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Original Article

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Abstract

An experiment was conducted to investigate the effect of Roselle calyx in two preparation forms in layer diets on egg production performance, egg quality and TBARS value in plasma and yolk. Two hundred and seventy, 33-week-old, CP Brown laying hens were randomly allocated into 6 treatments with 5 replications of 9 hens each. The dietary treatments were control diet, control diet supplemented with 250 mg/kg α-tocopheryl acetate, diet containing 1 and 2% of Roselle calyx crude extracts, and diet containing 2% and 4% Roselle calyx powder. Egg production performance was recorded, egg quality was measured and TBARS value in plasma and yolk were analyzed after fed experimental diets for 4 and 8 weeks. Yolk TBARS after storage for 10 and 20 days in refrigerator and room temperature were also determined. The results demonstrated that there was no significant difference in egg production performance and egg quality and TBARS value in plasma and yolk at the 4th and 8th week among treatment groups (p>0.05). Roselle calyx tended to decrease TBARS value in yolk (p>0.05) while TBARS value in yolk significantly increased when storage times increased (p<0.01) in both storage methods. In conclusion, both forms and levels of Roselle had no adverse effect on egg production and egg quality. As an antioxidant, Roselle could not clearly show antioxidant activity. This may be due to the concentration of phenolic compound level in supplemented Roselle. Storage time was an important factor to decrease egg quality and increase TBARS value in yolk in both storage methods.

Keywords: egg production performance, egg quality, *Hibiscus sabdariffa Linn.*, laying hen, Roselle, TBARS value

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บทคัดย่อ

ผลการเสริมกลีบเลี้ยงกระเจี๊ยบแดง (*Hibiscus sabdariffa Linn.*) ในอาหารไก่ไข่ต่อสมรรถภาพการผลิตไข่ คุณภาพไข่ และค่า TBARS ในเลือดและไข่แดง

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ศึกษาผลการใช้กระเจี๊ยบแดง 2 รูปแบบ ในอาหารไก่ไข่ต่อ สมรรถภาพการผลิตไข่ คุณภาพไข่ และค่า TBARS ในพลาสม่าและไข่แดง โดยใช้ไก่ไข่พันธุ์ซีพีบราวน์อายุ 33 สัปดาห์ จำนวน 270 ตัว สุ่มไก่ไข่ออกเป็น 6 กลุ่ม กลุ่มละ 5 ซ้ำๆ ละ 9 ตัว อาหารทดลองประกอบด้วย อาหารควบคุม อาหารควบคุมที่เสริม α-tocopheryl acetateในระดับ 250 มก./กก. อาหารที่ประกอบด้วยสารสกัดหยาบกระเจี๊ยบแดงในระดับร้อยละ 1 และ 2 และอาหารที่ประกอบด้วยกระเจี๊ยบแดงผงในระดับร้อยละ 2 และ 4 เก็บข้อมูลสมรรถภาพการผลิตไข่ คุณภาพไข่ และวัดค่า TBARS ในพลาสม่าและไข่แดงหลังจากที่ไก่ได้รับอาหารทดลองเป็นเวลา 4 และ 8 สัปดาห์ รวมทั้งวัดค่าTBARS ในไข่แดงหลังจากเก็บรักษาไข่เป็นระยะเวลา 10 และ 20 วันในตู้เย็นและอุณหภูมิห้อง ผลการทดลองพบว่าไม่มีความแตกต่างทางสถิติในเรื่องสมรรถภาพการผลิตไข่ คุณภาพไข่ และค่า TBARS ในพลาสมาและไข่แดงในสัปดาห์ที่ 4 และ 8 ระหว่างกลุ่มทดลอง ($p>0.05$) กระเจี๊ยบแดงมีแนวโน้มว่าจะช่วยลดค่า TBARS ในไข่แดงที่เก็บรักษา ขณะที่ค่า TBARS ในไข่แดงเพิ่มขึ้นอย่างมีนัยสำคัญเมื่อระยะเวลาในการเก็บรักษาเพิ่มขึ้นในทั้ง 2 วิธีการเก็บรักษา ผลการทดลองสรุปได้ว่าการใช้กระเจี๊ยบแดงทั้ง 2 รูปแบบ และ 2 ระดับ ไม่ส่งผลเสียต่อผลผลิตไข่และคุณภาพไข่ กระเจี๊ยบแดงไม่สามารถแสดงผลให้เห็นเด่นชัดในการเป็นสารต้านออกซิเดชั่น อาจเนื่องมาจากระดับความเข้มข้นของสารประกอบฟีนอลิกในกระเจี๊ยบแดงที่เสริม ระยะเวลาในการเก็บรักษาเป็นปัจจัยสำคัญที่มีผลต่อการลดลงของคุณภาพไข่และการเพิ่มขึ้นของค่า TBARS ในไข่แดงในทั้ง 2 วิธีการเก็บรักษา

คำสำคัญ: สมรรถภาพการผลิตไข่ คุณภาพไข่ *Hibiscus sabdariffa Linn* ไก่ไข่ ค่าTBARS กระเจี๊ยบแดง

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Introduction

The processes on keeping fresh eggs' quality from farm to table are very important (FAO, 2003). Egg yolk that contains high level of polyunsaturated fatty acid (Naber, 1979) is easily subjected to lipid oxidation. Not only free radical are produced in the metabolic process of the hen's body but also the storage environments and time are the causes of this phenomenon. Samli et al. (2005) reported a significant interaction between storage time and temperature on egg weight loss, specific gravity, air cell size, Haugh unit, albumen height, and pH. Van Elswyk et al. (1995) and Kang et al. (1998) indicated that storage temperature, relative humidity and light intensity significantly affected lipid oxidation which resulted in egg deterioration. In Thailand, as it is located in tropical area, storage during transportation from farms to customer destination is normally in ambient temperature condition, thus it cannot maintain its freshness quality. However, some commercial companies store eggs in air-conditioned vehicle during the transportation and keep them in the refrigerator for selling later which can increase retail price of the eggs. However, other methods should be considered such as reduce lipid oxidation by enhancing antioxidative status in plasma and yolk. Phenolic compound is a natural substance occurring in plants and has been confirmed on their antioxidant activities (Harborne, 1998; Kruawan and Kangsadalampai, 2006)

Roselle (Hibiscus sabdariffa Linn.) is a shrub plant that can be cultivated in all regions of Thailand. Roselle calyx contains phenolic compounds about 20.3-21.84 g/kg dry matter (Aphirakchatsakun et al., 2008; Kijparkorn et al., 2009). Phenolic compound in Roselle calyx is composed of anthocyanin (red pigment presented in the flowers), quercetin, l-ascorbic acid and protocatechuic acid, and clearly proved as an antioxidant (Tseng et al., 2000; Wang et al., 2000; Usoh et al., 2005; Kruawan and Kangsadalampai, 2006). Botsoglou et al. (1997) used Thyme powder which was also composed of phenolic compound at the level of 2,961 mg/kg diet and found that it could protect the occurrence of lipid oxidation in egg stored at 4°C for 60 days ($p<0.01$) while Galobart et al. (2001) reported no significant differences in antioxidant activity of phenolic

compound from Rosemary extract (500-1,000 mg/kg) and vitamin E (200 mg/kg) on Thiobarbituric acid value in eggs. The difference in results due to the sources and forms of phenolic compound were used. The incorporation of Roselle calyx for its antioxidant properties has not been reported in layer. Therefore, the objective for this study was to investigate the antioxidant effect of Roselle calyx in both dried powder and crude extract forms on egg production performance, egg quality, lipid oxidation in plasma and yolk, and oxidative activity in yolk of laying hen during the storage time in both storage methods.

Materials and Methods

Preparation of Roselle calyx: Dried Roselle calyx was brought from food markets and ground through 2 mm. screen by a cutting mill. A part of dried Roselle calyx was extracted with water according to the method of Chen et al. (2003). In brief, one kilogram of Roselle calyx powder was macerated with 15 l of hot water (95°C) for 2 hours then let cooled down at room temperature and filtrated through filter paper by suction. The filtrated was dried by a rotary evaporator under a vacuum at 60°C and 80 millibar pressure. The yield of the extraction was 47.0% and stored at 4°C before granulation. To facilitate a good dispersion of Roselle calyx extract in the diet, granule was produced by using corn as a carrier. Corn was ground to the particle size of 1190 micron and blended with Roselle calyx extracts in the ratio of 9:1 (w/w) in Hobart mixer for 15 min and incubated at 60°C for 2 hours to produce crude extract Roselle granule. Both dried powder and granule of Roselle were kept in room temperature before mixing in the diet. Samples were randomly collected for analyzing nutritional contents by proximate analysis (AOAC, 1990) and total phenolic compound according to the method of Duh and Yen (1997). Metabolizable energy of both forms was calculated using ME equation (AAFCO, 2000). The analysis results were shown in Table 1.

Table 1 Chemical composition of Roselle calyx powder and granule (g/100 g DM)

Nutrient composition	Roselle calyx	
	powder	granule
Crude protein	10.1	9.4
Ether extract	1.8	3.0
Crude fiber	12.6	1.9
Ash	8.7	3.0
Calcium	1.4	1.3
Total phosphorus	0.9	0.7
Total phenolic compound, g/kg	19.8	5.6
Calculated ME, kcal/kg	2,843.9	3,491.6

Animals and Management: Two hundred and seventy CP Brown laying hens, 33 weeks-old, were randomly allocated into 6 treatments with 5 replications. Three hens were reared in a cage size 40x41x32 cm and were regarded as 3 pens for one replicate. All hens were raised in evaporative cooling house and received 16 hour light per day. Feed and water were provided *ad libitum* throughout the experimental period (8 weeks). The temperature and relative humidity were recorded every day at 08:00 am and 14:00 pm. The average temperature and relative humidity of the entire experimental period were 24.6±0.2, 26.9±0.2°C and 84.4±0.2, 63.7±3.0%, respectively. The experimental protocol was proved by the Institutional Laboratory Animal Care and Use Committee of the Faculty of Veterinary Science, Chulalongkorn University.

Feed and Feeding: The laying hens were assigned to six dietary treatments: control diet, control diet supplemented with vitamin E (α-tocopheryl acetate) at the level of 250 mg/kg, diet composed of 1 and 2% Roselle calyx crude extract (10 and 20% Roselle calyx granules) and diet composed of 2 and 4% Roselle calyx powder. Diets were calculated to meet or exceed breed recommendation. All diets were analyzed for nutritional content by proximate analysis (AOAC, 1990) and total phenolic contents were calculated based on the chemical analysis of phenolic in both Roselle calyx forms (Table 2).

Data recording: Hen-day egg production, egg weight and mortality rate were daily recorded while feed intake was collected every 4 weeks. Body weight was determined at the beginning and the end of the experiment. Egg quality, specific gravity, Haugh Unit, pH in egg yolk and albumin were measured on weeks 4 and 8.

Sample collection: At the end of the 4th and 8th week of the experiment, 15 hens (3 hens /replicate) from each treatment group were randomly selected. Blood samples were collected from wing vein and were centrifuged (Nüve, NF 800 R, USA) at speed 308.7 G for 5 min and plasma was collected and stored at -80°C until analysis. Thiobarbituric acid-reactive substance (TBARS) in plasma was measured according to the methods of Feix et al. (1991). Two sets of 15 eggs from each treatment (3 eggs/replicate) were randomly sampled and kept for the measurement of egg quality and TBARS value in yolk. During the last six days of the experiment, another two sets of 15 eggs from each treatment (3 eggs /replicate) were randomly collected. The first set was stored at the room temperature (28.8±0.3°C) and the second set was stored in refrigerator (3.5±0.3°C) for the period of 20 days. TBARS values in yolk in both storage methods were measured by the method of Kang et al (2001) every 10 consecutive days.

Statistical analysis: Statistical analysis for all dependent variables was performed as a completely randomized design using one way analysis of variance (ANOVA) to determine the effect of treatments and storage time of each storage methods. Storage methods (refrigerator vs. room temperature) were not included for statistical analysis due to their effect on egg quality proven by many previous reports. Significant differences among treatment means were compared using Duncan's New Multiple Range Test at the significant difference level of $p<0.05$.

Table 2 Ingredient composition and chemical analysis of the experimental diet

Ingredients	Amount (kg/100 kg diet)[1]					
	T1	T2	T3	T4	T5	T6
Corn	57.4	57.4	46.9	36.6	54.9	52.6
Soybean meal (48%)	24	24	24.1	24	24.3	24.2
Rice bran	6.7	6.7	6.7	6.7	6.7	6.7
Rice bran oil	-	-	0.4	0.8	0.3	0.6
Mono-dicalcium phosphate	1.5	1.5	1.5	1.5	1.5	1.5
Calcium carbonate	9.5	9.5	9.5	9.5	9.5	9.5
Salt	0.4	0.4	0.4	0.4	0.4	0.4
DL-Methionine	0.2	0.2	0.2	0.2	0.2	0.2
Choline Chloride (60%)	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin premix [2]	0.1	0.1	0.1	0.1	0.1	0.1
Trace-mineral premix [3]	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin E (α-tocopheryl acetate)		0.025				
Roselle calyx extract 1% in granule form			10			
Roselle calyx extract 2% in granule form				20		
Roselle calyx powder 2%					2	
Roselle calyx powder 4%						4
Total	100.0	100.0	100.0	100.0	100.0	100.0
Chemical analysis (% DM)						
Crude protein	18.5	18.7	18.8	18.6	18.6	18.5
Ether extract	2.7	2.4	3.3	3.6	3.7	3.6
Crude fiber	1.5	1.5	1.5	1.5	1.6	1.9
Calcium	3.3	3.5	3.4	3.3	3.6	3.7
Total phosphorus	0.7	0.8	0.7	0.7	0.8	0.8
Calculated ME, kcal/kg	3,079	3,079	3,069	3,059	3,076	3,073
Calculated total phenolic compound, g/kg	-	-	0.56	1.13	0.40	0.79

[1]T1: control diet, T2: control diet supplemented with vitamin E (α-tocopheryl acetate at the level of 250 mg/kg), T3, T4: diet composed of 1 and 2% Roselle calyx extracts, T5, T6 diet composed of 2 and 4% Roselle calyx powder
[2]Vitamin provided per kg of diet : A 12,500 IU, D3 3,000 IU,E 10 mg, K 1.5 mg, B1 2 mg,B2 5 mg, B6 3 mg,B12 0.006 mg nicotinic acid 12.5 mg, folic acid 0.5 mg
[3]Trace-mineral provided per kg of diet : biotin 0.09 mg,D-Calcium Pantothenate 9.4 mg,Mn60 mg,Zn 50mg,Fe 40 mg, Cu 10 mg, I 2 mg, Co 2 mg, Se 1 mg

Table 3 Effect of Roselle calyx on egg production performance

Treatments[1]		Egg production (%)	Egg weight (g)	Feed intake (g/hen/day)	Feed / kg egg
1-4 weeks	T1	92.38	59.74	113.11	2.05
	T2	95.24	61.56	114.26	1.95
	T3	93.10	60.97	111.39	1.96
	T4	95.00	62.05	114.63	1.94
	T5	95.16	60.98	112.78	1.94
	T6	95.29	62.02	115.24	1.97
	SEM	0.649	0.344	0.826	0.017
	P-value	0.759	0.400	0.816	0.289
5-8 weeks	T1	92.62	61.27	114.73	2.02
	T2	90.08	61.89	114.01	2.01
	T3	94.13	62.56	114.87	1.95
	T4	93.49	62.42	115.44	1.98
	T5	94.37	62.44	113.89	1.94
	T6	89.84	62.50	115.03	1.95
	SEM	1.182	0.313	0.324	0.021
	P-value	0.820	0.847	0.753	0.835

[1]T1: control diet, T2: control diet supplemented with vitamin E 250 mg/kg, T3, T4: diet composed of 1 and 2% Roselle calyx extracts, T5, T6: diet composed of 2 and 4% Roselle calyx powder

Results

Chemical analysis of Roselle calyx and diets: Nutritional composition of Roselle calyx powder and granule on dry matter basis is presented in Table 1. Roselle calyx powder had higher nutritional content except ether extract, but lower ME than granule. Total phenolic compound in Roselle calyx powder was higher than granule. Chemical analysis of nutritional value in all diets was nearly the same except ether extract and crude fiber which were high and low in Roselle calyx granule compared to powder (Table 2).

Egg performance: There was no effect of Roselle calyx in both forms and levels on hen-day of egg production, egg weight, feed intake and feed per kg egg weight (p>0.05) in both raring periods (Table 3). In addition, weight changes (ranging of 0.08-0.12 kg) and survival rate (between 91-100%) during 8 weeks

of experimental period did also not differ among treatment groups (data not shown).

Egg quality: All parameters used for evaluation of egg quality are shown in Table 4. The result demonstrated that both forms and levels of Roselle calyx did not affect egg weight, specific gravity, Hough unit, pH in yolk and albumin in both periods (p>0.05).

Lipid oxidation in plasma and egg yolk: The thiobarbituric acid reactive substance (TBARS) value expressed as nmol of malondialdehyde (MDA) concentration/ml of plasma and expressed as mg of MDA concentration/kg of yolk is presented in Table 5. The TBARS values in plasma and egg yolk were not significantly different among the treatment groups at the end of the 4th week and 8th week of the experiment. However, the diet containing Roselle tended to significantly decrease TBARS values in plasma, but this occurrence was not as much as that was observed in the control and the control with vitamin E groups.

For both storage methods, Roselle calyx tended to decrease TBARS value in yolk. However, for storage time, TBARS value was significantly increased by the increase in storage times (p<0.05). An increasing in TBARS value was higher in room temperature than refrigerator after 10 days. No interaction between treatment groups and storage times were found (Table 6).

Table 4 Effect of Roselle calyx on egg quality

Treatments[1]	Egg weight (g)	Specific gravity	Haugh Unit	pH	
				yolk	Albumin
At 4th week					
T1	59.38	1.096	77.87	6.02	9.03
T2	62.32	1.100	75.73	5.96	9.03
T3	61.64	1.100	78.07	6.02	8.95
T4	64.15	1.097	72.47	5.94	9.07
T5	62.57	1.100	77.67	5.94	8.97
T6	61.42	1.099	71.73	6.00	9.00
SEM	0.500	0.001	0.967	0.017	0.020
P-value	0.133	0.181	0.204	0.514	0.601
At 8th week					
T1	62.34	1.093	74.87	6.15	9.06
T2	63.43	1.094	72.73	6.13	9.12
T3	64.63	1.095	75.67	6.10	9.09
T4	63.90	1.094	74.20	6.10	9.15
T5	62.80	1.095	74.13	6.08	9.11
T6	64.27	1.095	72.60	6.10	9.11
SEM	0.649	0.000	0.827	0.011	0.011
P-value	0.927	0.568	0.904	0.414	0.278

[1] T1: control diet, T2: control diet supplemented with vitamin E 250 mg/kg, T3, T4: diet composed of 1% and 2% dried Roselle calyx extracts, T5, T6: diet composed of 2% and 4% dried Roselle calyx powder

Discussion

Scott et al (1982) and Leeson and Summers (2001) stated that if bird received sufficient nutrient for maintenance and production, it would maintain normal egg production capacity. In the current study, both forms and levels of Roselle calyx contained in the diets had no effect on egg production performance (Table 3), weight changes and survival rate during the experimental period. These results implied that nutrient composition of diets was in the same value although the chemical analysis of diets showed a few different in ether extract and crude fiber. Egg quality parameters were also not affected among treatment groups (Table 4). It indicated that Roselle calyx powder could be used as an ingredient up to 4% in a diet without any adverse effect on production performance and egg quality. The highest level of total phenolic compound from Roselle calyx included in the diet was 1.13 g/kg of DM in 2% of Roselle calyx extract (Table 2), which tended to perform better production performance than control diet. Phenolic compound fed to bird at the highest level in the present study, which was about 70 mg/kg of body weight, did not show any significant difference on egg production performance and egg quality. In addition, the higher level at 145 and 290 mg/kg body weight reported by Florou-Paneri et al. (2006) showed similar non- significant difference. Concerning toxicity which its value has not been reported, the level of Roselle extract fed to bird in the present study, which was about 2.3 g/bird/day or 1.25 g/kg of body weight, are safe for feed supplementation in laying hen's diet.

Phenolic compound has been regarded as a good antioxidant substance as vitamin E which was widely accepted on its effectiveness on inhibition of lipid peroxidation in biological systems (Kang, et al., 1998; Lanari et al., 2004). In addition, Kruawan and Kangsadalampai (2006) reported that Roselle calyx contained high level of phenolic compounds (210.72 mg of gallic acid equivalents/g) and exhibited high antioxidant activity which was 2220 µmol/g by ferric reducing antioxidant power (FRAP) value and 93.12

% scavenging effect by a stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), which was supported by Tseng et al. (2000), Wang et al. (2000), Tee et al. (2002), and Usoh et al. (2005). The use of Roselle calyx in the present study failed to show the effect on reducing TBARS value in plasma and yolk (Table 5). Moreover, the vitamin E supplementation group also could not show any beneficial effect. These could imply that physiological stress that causes the increase in lipid peroxidation was not high enough in this study which could be confirmed by the stability of production performance (Spinu and Degen, 1993), or the physiological stress might occur but in a very low level that natural antioxidant defense system was able to combat it. It should be noted that for positive result of anti-oxidation substances from previous researches, inducing stress conditions in animal are required.

Table 5 Effect of Roselle calyx on TBARS values in plasma and yolk

Treatments[1]	TBARS values in Plasma		TBARS values in Yolk	
	4th week	*8th week*	*4th week*	*8th week*
T1	1.87	2.15	0.48	0.62
T2	1.71	1.79	0.52	0.61
T3	1.67	1.78	0.48	0.56
T4	1.72	1.58	0.53	0.55
T5	1.77	1.58	0.53	0.57
T6	1.81	1.56	0.51	0.59
SEM	0.072	0.098	0.009	0.016
P-value	0.975	0.518	0.612	0.793

[1] T1: control diet, T2: control diet supplemented with vitamin E (α-tocopheryl acetate) at the level of 250 mg/kg, T3, T4: diet composed of 1 and 2% dried Roselle calyx extracts, T5, T6: diet composed of 2 and 4% dried Roselle calyx powder

Table 6 Effect of Roselle calyx on TBRAS value on storage egg in both storage methods

		TBRAS value, mg MDA/kg yolk	
		Refrigerator	Room temperature
Main effect			
Treatments[1]	1	0.77	0.80
	2	0.73	0.73
	3	0.72	0.74
	4	0.75	0.75
	5	0.76	0.77
	6	0.71	0.73
Storage time, d	0	0.58[c]	0.58[c]
	10	0.78[b]	0.78[b]
	20	0.86[a]	0.91[a]
SEM		0.01	0.01
CV, %		21.60	21.83
Treatment (A)		0.5182	0.3360
Storage time (B)		0.0107	<.0001
A*B		0.7169	0.6037

[1] T1: control diet, T2: control diet supplemented with vitamin E 250 mg/kg, T3, T4: diet composed of 1and 2% dried Roselle calyx extracts, T5, T6 = diet composed of 2 and 4% dried Roselle calyx powder
a, b, c Means in a column with different superscripts are significantly different among treatment groups (p<0.05)

Yolk contains around 60 ppm of iron (Cotterill et al., 1977) and the range of iron for metal-catalyzed oxidation is 1 ppb to 500 ppm in egg as reported by Labuza (1971). This could imply that egg yolk is very susceptible to lipid oxidation. No clear evidences have been found and have proved that phenolic compound can transport to egg yolk. However, there were some studies demonstrating its antioxidant effect on egg yolk. Botsoglou et al. (1997) supplemented phenolic compounds from thyme powder at the level of 2,961 mg/kg. The result showed anti-oxidative activity in yolk compared to the control groups (p<0.01) and the authors postulated that phenolic compounds could be transported through the egg. However, Galobart et al. (2001), used phenolic compounds from rosemary crude extracted at the level of 0, 500 and 1,000 mg/kg and indicated that there was no significant difference on TBARS value in fresh egg. In the present study, the highest levels of phenolic compounds in powder and extract forms of Roselle were only 784.3 and 1,130 mg/kg DM diet which was nearly the level from the report of Galobart et al. (2001), but lower than Botsoglou et al.(1997). It might indicate that the added level of phenolic compounds in this study was too low; therefore, the significant difference of TBARS value due to the supplements in egg yolk could not be demonstrated. Moreover, egg yolk contains phosvitin, iron chelator, which can inhibit Fe2+-catalyzed oxidation of phospholipid and can act as a natural antioxidant (Lu and Baker, 1986) so the effect of supplements was not shown.

Roselle calyx which only tended to decrease yolk TBARS in both storage methods was due to the too low supplementation level of phenolic compounds as mentioned above. However, for

vitamin E, which is proved to be highly transported to egg yolk, there has still been no beneficial effect shown to reducing TBARS in egg yolk. The similar reason could be applied with the supplemented level of vitamin E in the present study according to previous reported by Hayat et al. (2010) and Grobas et al. (2002).

Conclusion

Roselle calyx extract and powder at the level of 1, 2 and 2, 4 % diet which gave phenolic compound up to 1,113 mg/kg diet, had no effect on egg production and egg quality. As an antioxidant, Roselle could not clearly show antioxidant activity while storage time was an important factor to increase yolk lipid oxidation. Further studies should be done at the higher levels of phenolic compound with the induction of physiological stresses that similarly occur in commercial layer production such as high temperature and low ventilation housing.

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