

Sano (*Sesbania javanica* Miq.) Flower as a Pigment Source in Egg Yolk of Laying Hens

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Abstract

The study was conducted to investigate Sano flower that naturally contains high concentration of yellow fat soluble pigment as a pigment source for egg yolk in layer diet. Dried Sano flower (Sano) was prepared and analyzed for nutritional composition and total carotenoids content. Six dietary treatments were: 1) corn-soy basal diet that contain total carotenoids from corn at 12 mg/kg diet, 2)-4) Sano diet that contain total carotenoids from Sano at 10, 15 and 30 mg/kg diet, 5) mixed carotenoids diet that contain total carotenoids from Sano at 10 mg/kg and synthetic pigment, canthaxanthin at 2 mg/kg diet, and 6) synthetic pigment diet that contain carotenoids from canthaxanthin at 2 mg/kg diet. One hundred and eight 30-week-old hens (Isa Brown) were randomly allocated into 6 treatment groups of 3 replications. Egg production, egg weight and feed intake were recorded. Eggs collected during the last six days of the experiment were used to determine yolk color using Roche color fan scale. Results demonstrated that Sano had high total carotenoids content. The major carotenoids were β -carotene follow by lutein and β -cryptoxanthin. There was no significant difference in egg production performances ($p>0.05$). All levels of Sano gave lower yolk color score than corn ($p<0.0001$). The combination between Sano and canthaxanthin obtained highest yolk color score ($p<0.0001$). Adding 10 mg/kg of total carotenoids from Sano in carotenoids-free diet or in combination with cantaxanthin can elevate approximately one unit of Roche's color fan scale. In conclusion, Sano has beneficial effect for enhancing yolk color.

Keywords: canthaxanthin, carotenoids, egg yolk color, laying hen, pigment, Sano, *Sesbania javanica* Miq.

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บทคัดย่อ

การใช้ดอกโสน (*Sesbania javanica* Miq.) เป็นแหล่งให้สีไข่แดงในไก่ไข่

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การใช้ดอกโสน ซึ่งมีรงควัตถุสีเหลืองปริมาณสูงและสามารถละลายได้ดีในไขมันเป็นแหล่งให้สีไข่แดงในอาหารไก่ไข่ ดอกโสนแห้ง ถูกเตรียมขึ้นและนำไปวิเคราะห์คุณค่าทางอาหารและปริมาณคาโรทีนอยด์ทั้งหมด อาหารทดลองมี 6 สูตร ดังนี้ 1) อาหารพื้นฐานที่ใช้ ข้าวโพดร่วมกับกากถั่วเหลืองโดยใช้ข้าวโพดในปริมาณที่ให้สารคาโรทีนอยด์ในระดับ 12 มก./กก. 2) อาหารที่ใช้โสนในปริมาณที่ให้สารคาโรทีนอยด์ในระดับ 10 15 และ 30 มก./กก. 3) อาหารที่ใช้โสนในปริมาณที่ให้สารคาโรทีนอยด์ในระดับ 10 มก./กก. ร่วมกับแคนทาแซนทีนในระดับ 2 มก./กก. และ 4) อาหารที่ใช้แคนทาแซนทีนเพียงอย่างเดียวในระดับ 2 มก./กก. ของอาหาร ไก่ไข่พันธุ์ช่าบราวน์ อายุ 30 สัปดาห์ จำนวน 108 ตัว แบ่งออกเป็น 6 กลุ่มๆละ 3 ซ้ำ เก็บข้อมูลผลผลิตไข่ น้ำหนักไข่ และปริมาณอาหารที่กิน เก็บไข่ทั้งหมดที่ผลิตได้ในช่วง 6 วัน สุดท้ายการทดลองเพื่อใช้วัดสีไข่แดงโดยใช้พัดสีของโรช ผลการทดลองพบว่าโสนมีปริมาณคาโรทีนอยด์ทั้งหมดอยู่สูง โดยมีเบต้าคาโรทีนเป็นส่วนประกอบหลักตามด้วย ลูทีนและเบต้าคริปโตแซนทีน ไม่พบความแตกต่างของสมรรถภาพการผลิต ($p>0.05$) ทุกระดับที่ใช้โสนให้สีของไข่แดงต่ำกว่าข้าวโพด ($p<0.0001$) การใช้โสนร่วมกับแคนทาแซนทีนให้สีของไข่แดงสูงที่สุด ($p<0.0001$) การเพิ่มคาโรทีนอยด์ทั้งหมดจากโสนในปริมาณ 10 มก./กก ในอาหารที่ปราศจากคาโรทีนอยด์ หรือใช้ร่วมกับแคนทาแซนทีนทำให้สีไข่แดงเพิ่มขึ้นประมาณ 1 หน่วยของพัดสีโรช สรุปได้ว่าการใช้ดอกโสนสามารถเพิ่มสีของไข่แดง

คำสำคัญ: แคนทาแซนทีน คาโรทีนอยด์ สีของไข่แดง ไก่ไข่ รังควัตถุ โสน *Sesbania javanica* Miq.

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Introduction

Egg yolk color is regarded as an important criterion for consumers especially when it is used in Thai traditional dessert. Consumers believe that the higher intensity yolk color infer to better egg quality. Natural feed ingredients in the ration, however, are insufficient to provide desire yolk color for consumer. The intensity of yolk color from hen fed corn-soy diet is usually ranked in number 7 from Roche's color fan scale whereas number more than 10 is more preferable in Thai fresh egg market (Kijparkorn and Muangcharoen, 1988). The intensify yolk color can be achieved by supplement diet by either natural or synthetic pigment. However, synthetic pigment is banned in some country (Karunajeewa et al., 1984), consequently, natural pigments from plants, such as carotenoids, are normally used. Marusich et al. (1960), Bornstein and Bartov (1966) and Smith and Perdue (1966) showed that the carotenoids content in egg yolk is related to the carotenoids distribution in the diet. Carotenoids compose of carotenes and xanthophylls. Carotenes contain no oxygen (pure hydrocarbon), usually are in orange color. In contrast, xanthophylls have oxygen (oxycarotenoids), so they are in yellow to red color (Lathcha, 1990). Natural pigments from marigold petal and red pepper or paprika are widely used. The supplementary dosage of these natural pigments to compete with the same color value of synthetic pigment are higher, for example, marigold (yellow pigment) requires 2.8-3

times higher dosage to equally compete with synthetic pigment (Pornpotsupakit, 1979, Hernandez et al., 1999; Santos-Bocanegra et al., 2004) and capsicum annum (red pigment) requires 2.5 time in laying hens and 3 times in ducks (Kijparkorn and Muangcharoen, 1992^a; 1992^b). According to their higher dose needed to enhance equal color intensity compare to synthetic, very high inclusion rate is needed. In consequent, it could increase production cost. Therefore, blending small amount of red synthetic, canthaxanthin, with yellow natural pigment is practically used to enhance egg yolk pigment in modern animal production industry (Belyavin and Marangos, 1989)

Sano (*Sesbania javanica* Miq.) is in the Fabaceae family (alt. Leguminosae) according to the GRIN taxonomy for plant (2007) and Thai Herb Database (2007). Sano is a tropical plant which can be found widely around the ditch or canal in Thailand during rainy season. The flower is yellow and is used in several Thai recipes. The inspiration occurred when frying Sano in oil; the yellow pigment can be dissolved. This pigment might be benefit to use as a pigment source in layer diet to intensify yolk color. In addition, using of Sano as a pigment source has not been scientifically published. Therefore the objectives of this study were to investigate the composition of carotenoids in Sano and their deposition efficiency in egg yolk when using either alone or in the combination with synthetic red pigment in laying hen.

Materials and Methods

Sano flower preparation: Fresh Sano flower was bought from local market, dried in oven at 60°C for 24 hours, and ground with hammer mill. Weight of final product was 14.06 kg after dried and 13.35 kg after ground per 100 kg of fresh flower. Ground sample was analyzed: nutritional value by proximate analysis (AOAC, 1990), amino acids content by sending sample to Degussa (Thailand) for amino acids analysis, and calculated metabolizable energy by the equation of AAFCO (2000). Sample of Sano and corn were sent to Industrial Technica Pecuaría S.A. (Spain) for analyzing total carotenoids content by HPLC method according to AOAC (1984) section 43.020 (a). All analytical data were presented in Table 1 and 2 and were used to formulate experimental diets.

Table 1 Chemical composition of Sano, % DM.

Nutrients	Sano, g/100g
Crude protein	23.46
Ether extract	2.27
Crude fiber	15.05
Ash	8.47
Calcium	0.83
Total phosphorous	1.02
Calculated metabolizable energy, kcal/kg*	2,790

*AAFCO (2000)

Table 2 Chemical analysis of pigment composition in Sano and corn.

Pigment	Sano	Corn
Total carotenoids, mg/kg	307	22
<i>Chromatogram of pigment carotenoids, %</i>		
β-Carotene	63.19	14.51
β-Cryptoxanthin	16.73	21.76
cis-Lutein	5.45	inappreciable
trans-Lutein	9.69	15.19
epoxide Lutein	3.74	6.14
trans-Zeaxanthin	1.21	42.41
Canthaxanthin	not detectable	not detectable

Animal and management: One hundred and eight Isa Brown hens, which were 30-week old, were randomly allocated into 6 treatment groups of 3 replications. One replication composed of six hens that were paired and caged in three of 40x40x35 cm wire-cage and all of them shared the same feeding trough. The birds were raised on two-tier battery system in open-side housing with 16 hours of daily light. Each bird was dewormed with peperazine at the recommended dose for 3 days before starting the experiment. Hens had free access to diet and water entire experimental period.

Experimental diets: To adjust egg yolk color to the minimum scale (number 1 or 2) of Roche color fan

scale, all hens were fed with low-pigment diet, which its main ingredients were broken rice and soybean, for two weeks before the experimental period (Pornpotsupakit, 1979; Marusich and Bauernfeind, 1981). At the end of the yolk color adjusted period, all eggs were determined yolk color by RCF to ensure that yolk color was in the desired lowest number. During experimental period, hens were fed with six isocaloric and isonitrogenous diets that met or exceeded the minimum nutrient requirement according to NRC standard (NRC, 1994). Six dietary treatments were: 1) corn-soy basal diet or control diet that contain total carotenoids from corn at the level of 12 mg/kg diet, 2)-4) Sano diet that contain total carotenoids from Sano at the level of 10, 15 and 30 mg/kg diet, 5) mixed carotenoids diet that contain total carotenoids from Sano at the level of 10 mg/kg and synthetic pigment, canthaxanthin (red synthetic pigment, Carophyll red®) at the level of 2 mg/kg diet, and 6) synthetic pigment diet that contain carotenoids from canthaxanthin at the level of 2 mg/kg diet. Diet composition and calculated analysis were shown in Table 3. Birds were fed with experimental diets for 20 days.

Data collection: Egg production, egg weight, temperature and relative humidity were recorded daily. Feed consumption per replicate was recorded at the end of the experiment. Each egg collected from day 15 through day 20 were measured for yolk color in the temperature controlled room with 40 watt of fluorescence light. Eggs were broken out on a clean Petri dish (90x15 mm) with white background underneath. After broken out, yolk color was measured against Roche color fan scale (Vuilleumier, 1969), which is accepted as a standard visual measuring device (Latscha, 1990). The same person and the same room condition were used when scoring the visual color of all eggs in this experiment. The internal egg quality was not determined in this experiment because many researches showed no significant difference by dietary pigments used (Guenther et al., 1973; Hasin et al., 2006; Olson et al., 2008).

Statistical analysis: All dependent variables were performed as a completely randomized design using one-way analysis of variance (ANOVA) to determine the effects of treatments (SAS, 1996). Dependent variables were egg production, egg weight and yolk color. Significant differences of variables among treatments were determined by Duncan's New Multiple Range Test at the level of $p < 0.05$ (Steel and Torrie, 1960).

Table 3 Composition of the experimental diets and calculated analysis in DM basis, %.

Ingredients	Corn	Sano10	Sano15	Sano30	Sano10 Canth2	Canth2
Sano	-	3.25	4.9	9.8	3.25	-
Carophyll red	-	-	-	-	0.002	0.002
Corn	54.5	-	-	-	-	-
Broken rice	-	54.5	54.5	54.5	54.5	54.5
Rice bran	4.5	5.5	4.2	0.5	5.5	7.9
Soybean meal 44%	25.5	23.3	22.9	21.6	23.3	24.2
Rice bran oil	4.4	2.4	2.5	2.7	3	3
Dical-PO ₄	1.8	1.8	1.8	1.8	1.8	1.8
Oyster shell	8.2	8.1	8.1	8.0	8.1	8.2
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Premix*	0.5	0.5	0.5	0.5	0.5	0.5
Methionine	0.1	0.1	0.1	0.1	0.1	0.1
Calculated analysis, DM basis						
ME, kcal/kg feed	2903	2902	2901	2900	2902	2902
Protein, %	16.53	16.50	16.50	16.50	16.50	16.51
Lysine	0.85	0.92	0.92	0.91	0.92	0.93
Methionine	0.37	0.39	0.39	0.38	0.39	0.39
Meth + cystine	0.64	0.68	0.68	0.67	0.68	0.68
Threonine	0.62	0.65	0.65	0.65	0.62	0.65
Tryptophan**	0.23	0.23	0.23	0.17	0.23	0.24
Fat, %	7.26	3.76	3.79	3.52	3.76	3.90
Fiber, %	3.5	8.05	8.11	8.25	8.05	7.94
Calcium, %	3.6	3.61	3.62	3.62	3.61	3.63
Avg-phosphorus, %	0.46	0.44	0.44	0.44	0.44	0.44
Carotenoids, mg/kg	12	10	15	30	12	2

*Premix/kg diet contained: vitamin A 8,000 IU, D3 1,500 IU, E 8 mg, K 2 mg, B1 0.5 mg, B2 3 mg, nicotinic acid 10 mg, D-calcium pantothenate 4.5 mg, pyridoxine 1 mg, biotin, 50 µg, folic acid, 0.5 mg, B12 5 µg, choline 150 mg, Cu 5 mg, Fe 40 mg, Mn 60 mg, Mo 1 mg, Se 0.1mg, Zn, 60 mg, Co 0.5 mg, Mo 0.75 mg, I 1 mg.

**Data not include tryptophan in Sano due to no analytical data available

Results

Sano was high in crude protein and crude fiber but low in ether extract and ash (Table 1). The calculated metabolizable energy was 2,790 kcal/kg. Amino acids composition of Sano based on dry matter basis was 1.11, 0.34, 0.63, 0.88, 0.96, 0.95, 1.46, 1.17, 0.61, 0.92, 0.92, 1.19, 1.37, 4.08 and 2.08 for lysine, methionine, methionine plus cystine, threonine, arginine, isoleucine, leucine, valine, histidine, phenylalanine, glycine, serine, alanine, aspartic acid and glutamic acid, respectively. Sano had higher in total carotenoids content than corn. The major carotenoids composition in Sano was β -carotene follows by lutein, β -cryptoxanthin, and zeaxanthin whereas corn was high in zeaxanthin, β -cryptoxanthin and lutein but low in β -carotene. Moreover, Sano had lower 'trans' forms than corn (Table 2).

The average morning and afternoon temperatures were 27.58±2.43 and 34.58±1.12°C and relative humidity were 74.84±8.27 and 53.63±1.95 % for entire experimental period. There was no significant difference in egg production, egg weight, feed intake, and feed per kg egg ($p>0.05$) (Table 4). Yolk color present in mode and average value were significant difference among treatment groups (Table 5). Increased in Sano concentration, egg yolk color increased. Sano at the concentration of carotenoids 30

mg/kg showed lower yolk color than corn at 12 mg/kg. The combination of Sano at the level of 10 mg/kg with canthaxanthin at the level of 2 mg/kg gave highest yolk color score.

Discussion

Sano, compared to corn, had higher carotenoids content and its major composition was β -carotene follows by lutein and β -cryptoxanthin whereas major carotenoids content in corn was Zeaxanthin and follows by β -cryptoxanthin and lutein. In contrast, Dua et al. (1967), Krinsky, (1990) and Karunajeewa et al. (1984) reported that corn had high in lutein follow by zeaxanthin. The variation of these carotenoids content might due to the variation of corn's breed (Dua et al., 1967) and analytical techniques (Guenther et al. (1973). Kohler et al. (1967) reported that lutein, zeaxanthin, and cryptoxanthin, are hydroxyl carotenoids which found in nature, especially in green plant and in most flowers and fruits of yellow and red color. Moreover, according to Braunlich (1974), these chemicals can be produced from the oxidation of β -carotene. The carotenoids content of corn from this experiment was in the same range as reported by Scott et al (1967) and in reviewed of Karunajeewa et al. (1984), which were between 20-25 and 8-50 mg/kg, respectively.

Table 4 Effect of Sano on production performances

Observation	Corn	Sano10	Sano15	Sano30	Sano10 Canth2	Canth2	P-value
Egg production (%)	79.2±27	81.4±10	85.0±8.2	74.2±11	84.4±5.1	85.8±6.5	0.893
Egg wt (g/egg)	55.9±1.3	55.0±1.8	56.5±2.8	56.5±0.3	56.9±2.2	55.7±1.7	0.847
Feed intake (g/d)	112±11	112±3.8	112±6.7	107±3.1	111±2.3	113±3.2	0.848
Feed/kg egg	2.3±0.7	2.1±0.3	2.0±0.2	2.1±0.3	2.0±0.1	2.0±0.1	0.791

^{1/} Mean±SD

Table 5 Effect of pigment sources on egg yolk color using Roche color fan scale

Observation	Corn	Sano10	Sano15	Sano30	Sano10 canth2	Canth2	P-value
Egg Number	76	83	89	73	85	90	0.663
Egg wt (g/egg) ^{1/}	55.6±1.5	56.0±1.6	54.7±2.9	55.5±0.5	57.0±2.5	55.3±1.5	0.742
Yolk color ^{1/}							
Mode	6.3±0.6 ^c	3.3±0.6 ^e	4.7±0.6 ^d	5.3±0.6 ^d	9.0±0.0 ^a	8.0±0.0 ^b	0.0001
Average	6.2±0.4 ^c	3.4±0.4 ^f	4.4±0.2 ^e	5.6±0.2 ^d	9.8±0.3 ^a	8.5±0.2 ^b	0.0001

^{1/} Mean±SD

No significant difference in production performance was shown; this implied that all diets had the same quality although the diet composition was not analyzed. Many researchers present that the pigment supplementation had not been associated with the changes in production performance (Angeles and Scheideler, 1988; Gonzalez et al., 1999; Garcia et al., 2002; Soto-Salanova, 2003). In this experiment, the high level of Sano, however, had a trend to decreased egg production. One reason that could be used to explain this is Sano is in the Leguminosae family, which some anti-nutritive substances have been reported (Leeson and Summer, 2001). These substances are non-protein amino acids that occur in conjugated form and usually interfere with the metabolism of structurally related essential amino acids. Since this experiment was run in a relatively short period, the significant toxic effect was not shown. Thus the further studies about Sano should be concerned in this point.

Because of the average egg weight for measuring yolk color was not different, the difference in egg yolk color was considered as treatment related. Roche color fan scale, a subjective method, was selected to determine yolk color. Baiao et al. (1999) reported that pigmentation values obtained by reflectance colorimetric methods were consistent with visual value obtained by the Roche color fan and both methods yielded essentially identical results in practical application. Egg yolk color in current study changed significantly ($p < 0.0001$) according to the sources and concentration of pigment sources (Table 5). Marusich et al. (1960), and Bornstein and Bartov (1966) reported that there was linear relationship between dietary carotenoids and visual scoring of egg yolk. Sullivan and Holeman (1962) indicated that utilized xanthophyll at the level of 22-26.4 mg/kg diet was enough to provide egg yolk color. The yolk color value presented in both mode and mean values from all eggs gave nearly the same number. Sano showed beneficial result when increased the concentration of total carotenoids from 10 to 30 mg/kg diet. Egg yolk color increased about one unit of Roche color fan score for every 10 mg/kg of total carotenoids from Sano increased. However, the highest level of carotenoids from Sano (30 mg/kg) gave lower yolk color than corn-soy basal group that contained only 12 mg/kg of total carotenoids from corn. This result was due to the difference in ability of carotenoids components to deposit into hen's organs from both pigment sources (Fletcher, 1992).

Sano that gave lower egg yolk color score than corn might due to its high level of β -carotene but

low level of zeaxanthin and lutein. Ganguly et al (1953) and Williams et al. (1963) demonstrated that higher concentration of lutein and zeaxanthin from diet were found in blood, liver, ovary and egg compared to α - and β -carotene in laying hen. Smith and Perdue (1966), Gonzalez et al. (1999) and Karadas et al. (2006) reported that in egg yolk lutein has highest deposition efficiency and follow by zeaxanthin and β -cryptoxanthin, respectively. Marusich and Bauernfeind (1981) showed that zeaxanthin deposited in yolks with an efficiency of 56% whereas cryptoxanthin is poorly absorbed, it was only 10% as efficient when compared with lutein (Hamilton, 1992). Moreover, carotenoids composition in corn especially zeaxanthin is highly absorbable and present a deep orange color (Scott et al., 1967). Na et al. (2004) indicated that the absorption and accumulation of β -carotene in yolk of laying hen was poorer than zeaxanthin and lutein in maize. In contrast to zeaxanthin, most of β -carotene is converted to vitamin A in the intestinal mucosa (Cheng and Deuel, 1950; Latscha, 1990; Hencken, 1992), and small amount of unchanged β -carotene escapes and enters the bloodstream (Ganguly et al (1953). Damron et al. (1984) and Jiang et al. (1994) reported that β -carotene could deposit in yolk when it was only pigment source in the laying hen diet. As a result, Isikawa et al. (1999) found that the efficiency in yolk deposition was low even when 12 mg/kg β -carotene derived from β -carotene beadlet was added in corn-soy diet. Moreover, Hencken (1992) stated that 60-90% of carotenoids in natural feedstuffs occur in 'tran' form and had better pigmenting efficiency than 'cis' form. Therefore in present experiment that highest carotenoids level from Sano diet could not show higher yolk color score partly due to it had lower 'tran' form of carotenoids compared to corn diet. In addition to lower efficiency of β -carotene deposition in yolk and its high crude fiber, which main components are cellulose and lignin that could form an indigestible cover around carotenoids and limit their availability also are the additional reason.

For synthetic pigment, the group that use only canthaxanthin at 2 mg/kg gave higher yolk color score than all of natural pigment diets, Sano and corn, except the group that received combination between Sano and canthaxanthin. Na et al. (2004) reported that polarity of pigment can affect its absorption into the blood and accumulation into egg yolk. Thus the synthetic canthaxanthin that are more polarity gave higher egg yolk color even though it was used in the smaller amount. In addition, Hencken (1992) reported that the deposition rate of canthaxanthin was higher

than zeaxanthin did in the egg yolk (30-40% V.S. 25%). The pigments combination between yellow carotenoids from Sano at the level of 10 mg/kg and red carotenoids from canthaxanthin at the level of 2 mg/kg in present experiment showed similar yolk color result (9.8 RCF scale) as Castello and Tirado (1988) in which their layer hens were fed with yellow pigment from 40% corn in basal diet that composed of 6.92 mg/kg xanthophyll combined with the same source and level of red carotenoids for 21 days. Therefore, the natural pigment from Sano showed an additive effect on synthetic pigment to increase yolk color score for one more unit.

In conclusion, Sano had beneficial effect on increasing yolk color score. Adding 10 mg/kg of total carotenoids from Sano in carotenoids-free diet or in combination with cantaxanthin can elevate approximately one unit of Roche's color fan scale. Since Sano has high content of β -carotene, other aspects may be considered such as the role of provitamin A activity antioxidant action and immune response system. Moreover, Sano is high in protein so it should be use as a protein feedstuff in poultry diet but anti-nutritional substance need to be considered when using.

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