Effects of Dietary Lycopene or Tomato Paste on Laying Performance and Serum Lipids in Laying Hens and on Malondialdehyde Content in Egg Yolk upon Storage

Byoung-Ki An[1], Won-Don Choo[1], Chang-Won Kang[1], Jienny Lee[2] and Kyung-Woo Lee[1]

[1] Poultry Nutrition Laboratory, Department of Animal Science and Technology, Konkuk University,
120 Neungdong-ro, Gwangjin-gu, Seoul 05029, South Korea
[2] Animal and Plant Quarantine Agency, Gimcheon-si, Gyeongsangbuk-do 39660, South Korea

The present study was undertaken to investigate the effect of dietary, synthetic lycopene or tomato paste on laying performance and egg qualities in laying hens, and on lipid oxidation of stored eggs. One hundred and sixty 38-week-old Hy-line Brown laying hens were randomly housed in cages (two birds per cage, five cages per replicate) equipped with nipples and a trough-type feeder and subjected to one of four experimental diets. Each treatment had four replicates. A corn and soybean meal base diet was added with or without either synthetic lycopene to contain 10 or 20 mg per kg of diet, or with 17 g of tomato paste per kg of diet. The feeding trial lasted four weeks. Feed intake did not differ between dietary treatments. Laying hens fed diets containing lycopene or tomato paste laid lighter eggs ($P<0.05$) compared with those fed on the control diet. Egg production was higher ($P<0.05$) in tomato paste-fed layers, but lower ($P<0.05$) in those fed on a diet containing 20 mg/kg of lycopene compared with the control diet-fed counterparts. Dietary lycopene did not affect egg quality, except for yolk color, nor did serum lipid profiles. Malondialdehyde (MDA) content in serum samples and eggs that had been stored at 24℃ for four weeks was reduced ($P<0.05$) by lycopene or tomato paste. Adding lycopene or tomato paste into a diet of laying hens increased the incorporation of lycopene into the liver and egg yolk. Collectively, the present study shows that addition of low levels of lycopene or tomato paste into the layers' diet is an effective nutritional strategy to enhance oxidative stability of fresh eggs.

Key words: laying hens, lycopene, malondialdehyde, serum lipids, tomato paste

Introduction

Lycopene (LP) is a red pigment that is a naturally present carotenoid in fruits and vegetables. Tomatoes are known to be the major LP source with a content of 3,100−8,600 μg per 100 g of tomatoes or their products (Stahl and Sies, 1996). The most well-known biological effect of LP is its action as a cholesterol-lowering or antioxidant agent (Di Mascio et al., 1989), thus preventing the oxidation of low-density lipoprotein, associated with decreasing the risk of atherosclerosis and coronary heart disease in humans (Agarwal and Rao, 1998; Fuhrman et al., 2000; Palozza et al., 2012; Muller et al., 2016). In studies with chickens, dietary LP has been shown to improve meat quality via inhibiting lipid peroxidation and through lowering serum lipids (Leal et al., 1999; Botsoglou et al., 2004; Sahin et al., 2006a, b; Bou et al., 2009; Sun et al., 2014a; Sun et al., 2015).

In the regression analysis reported by Olsen et al. (2008), the quadratic pattern of LP incorporation into eggs from laying hens was noted, and the optimal dietary lycopene occurred at the level of 420 mg LP/kg of diet. Sun et al. (2014a) reported that dietary LP with increasing levels up to 80 mg/kg within the diet linearly increased LP contents in the serum, liver, and egg yolk of breeding hens. Transfer efficiency of dietary LP into egg yolk has been found to range from 0.13% to 4.5% (Kang et al., 2003; Olsen et al., 2008, Rotolo et al., 2010). Finally, it is known that LP incorporation into the eggs occurs immediately after feeding an LP-added diet to laying hens and the LP concentration in eggs being kept constant during the feeding trial (Sun et al., 2014a).

Previous studies focused on the impact of LP on the blood lipid profiles, hosts' antioxidant systems or oxidative sta-

bility of poultry meats (Dotas *et al*., 1999; Olsen *et al*., 2008; Sahin *et al*., 2008; Akdemir *et al*., 2012; Sun *et al*., 2014a). However, there is a lack of research into the oxidative stability of eggs under long-term storage conditions. In addition, the effect of low levels of LP on laying performance, egg qualities, blood lipids, and lycopene levels in tissue and eggs has not been formerly tested, which prompted us to design the current study. In this study, synthetic LP or tomato paste (TP) was added into the diet of laying hens to reach 5, 10 or 20 mg LP per kg of diet to evaluate the aforementioned LP effects. Recently, we confirmed that dietary LP or TP was deposited into the serum and liver and prevented the copper-mediated oxidation of low density lipoprotein (LDL) in broiler chickens (Lee *et al*., 2016).

Materials and Methods

Animals, Diets, and Experimental Design

One hundred and sixty 38-week-old Hy-line Brown laying hens were purchased from a local laying farm and randomly housed into two-tier battery cages, with two birds per cage, equipped with nipples and a trough-type feeder. Upon arrival, they were adapted to the new facility for two weeks and fed a corn and soybean meal based diet (Table 1). Following the two-week adaptation period, they were randomly subjected to one of four dietary treatments. We confirmed that there was no difference in egg production rate between the four treatment groups at the initiation of the experiment, of which we used the routine management procedure used in experiments with laying hens (data not shown). In addition, all layers laid during the experimental period. Each treatment had four replicates with ten birds per replicate (two birds per cage, five cages per replicate). A corn and soybean meal base diet was used as a control diet (Table 1). To formulate the experimental diets, the control diet was added to either synthetic LP (DSM Nutritional Products Inc., Basel, Switzerland) to reach 10 (LP10) or 20 mg (LP20) per kg of diet, or with 17 g of TP (TP17) (Heinz) per kg of diet, combined with an equal amount of the control diet. TP contained 300 mg of lycopene per kg according to the manufacturer's specification (Lee *et al*., 2016). Experimental diets were prepared weekly and stored in an air-tight plastic bag at 4℃. Feed and water were provided *ad libitum* and the feeding trial lasted four weeks. The lighting program consisted of a 17 h light and 7 h dark cycle. The temperature of the facility was maintained at 20℃ during the experimental period. All experimental protocols were approved by the Animal Care Committee of Konkuk University.

Sampling

Feed intake was measured on a weekly basis. Eggs were collected daily and weighed. Egg production was calculated by dividing total eggs laid by laying hens, and only intact eggs were used to calculate average egg weight. During the last three days of the experiment, intact eggs were collected, stored in a cool room and randomly re-grouped into two sets of five eggs per replicate. The first egg set was used to analyze egg quality on the day of sampling. The second egg set was stored at 24℃ in an incubator for four weeks and

Table 1. **Ingredients and composition of the basal diet**

Ingredients	g/100 g
Corn	61.96
Dehulled lupin	3.00
Dehulled soybean meal	0.30
Soybean meal	18.00
Rapeseed meal	1.50
Corn gluten meal	2.00
Fish meal	0.70
Yellow grease	2.20
Limestone	9.10
Tricalcium phosphate	0.60
Salt	0.27
DL-methionine	0.11
Choline-Chloride	0.07
Vitamin and mineral premix[1]	0.16
Phytase	0.03
Total	100.00
Calculated nutrient content	
Crude protein[2], %	16.45
Crude fat, %	4.76
Crude fiber, %	3.46
Crude ash, %	12.22
Ca, %	3.93
Available P, %	0.38
AMEn, Kcal/kg	2,852

[1] Vitamin and mineral premix provided following nutrients per g of diet: Vit. A, 18,000 IU; Vit. D3, 5,000 IU; Vit. E, 40 mg; Vit. K3, 3.0 mg; Vit. B$_1$, 1.0 mg; Vit. B$_2$, 10 mg; B$_6$, 3 mg; Vit. B$_{12}$, 0.020 mg; Niacin, 132.0 mg; Biotin, 0.12 mg; Folacin, 1.0 mg; Pantothenic acid, 14.0 mg, Fe, 72 mg; Mn, 90 mg; Zn, 74 mg; I, 1.8 mg; Se, 0.36 mg; Cu, 4.8 mg.
[2] Analyzed crude protein value.

used to measure malondialdehyde (MDA) contents.

At the end of the experiment, two birds per replicate were euthanized by overdose of carbon dioxide, and sampled for blood. Sera were obtained by gentle centrifugation at 1,700 *g* and stored at −20℃ until later use. Immediately after blood sampling, birds were eviscerated and their liver was sampled, weighed, and stored at −70℃ until analysis.

Measurement of Egg Quality

Eggshell strength and shell thickness without shell membrane was measured using a strength meter and gauge (FHK, Fujihira Ltd., Tokyo, Japan). Average shell thickness was calculated by measuring three spots on the eggshell at the blunt and point ends, and the equator. Yolk color was visually scored using the DSM yolk color fan (DSM Nutritional Products, Basel, Switzerland), on a 1−15 scale, ranging from 1 (light yellow) to 15 (deep orange). Albumen height was measured by an egg multi tester (Technical Services and Supplies Ltd., York, England) and used to calculate Haugh unit using the formula as described by Roush (1981).

Measurements of Total Cholesterol, Triglyceride, and Lipoproteins in Serum Samples

Total lipid and cholesterol, triglyceride, and high-density

lipoprotein (HDL) cholesterol in serum samples were analyzed using an automatic blood analyzer (Hitachi 7600−110, 7170).

Measurement of Malondialdehyde Contents in Sera and Egg Yolks

Lipid peroxidation in serum samples, as well as in eggs which had been stored at 24℃ in the incubator for four weeks, was estimated by measuring the MDA level as described by Buege and Aust (1978), with minor modifications. In brief, 500 μl of serum samples were added to 2 ml of thiobarbituric acid/thiobarbituric acid reagent (15% [w/v] trichloroacetic acid, 0.375% [w/v] thiobarbituric acid in 0.25 N hydrochloric acid), incubated at 60℃ for 15 min, cooled in the refrigerator and centrifuged at 1,000 g for 10 min. The supernatant was then removed, the absorbance was read at 532 nm, and the TBA solution was used as a blank. For the MDA measurement in eggs, egg yolk was obtained by gentle suction using a disposable syringe and subsequently homogenized. One gram of homogenized egg yolk was mixed with 10 ml of thiobarbituric acid/thiobarbituric acid reagent and steps followed as described above. The MDA concentration was determined by the specific absorbance coefficient (1.56 $\times 10^5 \mu$mol cm^{-3}). The produced MDA is expressed at nM per ml of serum sample or gram of fresh yolk.

LP Measurement in Liver and Egg Yolk

LP contents in liver and egg yolk were extracted using the method of Boileau $et\ al.$ (2000) and analyzed by the method of Wei $et\ al.$ (2001) using high performance liquid chromatography (HPLC). In brief, approximately 0.1 g of liver tissue or egg yolk was homogenized thoroughly, dissolved in 6 ml of a potassium hydroxide/ethanol (1:5) solution containing 1 g/l butylated hydroxytoluene (BHT) and saponified at 60℃ for 30 min. LP was extracted twice under yellow light using equal volumes of hexane (6 ml) plus 2 ml of distilled water. The extracts were dried and stored at −20℃

for no longer than 2 d before LP measurement by HPLC. The HPLC system included Waters 510 pumps, a Waters 717 plus auto sampler, a Waters 486 Tunable Absorbance detector, and Waters Nova-Pak (5 μm, 3.9 cm$\times$300 mm) C18 column. The mobile phase was methanol:acetonitrile:chlorform (47:47:6, v/v/v) and the flow rate was 1.0 ml/min. The HPLC was controlled by Waters Millennium chromatography software and the lycopene peak was monitored at 472 nm. LP concentration was calculated using a calibration curve prepared with the pure LP standard (L-9879, Sigma Co., St. Louis, MO, USA).

Statistical Analysis

Replicates were considered as an experimental unit. Data obtained in this study was evaluated by one-way analysis of variance using the general linear model procedure of SAS (SAS, 2002). If the F-test for treatment effect was significant, differences between treatment means were determined using the Duncan's multiple range test (Duncan, 1955). Differences were considered significant at a value of $P < 0.05$.

Results

Feed intake was not affected by dietary treatment (Table 2). Laying hens fed LP or TP diets laid lighter ($P < 0.05$) eggs compared with those fed the control diet. Egg production was higher ($P < 0.05$) in TP-fed laying hens, but lower ($P < 0.05$) in those fed a LP20 diet compared with the control diet-fed counterparts. Egg qualities, i.e., eggshell strength, eggshell thickness, and Haugh unit were not affected ($P > 0.05$) by dietary treatments (Table 3). However, dietary LP, but not TP, intensified ($P < 0.05$) yolk color, compared with the control group. No serum lipids were altered by the dietary treatments (Table 4). The MDA contents in serum samples decreased ($P < 0.05$) in both LP20 and TP17 groups, compared with the control group (Fig. 1A). When eggs were stored at 24℃ for four weeks, the MDA

Table 2. **Effect of dietary lycopene or tomato paste on laying performance in laying hens[1,2]**

Items	Control	LP10	LP20	TP17
Feed intake, g/d/bird	118.9±1.83	116.7±2.89	116.8±1.08	120.1±0.98
Egg weight, g	65.9±0.05[a]	63.3±0.27[b]	63.6±0.36[b]	63.1±0.24[b]
Egg production, %	88.9±0.44[b]	86.7±0.93[bc]	85.2±0.94[c]	92.4±0.76[a]

[1] LP10＝lycopene at 10 mg/kg of diet; LP20＝lycopene at 20 mg/kg of diet; TP17＝tomato paste at 17 g/kg of diet.
[a-c] Mean±SE (N＝4/treatment) within the same row with no common superscripts differ significantly ($P < 0.05$).

Table 3. **Effect of dietary lycopene or tomato paste on egg quality in laying hens[1,2]**

Items	Control	LP10	LP20	TP17
Eggshell strength, kg/cm^2	4.54±0.23	4.74±0.52	4.50±0.29	4.39±0.31
Eggshell thickness, mm/100	35.6±1.07	35.9±1.03	35.0±1.04	35.4±0.98
Haugh unit	82.4±1.07	81.2±1.15	82.4±1.53	83.2±0.94
Yolk color	7.56±0.20[b]	9.44±0.30[a]	10.03±0.16[a]	8.08±0.23[b]

[1] LP10＝lycopene at 10 mg/kg of diet; LP20＝lycopene at 20 mg/kg of diet; TP17＝tomato paste at 17 g/kg of die.
[a,b] Mean±SE (N＝4/treatment) within the same row with no common superscripts differ significantly ($P < 0.05$).

Table 4.　**Effect of dietary lycopene or tomato paste on serum lipid contents in laying hens[1,2]**

Items	Control	LP10	LP20	TP17
Total lipid, mg/dl	975.1±76.5	1291.4±142.6	974.5±96.5	1218.6±97.2
Triglyceride, mg/dl	617.3±84.9	862.1±131.0	636.9±87.7	936.3±124.1
Total cholesterol, mg/dl	85.8±4.3	99.1±8.3	82.3±4.2	96.9±9.5
HDL cholesterol, mg/dl	5.6±0.7	5.0±0.9	6.6±0.8	7.1±1.3

[1] LP10＝lycopene at 10 mg/kg of diet; LP20＝lycopene at 20 mg/kg of diet; TP17＝tomato paste at 17 g/kg of diet.
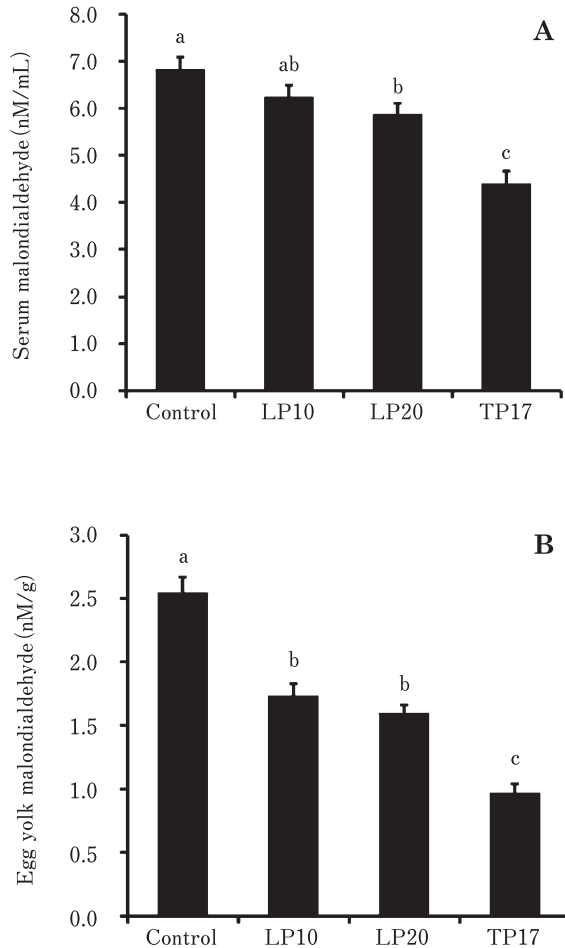[2] Values are mean±SE (N＝4/treatment).

Fig. 1.　**Malondialdehyde content within the serum sample (upper panel A) or egg yolk (lower panel B) of laying hens fed diets containing lycopene at 10 (LP10) or 20 (LP20) mg/kg diet, or tomato paste (TP17) at 17 g/kg of diet.** Each bar represents the mean±SE value (N＝4/treatment). Bars not sharing the same letter are significantly different according to the Duncan's multiple range test (*P*＜0.05).

5).　As expected, LP was not detected in egg yolks and livers of the control diet-fed laying hens (Table 5).

Discussion

LP is a carotenoid that represents the red coloring of tomatoes and its products, and is known to have strong antioxidant effects. The latter biological LP property led us to test whether LP or tomato-related products incorporated into the diet of chickens could affect lipid metabolism and the oxidative stability of meats (Lee *et al*., 2016). In this study, we further explored whether dietary LP or TP could affect lipid peroxidation of eggs under long-term storage conditions. However, it should be noted that our study is not intended to propagate the storage of raw eggs at room temperature, although consumers in Asian (Koppel *et al*., 2014) or European (Koppel *et al*., 2015) countries might buy eggs stored at room temperature from various sources such as farmers, local markets, or stores. It has been reported that egg quality influences consumer acceptance and this decreases as the storage time at room temperature increases (Liu *et al*., 2017). Here, long-term storage of eggs at room temperature in our study and elsewhere (Mohiti-Asli *et al*., 2008) was designed to confirm the presence of diet-origin antioxidants, which may have been incorporated into the egg yolk.

In this study, both egg weight and egg production was significantly lower in the LP20 group compared with the control group. TP-fed laying hens laid lighter eggs but produced more eggs than the control group. The effect of LP or tomato-related products on laying performance has been at best, inconsistent. For example, no effect of dietary LP on laying performance in Chinese native breeding hens (Sun *et al*., 2014a) or commercial hybrid laying hens (Kim *et al*., 2008; Rotolo *et al*., 2010) was reported. However, Sahin *et al*. (2008) and Akdemir *et al*. (2012) noted that dietary LP or tomato-related products increased feed intake and egg production with the increasing LP supplementation in Japanese quails or laying hens. Sahin *et al*. (2006b) observed that dietary LP at 100 ppm did not affect feed intake and egg weight, but increased egg production in Japanese quails. Egg qualities, i.e., eggshell strength, thickness, or Haugh unit were not affected by dietary treatments as reported previously (Kim *et al*., 2008; Rotolo *et al*., 2010; Akdemir *et al*., 2012). In contrast to our findings, dietary LP at 100 or 200 ppm linearly increased Haugh unit in quails (Sahin *et al*., 2008). Here we confirmed the well-established LP effect on

contents (*P*＜0.05) in laying hens fed LP or TP-added diets was significantly lower (*P*＜0.05), compared with those fed the control diet (Fig. 1B). Dietary supplementation of LP or TP increased LP contents in both liver and egg yolks (Table

Table 5.　**Lycopene contents in egg yolk and liver of laying hens fed diets containing lycopene or tomato paste**[1,2]

Items	Control	LP10	LP20	TP17
Egg yolk, μg/g	ND	0.80 ± 0.08^a	0.88 ± 0.06^a	0.43 ± 0.05^b
Liver, μg/g	ND	3.64 ± 0.35^b	6.46 ± 0.84^a	0.70 ± 0.09^c

[1] LP10＝lycopene at 10 mg/kg of diet; LP20＝lycopene at 20 mg/kg of diet; TP17＝tomato paste at 17 g/kg of diet; ND＝not detected.
[a–c] Mean±SE (N＝4/treatment) within the same row not sharing a common superscript differ significantly ($P<0.05$).

intensifying yolk color, which is the consequence of direct incorporation of LP into the egg yolk, as seen in this study and reported elsewhere (Kim *et al*., 2008; Sahin *et al*., 2008; Rotolo *et al*., 2010; Akdemir *et al*., 2012; Sun *et al*., 2014a).

It is known that LP-enriched eggs were inversely associated with a decrease in MDA contents in egg yolks (Sahin *et al*., 2008) due to the role of LP as a natural antioxidant (Di Mascio *et al*., 1989). Similarly, we found that a decreased serum or egg yolk MDA content was observed in both LP20 and TP17 groups, compared with the control group. It is of note that TP was more effective than LP in lowering the MDA content within serum or stored eggs. This superior TP effect could be linked to the presence of additional natural antioxidants such as vitamin C, vitamin E, lutein, β-carotene, or zeaxanthin in TP (Gama *et al*., 2006; Lee *et al*., 2016), which potentiates its antioxidant property. Similarly, it was reported that tomato-fed laying hens contained significant amounts of carotenoids and vitamins in their serum and egg yolk, with concomitant reduction in MDA contents compared with the control group (Akdemir *et al*., 2012).

In this study, dietary LP or TP did not affect serum lipids, of which our observation is in line with the previous finding by Sevcikova *et al*. (2008). In contrast to our finding, a lipid lowering effect by dietary LP was reported. For example, dietary LP at levels of 50 to 200 ppm decreased triglyceride and total cholesterol concentrations in serum samples (Sahin *et al*., 2006a) and total cholesterol concentrations both in serum and egg yolk (Sahin *et al*., 2006b) in quails. Sun *et al*. (2014b) reported that dietary LP in the range 20 to 80 mg/kg diet lowered serum total cholesterol, but not triglyceride, in breeding hens. The explanation for the lack of LP or TP effect on serum lipids is not readily available. It is likely that the relatively short period of feeding, or fluctuation pattern of blood lipids may in part explain the lack of an effect. Indeed, Sun *et al*. (2014a) found an age-dependent effect of LP on cholesterol, i.e., dietary LP lowered serum total and HDL cholesterols at 35 days, but not at 21 or 28 days in breeders.

Earlier studies showed the transfer efficiency of dietary LP into egg yolk ranged from 0.13 to 4.5% (Kang *et al*., 2003; Olsen *et al*., 2008, Rotolo *et al*., 2010). In this study, if egg yolk is assumed to be 23.5% of an egg, then the transfer efficiency of diet-origin LP (5 to 20 mg/kg diet) into the egg yolk can be calculated to range from 0.56 to 1.06%, of which values are within the reported ranges.

Based on our findings (i.e., laying performance, egg qual-

ities, serum lipid contents, serum MDA, and egg yolk MDA), no clear difference in laying hens fed either 10 or 20 mg lycopene was noted, indicating no additive LP effect. It is not clear why laying hens fed 20 mg LP/kg did not exhibit superior performance or egg quality compared with those fed 10 mg/kg. Conversely, a dose-dependent LP effect was observed in terms of liver LP contents, in that this was higher in laying hens fed 20 mg/kg than those fed 10 mg/kg LP. Higher LP transfer into the liver is considered beneficial, as a higher systemic LP availability would improve the antioxidant capacity of laying hens under stress.

In conclusion, dietary LP and TP reduced egg weight, but increased yolk color, thus can be associated with a direct diet-origin LP incorporation into the egg yolk. The decreased MDA contents within the serum and egg yolk was noted in LP and TP groups, confirming the antioxidant characteristics of layers within these groups. It seems that an inclusion of LP equal or less than 20 mg/kg diet can be considered as a beneficial nutritional strategy to improve the oxidative stability of fresh eggs.

Acknowledgments

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