863	4734	4711	4849	41.625	0.263
Total egg weight (g/hen/ 42d ay)	2448	2604	2522	2543	2531	27.150	0.512
Average egg weight (g/hen/ 42 day)	58.29	62.01	60.05	60.55	60.27	0.646	0.512
Egg production (number/ 42 day)	38.11 ^b	40.23 ^a	40.00 ^a	40.36 ^a	39.79 ^{ab}	0.272	0.080
Feed conversion ratio	1.94	1.87	1.88	1.87	1.94	0.022	0.717

SED, Standard error of difference between means; ^{a,b}Means bearing different superscript in the same row are significantly different (p<0.05).

significant effect on feed intake and egg weight. Coh *et al.* (2013), showed that adding of 5-10% dried distillers grains and 0.011-0.021% synthetic pigment canthaxanthin in laying hens diet has a significant effect on egg production but they do not have any effect on feed intake. On the contrary, results of this study are inconsistent with results of Titcomb *et al.* (2019). They used 0.05% marigold-fortified powder, 0.35 and 0.40% orange-carrot leaf-fortified and red-carrot leaf-fortified in Comb White Leghorns laying hens diets and reported that feed intake was significantly less in the leaf treatment groups (p<0.05). Also, Grčević *et al.* (2016), added 0, 200, 400 mg/kg lutein in layer hens' diets and observed that, there was no significant difference

between treatments group in egg production. Altuntaş and Aydın (2016), supplemented 10 g/kg or 20g/kg marigold in layers hens' diet for 42 days and they did not observe any significant changes in egg production (p>0.05), but marigold changed egg weights of layer hens.

Egg quality: Results obtained from this experiment indicated that using dietary lutein had significant effects on shell weight (%), egg width, egg shape index, albumen width, yolk height, yolk index and shell thickness (p<0.05). Also, different levels of lutein did not have significant effect on egg weight, albumen weight (g and %), yolk weight (g and %), shell weight (g), egg length, albumen length, albumen height, albumen index, yolk width, Haugh

Table 3. Effect of different levels of dietary lutein on egg quality at 41- 46 weeks of age

Parameter	Treatment					SED	p
	Control	100 ppm	200 ppm	400 ppm	800 ppm		
Egg weight (g/number)	64.05	62.76	63.05	63.26	64.01	0.437	0.845
Albumen weight (g)	40.32	39.27	39.57	39.66	40.21	0.353	0.865
Albumen weight (%)	62.90	62.53	62.74	62.65	62.77	0.22	0.987
Yolk weight (g)	16.46	16.55	16.21	16.39	16.47	0.134	0.943
Yolk weight (%)	25.74	26.40	25.72	25.95	25.78	0.204	0.811
Shell weight (g)	7.28	6.94	7.27	7.21	7.33	0.064	0.246
Shell weight (%)	11.36 ^{ab}	11.07 ^b	11.54 ^a	11.41 ^{ab}	11.45 ^{ab}	0.060	0.150
Egg width (mm)	44.31 ^{ab}	44.65 ^a	43.34 ^b	44.34 ^{ab}	44.15 ^{ab}	0.167	0.142
Egg length (mm)	57.77	57.49	57.83	57.93	57.71	0.161	0.925
Egg shape index	76.73 ^{ab}	77.70 ^a	74.96 ^b	76.56 ^{ab}	76.55 ^{ab}	0.323	0.115
Albumen width (mm)	79.54 ^{ab}	78.96 ^{ab}	75.88 ^b	79.58 ^{ab}	81.41 ^a	0.569	0.058
Albumen length (mm)	90.68	91.30	89.06	90.36	91.62	0.602	0.708
Albumen height (mm)	6.59	6.32	6.70	6.43	6.28	0.102	0.662
Albumen index	7.82	7.44	8.16	7.67	7.29	0.161	0.488
Yolk width (mm)	40.32	40.31	40.44	40.62	40.74	1.158	0.897
Yolk height (mm)	18.47 ^a	18.54 ^a	18.31 ^{ab}	18.35 ^{ab}	17.91 ^b	0.074	0.125
Yolk index	45.83 ^a	46.06 ^a	45.31 ^{ab}	45.21 ^{ab}	43.97 ^b	0.224	0.066
Haugh unit	79.02	77.83	80.38	78.13	77.17	0.665	0.607
Shell strength (kg/cm ²)	4.58	4.53	4.66	5.00	5.05	0.078	0.121
Shell thickness (µm)	385.80 ^{ab}	375.12 ^b	387.02 ^{ab}	391.78 ^a	389.92 ^a	1.949	0.072
Blunt end (µm)	308.26	376.07	387.17	390.60	388.10	2.274	0.229
Equator (µm)	387.93 ^{ab}	374.81 ^b	389.03 ^a	390.15 ^a	393.99 ^a	2.118	0.063
Sharp end (µm)	389.20	374.49	384.85	394.60	387.67	2.264	0.074

SED, standard error of difference between means; ^{a,b}Means bearing different superscript in the same row are significantly different (p<0.05).

Table 4. Effects of different levels of dietary lutein on yolk colour

Week	Yolk colour	Treatment					SED	p
		Control	100 ppm	200 ppm	400 ppm	800 ppm		
Week 41	l	60.28	60.00	59.81	60.59	59.69	0.264	0.844
	a	11.46	11.55	12.06	11.34	11.80	0.099	0.191
	b	56.44 ^b	60.36 ^a	59.14 ^{ab}	59.55 ^{ab}	59.68 ^{ab}	0.508	0.200
Week 42	l	59.77	59.52	59.22	59.81	60.00	0.256	0.918
	a	11.54 ^b	11.67 ^b	11.91 ^{ab}	11.37 ^b	12.37 ^a	0.090	0.033
	b	58.47	58.28	59.37	59.53	60.24	0.360	0.512
Week 43	l	59.99	60.01	60.40	59.33	59.49	0.296	0.828
	a	11.96 ^{ab}	11.96 ^{ab}	11.95 ^{ab}	11.22 ^b	12.46 ^a	0.163	0.290
	b	57.82 ^{ab}	58.14 ^{ab}	60.09 ^a	55.72 ^b	60.18 ^a	0.527	0.086
Week 44	l	60.06 ^{ab}	57.92 ^b	60.55 ^{ab}	60.82 ^{ab}	62.78 ^a	0.486	0.080
	a	11.37 ^{ab}	10.59 ^b	11.30 ^{ab}	11.57 ^{ab}	11.93 ^a	0.134	0.067
	b	58.54 ^{ab}	55.86 ^b	59.85 ^a	62.63 ^a	61.48 ^a	0.517	0.003
Week 45	l	59.72 ^{ab}	61.00 ^a	60.25 ^{ab}	60.77 ^{ab}	58.94 ^b	0.247	0.149
	a	11.72	12.14	11.85	11.09	11.86	0.137	0.111
	b	60.53	60.62	62.42	60.83	62.73	0.542	0.585
Week 46	l	58.00	58.52	59.50	59.02	58.44	0.251	0.455
	a	12.61 ^b	13.10 ^{ab}	13.66 ^a	13.48 ^a	13.75 ^a	0.111	0.022
	b	61.45	61.22	62.03	62.93	62.57	0.624	0.917
Week 47	l	59.03 ^a	56.40 ^b	56.98 ^b	58.09 ^{ab}	57.72 ^{ab}	0.245	0.022
	a	13.52	12.51	12.70	12.64	13.49	0.142	0.103
	b	61.47	58.85	59.66	61.63	62.53	0.581	0.366
Average	l	59.52	59.01	59.48	59.74	59.68	0.152	0.634
	a	12.00 ^b	11.86 ^b	12.21 ^{ab}	11.78 ^b	12.58 ^a	0.075	0.037
	b	59.31 ^b	58.89 ^b	60.34 ^{ab}	60.48 ^{ab}	61.33 ^a	0.268	0.068

SED, Standard error of difference between means; l, (lightness); a, redness; b, yellowness; ^{a,b}Means bearing different superscript in the same row are significantly different ($p < 0.05$).

unit and shell strength ($p > 0.05$). According to the results of this experiment, the amount of shell weight (%), egg width (mm), egg shape index, albumen width (mm), yolk height (mm) and yolk index in group fed lutein containing diets were higher than the control group. Also, lutein added to the diet could change the egg shell thickness; so the highest egg shell thickness were in the groups of 800, 400 and 200 ppm lutein, respectively. It should be noted that, in the thickness measurement of different parts of the egg shell, only the thickness of the equatorial part showed statistical difference between the groups.

The results of this experiment are in agreement with Abou-Elkhair *et al.* (2018), who stated that supplementation of 5 g/kg fennel seeds, black cumin seeds, and hot red peppers in laying hens' diet changed egg shape index and shell weight (%) but not have a significant effect on albumen weight (%) and albumen index. According to some studies, supplementation of different levels of marigold powder and heterotrophic *Chlorella* in laying hens' diet does not have significant effect on egg weight, yolk weight, and Haugh unit (Kotrbaek *et al.* 2013, Sujatha *et al.* 2015). Coh *et al.* (2013) indicated that adding 5-10% dried distillers' grains and 0.011-0.021% synthetic pigment canthaxanthin in laying hens' diet does not have significant effect on Haugh unit and shell strength. Also, there is a discrepancy between the results of this experiment

and other experimental results. Greevic *et al.* (2019) stated that supplementation of 1 and 2 g/kg marigold extract in laying hens' diets does not have a significant effect on egg width, egg shape index, but changed egg weight, albumen weight, yolk weight, albumen height and Haugh unit. It was reported that supplementation of quails' diet with 30 mg/kg piperine increased Haugh unit (Hilmi *et al.* 2015).

Yolk colour: Using lutein in laying hens' diets had a significant effect on egg yolk colour during the experiment ($p < 0.05$) (Table 4). Also, it can be seen that, while changing the amount of yolk colour, the highest values are related to some groups fed a diet containing lutein. Results obtained from this study indicated that the group fed with diet containing 800 ppm lutein produced yellower and redder egg yolk compared to other treatment groups.

Various results have been reported in many studies on the use of phytochemical products to change the egg yolk colour. A study was conducted to evaluate the effect of using 0.06% paprika extract and 0.01% marigold extract on egg quality in Japanese quail and results showed that inclusion of these feed additives can change egg yolk colour (Oliveira *et al.* 2020). Rezaei *et al.* (2019) used 20, 25, 40 ppm marigold flower and synthetic pigments for eight weeks in layer hens' diets and reported that inclusion of these feed additive in diets changed egg yolk colour. Skrivan *et al.* (2016) stated that supplementation of hens'

diet with 0, 150, 350, 550, 750 and 950 mg/kg marigold flower extract changed l, a and b amounts in egg yolk. Also, it was reported that increasing doses of marigold flower extract caused increase of DSM yolk colour fan, redness, yellowness, ratio of redness and yellowness (Skrivan *et al.* 2015).

It can be concluded that adding of lutein in layer hens' diets significantly increased egg production and numerically improved feed conversion ratio. Use of lutein increased egg yolk colour (a and b) values and egg shell thickness. The results suggested that using lutein in layer hens' diet has the potential to increase egg quality in terms of yolk pigmentation.

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