Vol.9, No.3 pages 73-83 Food Agricultural Sciences and Technology (FAST) Research Article

Influence of golden shower (*Cassia fistula* L.) petals supplementation in laying hen diet to enhance egg-yolk color and customer acceptance

Khakhanang Ratananikom[1], Thanitpan Pongjungmit[2], Kantapon Premprayoon[3], Chuleeporn Bungthong[4], Pianpan Supakot[4], Jatupat Samappito[4], Jittawan Kubola[4*]

[1] *Department of Public Health, Faculty of Science and Health Technology, Kalasin University, Kalasin, Thailand.*

[2] *Department of Animal Production, Faculty of Agricultural Technology, Kalasin University, Kalasin, Thailand*

[3] *Department of Agricultural Machinery Engineering, Faculty of Engineering, Rajamangala University of Technology Isan, Khon Kaen Campus, Khon Kaen, Thailand*

[4] *Department of Food Innovation and Processing, Faculty of Agricultural Technology, Buriram Rajabhat University, Buriram, Thailand*

[*] *Corresponding author: jittawan.kb@bru.ac.th*

Received: 5th September 2023**, Revised:** 9th November 2023**, Accepted:** 10th November 2023

Abstract - The objective of this study was to investigate the dietary supplement of golden shower (*Cassia fistula* L.) petals on egg quality and customer acceptance. Sixty Hybrid breed laying hens at 40-week old were divided into 4 treatments, with 3 replicates of 5 laying hens each. Each group was randomly taken experimental diet, supplemented with golden shower at 0, 5, 10 and 15 g/kg for 8 weeks. Intensity of yolk color increased according to golden shower supplementation. The redness score (a*) of yolk from the experimental group, fed with golden shower at 15 g/kg was significantly higher than control group (10.05±0.44 and 6.79±0.94, respectively) ($P<0.01$). This data agreed with the total carotenoid content in yolk. The highest total carotenoid content was found from the experimental group, fed with golden shower at 15 g/kg (31.86±2.19 µg/g of yolk dry weight). Moreover, the golden

Citation: Ratananikom, K., Pongjungmit, T., Premprayoon, K., Bungthong, C., Supakot, P., Samappito, J., & Kubola, J. (2023). Influence of golden shower (Cassia fistula L.) petals supplementation in laying hen diet to enhance egg-yolk. *Food Agricultural Sciences and Technology, 9*(3), 73-83.

shower supplementation did not bring negative effect on acceptance test. On the other hand, the significant higher 9-point hedonic on yolk color from the experimental group, fed with golden shower at 15 g/kg was 7.77 in total of 9.0, indicating that consumers liked yolk color of the experimental group, fed with golden shower at 15 g/kg very much when compared with control group ($P<0.01$). This study highlights the potential of golden shower petals as a natural colorant that can be beneficially introduced into animal feed to enhance yolk color and contribute satisfy consumer preference.

Keywords: Golden shower, egg yolk color, carotenoids, acceptant test

1. Introduction

In the contemporary landscape of commercial egg production, emphasis has shifted towards enhancing egg quality to align with consumer preferences. Alongside factors such as freshness, cleanliness, size, and nutritional content, yolk color has emerged as a crucial factor to consider. Consumer expectations lean towards darker yolk shades. The vibrancy of yolk hue not only influences the market perception of eggs but also resonates with the belief that a more intense yolk color signifies superior egg quality (Englmaierová *et al*., 2014). Visual assessment of egg yolk color is commonly determined by yolk color fan, developed by DSM Nutritional Products, Basel (Switzerland) which consists of 15 blades (Grashorn, 2016). According to the study from Grashon (2016), European consumers exhibit a preference for egg yolk coloration falling within the range of 9 to 14, albeit with distinctions between nations in the northern and southern regions. Specifically, countries in the southern part lean towards a predilection for richly pigmented yolks (11-14), whereas their counterparts in the northern countries tend to favor lighter hues (9-10) (Grashorn, 2016). In addition, Asian consumers preferred yolk coloration ranged from 10 to 14 (Galobart *et al*., 2004). The research conducted by Bovškova *et al*. (2014) also highlighted that eggs with yolk color scores ranging from 8 to 14, as assessed using yolk color fans, are generally favored by consumers (Bovšková *et al*., 2014).

Yolk color is predominantly influenced by the types and levels of carotenoid pigments found in their diet. However, laying hens lack the ability to produce the pigments of egg yolk internally and instead rely on storing pigments acquired through their dietary intake (Englmaierová *et al*., 2014). In other word, yolk color is dietary response. Improvement of yolk color intensity can be achieved by supplement diet with either synthetic or natural pigments (Bovšková *et al*., 2014). Synthetic pigments, however, are often imported, expensive and have been banned in some countries, such as Sweden because of their health and safety matters (Lokaewmanee *et al*., 2010). Consistent with the increasing demand for organic eggs, laying hens are fed with feedstuffs from natural origin, thus synthetic pigments such as β-apo-8-carotenoic acid ethyl ester and canthaxanthin known as Carophyll®Yellow and Carophyll®Red are not allowed (Skřivan *et al*., 2015). As a result, pigments derived from natural sources have garnered significant attention due to their lack of adverse effects on health and cost-effectiveness. Within the realm of natural pigments, carotenoids have been widely acknowledged as effective natural colorants for enhancing yolk color intensity (Spasevski *et al*., 2018). The natural sources of carotenoids that significantly contribute to the vibrant coloring of egg yolks include red peppers (Lokaewmanee *et al*., 2011), paprika (Lokaewmanee *et al*., 2010), tomatoes (Akdemir *et al*., 2012), carrots (Hammershøj *et al*., 2010), algae (Fredriksson *et al*., 2006; Ratananikom *et al*., 2019), basil (Kljak *et al*., 2021), and marigold (Sujatha *et al*., 2015).

Golden shower (*Cassia fistula* L.) belongs to the Fabaceae family. This flowering plant can be usually found in tropical countries. In Thailand, it is known as golden shower. It is a medium-sized perennial tree, flowering in from February to May. The flowering style is a hanging bouquet with many flowers with yellow petals. It is cultivated not only for ornamental reasons but also due to its nutritional and biological properties. The study from Ratananikom *et al*. (2021) indicated that golden shower contains high total carotenoid content (363.88±12.74 μg/g dry weight of sample) and its significant carotenoid pigment is β-carotene. Also, it showed high free radical scavenging activities against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals, which delay the oxidative mechanism (Ratananikom *et al*., 2021). The inspiration occurred during Songkran festival in Thailand; tons of blooming golden shower are purposely used in this ceremony and discharged as waste afterward. Turning waste into pigment source for laying hen diet to intensify yolk color might be useful in terms of zero-waste approach. In addition, using golden shower as a pigment source has not been scientifically published. Therefore, this study aimed to evaluate the effect of golden shower supplementation into laying hen diets on egg quality and customer acceptance.

2. Material and methods

2.1 Sample collection

Golden shower petals were collected from Kalasin University, Mueang District, Kalasin Province, Thailand. They were dried by a hot air oven at 60°C for 24 hours, then ground into a fine powder. The dried golden shower powder was stored in an airtight bag for further study. The golden shower petals used in this study were analyzed for the total carotenoid content according to the method described by Ratananikom *et al*. (2021). The carotenoid content found in the golden shower petals were 350.42±10.18 μg/g dry weight of sample.

2.2 Animal study

All the experimental producers involving animals were proved by the Institutional Laboratory Animal Care and Use Committee of Kalasin University (the ethics approval number: KSU-AE-004/2566). Sixty Hybrid breed laying hens at 40-week old were divided into 4 treatments, with 3 replicates of 5 laying hens each. Each group was randomly taken experimental diet, supplemented with golden shower for 8 weeks.

For the dietary formulation, the control group, which is based on a maize-soybean diet as shown in Table 1, was prepared following the guidelines outlined in Skřivan *et al*. (2015) (Skřivan *et al*., 2015). Four experimental diets were formulated by supplementing them with four different levels of golden shower, specifically at 0, 5, 10, and 15 g/kg of the diet. The nutritional information for the experimental diets can be found in Table 2.

All laying hen treatments were raised in open-housing system and each bird was individually housed in a laying cage. During the whole experiment periods, they were fed twice a day at 110 g and fully provided clean water as well as 16-hours constant light were maintained, according to method described by Lokaewmanee *et al*. (2009). Egg quality parameters were measured daily. For egg weighting, whole egg weight was measured, and yolk weight was measured after separation yolk and albumin. Albumin weight was obtained by subtracting yolk and eggshell from whole egg weight. Eggshell thickness, yolk height, and albumen height were measured by using Vernia caliper. To analyze yolk color, separated yolk was homogenized and yolk color was measured according to CIE Lab color scale. Color was expressed as the brightness (L*), redness (a*), and yellowness (b*) using Hunter Lab, model Ultra-Scan Pro (Hunter Lab, USA).

Table 1. Ingredient composition of the basal diet

Ingredients	Amount (g/100 g diet)
Fish meal	10
Corn	35
Soybean meal	15
Broken rice	15
Rice bran	15
Limestone	2
Shells	7
Premix[1]	0.5
Salt	0.5

[1] Concentrate mixture including (per kg of diet): vitamin A 32000 IU; vitamin D_3 6400 mg; vitamin E 8 mg; vitamin K_3 3 mg; vitamin B_1 4 mg; vitamin B_2 8 mg; vitamin B_6 4 mg; vitamin B_{12} 0.02 mg; nicotinamide 16 mg; pantothenic acid 4 mg; choline 400 mg; folic acid 0.4 mg; manganese 68.8 mg; iron 140 mg; cobalt 0.4 mg; copper 4 mg; zing 40 mg; methionine 80 mg.

Table 2. Nutrition values of experimental diet

Nutrition values (%)	Level of golden shower supplementation in diets (g/kg)			
	0	5	10	15
Dry matter	90.27±0.60	90.13±0.72	90.36±0.32	90.17±0.66
Ash	9.72±0.04	9.08±0.08	9.59±0.61	9.84±0.53
Crude fiber	3.11±0.11	4.38±0.09	4.41±0.25	3.62±0.36
Crude fat	2.04±0.20	2.54±0.06	2.35±0.02	2.45±0.07
Crude protein	17.15±0.58	17.18±0.01	17.18±0.01	17.17±0.01
Nitrogen free extract[1]	58.24±0.20	56.94±0.09	57.83±0.08	57.09±0.02

Data was expressed as mean ± standard deviation, n=3.
[1] Nitrogen free extract (% = 100 – (% Moisture + % ash + % crude fiber + % crude fat + % protein)

2.3 Carotenoid analysis

Yolk samples underwent desiccation through lyophilization. Carotenoids were extracted, and the total carotenoid content were assessed utilizing colorimetric method by spectrophotometer (Britton, 1995a; Britton, 1995b). A mixture of acetone and distilled water was employed to extract carotenoids from the samples, followed by the addition of hexane. This concoction was gently agitated and allowed to separate fully. The ensuing hexane layer was isolated and subjected to evaporation through a vacuum evaporator. The absorbance of the pigment extracts was gauged at 450 nm using petroleum ether as the solvent. The overall content of carotenoids was computed based on the absorption coefficient ($E^{1\%, 1cm}$) set at 2500.

2.4 Acceptant test

The consumer sensory acceptance test on yolk color was conducted in the 8th week of the experiment with 30 people and used a 9-point hedonic scale, described by Caner (2005). Each panelist was asked to evaluate the acceptability of yolk color. The rating score was explained as following;

1 means “dislike extremely”
2 means “dislike very much”
3 means “dislike moderately”
4 means “dislike slightly”
5 means “neither like nor dislike”
6 means “like slightly”
7 means “like moderately”
8 means “like very much”
9 means “like extremely”

2.5 Statistical analysis

The experimental design was completely randomized design (CRD). Data with a normal distribution were analyzed using one-way analysis of variance (ANOVA), followed by least significant difference (LSD) with a significance level of α = 0.05.

3. Results

3.1 Egg qualities

Table 3 shows egg quality results. Golden shower supplementation did not change egg qualities. Egg weight, yolk weight, albumin weight, eggshell weight, eggshell thickness, yolk height and albumin height were not significantly among treatments (P>0.05) (Table 3).

Table 3. Effect of golden shower supplementation on egg qualities

Egg qualities	Level of golden shower supplementation in diets (g/kg)			
	0	5	10	15
Egg weight (g)	53.73±2.87	53.57±2.51	55.24±1.70	54.39±1.67
Yolk weight (g)	13.37±0.54	13.22±0.82	13.85±0.58	13.90±0.64
Albumen weight (g)	35.16±2.30	35.10±2.01	35.94±1.38	35.32±1.28
Eggshell weight (g)	5.21±0.20	5.25±0.36	5.44±0.12	5.17±0.17
Eggshell thickness (mm)	0.31±0.02	0.30±0.01	0.31±0.01	0.30±0.01
Yolk height (mm)	13.88±0.10	13.99±0.09	13.00±0.08	13.84±0.12
Albumen height (mm)	7.92±0.03	7.87±0.05	7.23±0.05	7.03±0.07

Data was expressed as mean ± standard deviation.

3.2 Yolk color

Table 4 presents yolk color which was expressed as brightness (L*), redness (a*) and yellow (b*). The redness was significantly different among treatments (P<0.01), but no significant differences was observed in terms of brightness and yellowness. (P>0.05). The redness exhibited a proportional increase corresponding to the quantity of golden shower supplementation in the diet. Laying hens fed by diet with 15 g/kg of golden shower supplementation gave highest redness score as 10.05±0.44. The next lower redness scores were found from laying hens fed by diet supplemented with 10 and 5 g/kg of golden shower as 8.71±0.33 and 8.58±0.29, respectively. The control group (no golden shower supplementation in diet) gave lowest redness score as 6.79±0.94.

Table 4. Effect of golden shower supplementation on yolk color

Level of golden shower supplementation in diets (g/kg)	Hunter Lab		
	L*	a*	b*
0	72.66±0.34	6.79±0.94[c]	63.38±0.27
5	73.47±0.94	8.58±0.29[b]	64.81±0.60
10	72.67±0.77	8.71±0.33[b]	65.25±0.60
15	73.51±0.87	10.05±0.44[a]	65.74±0.79

Data was expressed as mean ± standard deviation.
Means with different letter within a column of each group are significantly different (P<0.05).

3.3 Carotenoid content in egg yolk

Total carotenoid contents were correlated with the results of yolk color (Figure 1). Total carotenoid content improved as the amount of golden shower supplementation in diet. The control group exhibited the lowest total carotenoid content as 24.66±1.76 µg/g of yolk dry weight, but the significantly highest total carotenoid content was found from the treatment with 15 g/kg of golden shower supplementation in diet as 31.86±2.19 µg/g of yolk dry weight.

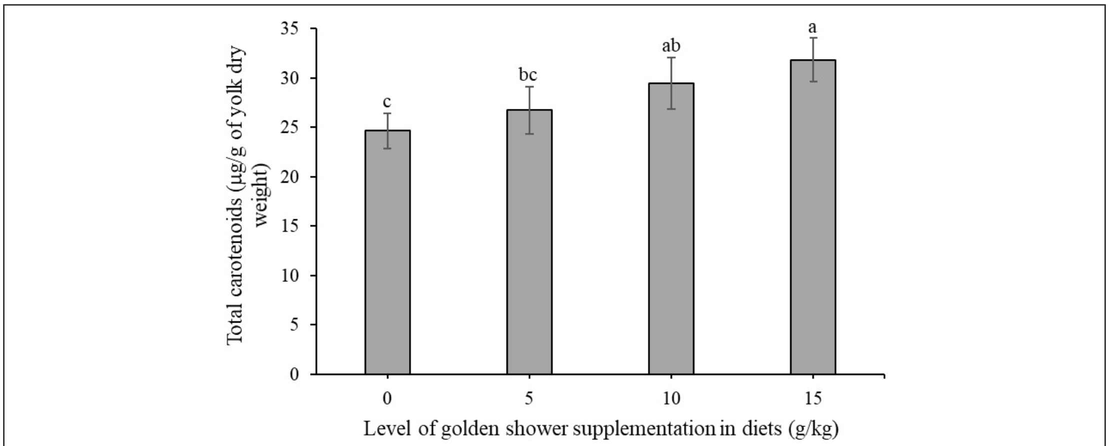

Figure 1. Total carotenoids in yolk

3.4 Consumer sensory acceptance on yolk color

Figure 2 shows results of 9-point hedonic score on yolk color. The 9-point hedonic score ranged from 6.30-7.77 from a full score of 9.00. The highest score was found from the treatment with 15 g/kg of golden shower supplementation in diet as 7.77±1.05 which meant that consumer liked yolk color very much. The score from treatment with 10 and 5 g/kg of golden shower supplementation in diet were 7.20±1.52and 6.30±1.76, respectively which meant that

consumers moderately liked and little liked color of yolk, respectively. The lowest score was found from control group as 6.30±1.76, which was classified as slight preferences.

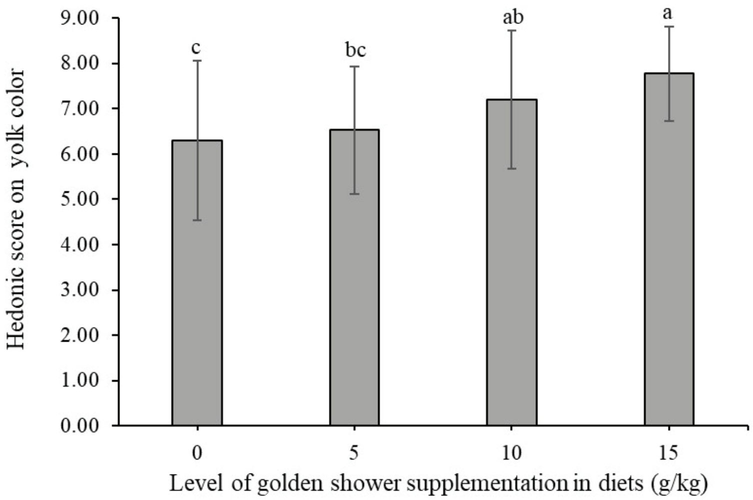

Figure 2. The 9-point hedonic score on yolk color

4. Discussion and conclusion

4.1 Egg qualities

Supplementation of golden shower in laying hen diets at 5, 10 and 15 g/kg did not bring negative feedback on egg qualities, including egg weight, yolk weight, albumin weight, eggshell weight, eggshell thickness, yolk height and albumin height. This result was probably as a result of supplementations on golden shower into feed diets did not contribute the difference in nutritive values among the experimental diets, suggesting that all experimental groups received similar nutrients for living. Therefore, egg qualities among the experimental groups were not in significantly difference. This finding agreed with the supplementation of paprika extract and mixed extract between paprika and marigold into laying hen diets. Addition of 0.1% of paprika extract and mixed extract between paprika and marigold into the laying hen diets of the 48-week-old Charoen Pokphand brown for 3 weeks had no effect on all aspects of egg quality changes, including egg weight, eggshell weight, egg yolk weight, egg white weight, and eggshell thickness, except for one factor: yolk color (Lokaewmanee *et al*., 2010). Furthermore, our finding was supported by the studies from Sukkhavanit *et al*. (2011) and Sujatha *et al*. (2015) which examined the effects of supplementation on 2 flower petals which were roselle and marigold into laying hen diets. It was found that the addition of both types of flower petals did not have any negative effects on the productive performance and quality of eggs (Sukkhavanit *et al*, 2011; Sujatha *et al*., 2015).

4.2 Yolk color and total carotenoids in yolk

A significant change was found on yolk color of laying hens fed with golden shower supplementation at 0, 5, 10 and 15 g/kg. Redness (a*) of yolk in the experimental groups with golden shower supplementation at all levels increased significantly and differed from the control group ($P<0.05$). It is noted that the a* value increased proportionally in relation to the total carotenoid content in yolk. The yolk color is directly influenced by the quantity of specific carotenoids and the proportion of yellow to red carotenoids present in the feed. Precise measurement of this color is achieved through reflective photometry, and the resulting data is expressed using the L*, a* and b* system established by the International Commission on Illumination. However, an approximate visual assessment of yolk color can be observed by a fan with 15 blades, developed by DSM Nutritional Products of Basel, Switzerland, which directly correlates with the composition and ratios of yellow and red carotenoids in the diet. The fan scores and the measured L*, a* and b* values exhibit a correlation. As the fan number increases, the brightness (L*) decreases, while the redness (a*) increases. The indicator of yellowness (b*) shows an increase up to fan number 9 and then declines. These trends align with human perception of yolk color (Grashorn, 2016)

According to the restricted metabolism, laying hens are unable to in situ synthesize pigment. They must be given pigment from diets they eat, metabolizes and accumulated in yolk afterward. Therefore, the source of diets that laying hens eat is a very important factor to intensify yolk color. Previous studies have proved that carotenoids are pigments or coloring substances that can accumulate in yolk which results in increasing color intensity of yolk. Carotenoids can be found in natural plants such as corn, pumpkin, marigold petals, peppers, blue-green algae, etc. The structure of the carotenoid is divided into two main groups; carotene and xanthophyll of which laying hens were able to absorb and use up to 20-60% of these pigments from diets to accumulate in yolk (Kilner, 2006). In other word, the increase in yolk color is influenced by carotenoid accumulation. In this study, yolk color increased corresponded to total carotenoid content in yolk which was found to increase with the level of golden shower supplementation in the diet. This data reflects that golden flower petals are a good source of carotenoids that laying hens can digest, absorb and use to accumulate in yolk. Other studies have found the same ideas as supplementation of flower petals such as roselle, marigold, safflower and nettle in laying hen diets would level up yolk intensity and carotenoid contents (Rowghani *et al*., 2006; Sukkhavanit *et al*., 2011; Loetscher *et al*., 2013; Lu *et al*; 2013; Altuntaş & Aydin, 2014; Sujatha *et al*., 2015; Kljak *et al*., 2021).

4.3 Consumer sensory acceptance on yolk color

Considering the level of consumer acceptance of yolk color resulting from the inclusion of golden shower in the laying hen diets, it was observed that the addition of golden shower supplementation did not have a negative impact on consumer acceptance. Conversely, the average acceptance score for yolk color exhibited a relative increase corresponding to the level of golden shower supplementation in the diet. The most elevated acceptance score for yolk color was noted among the laying hens that were fed with golden shower supplementation at the level of 15 g/kg was found as 7.77 out of a total of 9.00. The results indicated that supplementation of golden shower in laying hen diet made the yolk color more matched the customer needs. However, it was found that factors, including culture and tradition influent on the popularity of the yolk color form consumers. Consumers in European countries such as Germany, the Netherlands, Spain and Belgium enjoyed a 13 to 14 -point score of yolk color when measured with Roche color fans. Meanwhile, consumers in Ireland, Swedes and England often consume yolks with scores of yolk color ranging in 8-9 (Bovšková *et al*., 2014). Nevertheless, the data obtained in this study on consumer sensory acceptance of yolk color reflected that consumers prefer eggs with a non-pale yolk color.

In summary, the addition of golden shower petals to laying hen diets does not impact changes in egg quality. However, the addition of golden shower into laying hen diets, especially golden shower supplementation at 15 g/kg of feed diet can directly enhance the color intensity of the yolk and increase the accumulation of carotenoids in the yolk. In addition, improvement in yolk color intensity as a result of golden shower supplementation also enhances the consumer acceptance scores on yolk color, especially golden shower supplementation at 15 g/kg of feed diet. Therefore, golden shower petals can be applied as useful natural colorant for egg production and improve satisfy consumer preference.

5. Acknowledgement

This research was funded by Thailand Science Research and Innovation, grant number BRF 011/2564, Kalasin University.

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