Journal of Bioscience and Bioengineering
VOL. 120 No. 5, 539–543, 2015

ELSEVIER

www.elsevier.com/locate/jbiosc

Effects of recombinant lycopene dietary supplement on the egg quality and blood characteristics of laying quails

Wei-Ting Hsu,[1] Chung-Jen Chiang,[2] Yun-Peng Chao,[3,4,5] Chi-Huan Chang,[1] Li-Jen Lin,[5] Bi Yu,[1] and Tzu-Tai Lee[1,*]

Department of Animal Science, National Chung Hsing University, 250 Kuo Kuang Road, Taichung 402, Taiwan,[1] Department of Medical Laboratory Science and Biotechnology, China Medical University, Taichung 404, Taiwan,[2] Department of Chemical Engineering, Feng Chia University, Taichung 40724, Taiwan,[3] Department of Health and Nutrition Biotechnology, Asia University, Taichung 41354, Taiwan,[4] and School of Chinese Medicine, China Medical University, Taichung 404, Taiwan[5]

Received 14 January 2015; accepted 16 March 2015
Available online 19 June 2015

This study was conducted to determine the effect of dietary supplement of bacterial lycopene (BL) produced by *Escherichia coli* on the egg quality and blood characteristics of laying quails. The antioxidant activity measurement showed that BL exhibited 100% 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging capacity at a concentration of 4.65 μg/ml, which was more effective than butylated hydroxytoluene (BHT) and commercial lycopene (CL). Moreover, seven dietary groups of laying quails consisting of 10 100-day-old quails (*Coturnix coturnix japonica*) each were fed with the basal diet supplemented with BL, CL or canthaxanthin (CA) for 4 weeks. Consequently, the triglyceride content of yolk was significantly lower in the group with BL and CL supplement. The serum malondialdehyde (MDA) level of the BL- and CA-supplemented groups at 18 mg/kg was lower than the control group. In conclusion, BL has a high antioxidant activity and is promising as a feed additive in the diet of laying quails.

[**Key words:** Recombinant lycopene; Laying quail; Antioxidant activity; Feed additive; Egg quality]

Free radicals can snatch electrons with non-radicals and surrounding molecules to produce more free radicals (1). This chain reaction eventually causes a damage to proteins, which in turn leads to the serious aging and pathological changes in the organism (2). Under the physiological condition, living organisms normally produces reactive oxygen species (ROS). ROS are removed by the *in vivo* antioxidant system. However, free radicals result in oxidative damage if excessive ROS accumulate (3). Maintaining a proper balance of free radicals *in vivo* appears to be important for the organism's health (4).

First separated from *Tamus communis* L, lycopene is one of carotenoids and commonly found in fruits and vegetables (5). Some carotenoids have cyclic structures and are the precursor for the synthesis of vitamin A (6). Lycopene has a straight-chain structure with 11 consecutive conjugated double bonds, making it effective to bind with electrons or free radicals (7). Its strong antioxidant capacity elicits an inhibitory effect on low-density lipoprotein (LDL) oxidation and HMG-CoA reductase activity, affects cholesterol biosynthesis, and reduces blood triglyceride and cholesterol levels in human (7,8). Moreover, rats fed with lycopene exhibited a reduction in the serum malondialdehyde (MDA) content while their lipid peroxidation and oxidized LDL generation were inhibited (9–11). The serum MDA content was also largely reduced in the lycopene-fed birds (12). The quails supplemented with lycopene and vitamin E showed a higher antioxidant capacity (13,14). In addition, canthaxanthin (CA) is a phytochemical and has various physiological functions, such as pigmentation and antioxidation (15). Like lycopene, it has been applied in poultry industry (16).

We have reported the lycopene production in *Escherichia coli* (17). However, little information is available regarding the effect of recombinant lycopene in quail diets. This issue was addressed by using recombinant lycopene as a supplement in the quail diet. A systematic investigation was conducted by characterizing the blood lipid traits and serum antioxidant enzymes in the quails, as well as the lipid content of their egg yolks. To our best knowledge, this is the first study reporting the usefulness of recombinant lycopene as a dietary supplement for the poultry.

MATERIALS AND METHODS

Recombinant lycopene production Recombinant lycopene was produced by *E. coli* as per our reported methods (17) (Fig. 1). In brief, the lycopene-producing *E. coli* strain BL21-CrtD1 was grown in shake flasks containing Luria–Bertani medium with 0.4% glucose. The bacterial growth was followed by measuring at 600 nm (OD_{600}). With an initial cell density of 0.1 at OD_{600}, the producer strain was incubated at 37°C and induced by adding 20 μM of IPTG upon reaching 0.4 at OD_{600}. Bacterial cells were cultured for 36 h and then harvested by centrifugation. To determine the lycopene content, cells were rinsed with saline water and then immersed in 1 ml acetone at 55°C in the dark for 10 min, followed by centrifugation at 10,000 ×*g* for 10 min. The supernatant was recovered and measured at 470 nm with commercial lycopene (CL) (Sigma, USA) used as a standard. Meanwhile, harvested cells were washed twice and resuspended in 0.1 M phosphate buffer (pH 7.5). Cells were subsequently frozen at −50°C for 72 h and stored at −20°C until use.

* Corresponding author. Tel.: +886 4 22840366; fax: +886 4 22860265.
E-mail address: ttlee@dragon.nchu.edu.tw (T.-T. Lee).

1389-1723/$ – see front matter
http://dx.doi.org/10.1016/j.jbiosc.2015.03.016

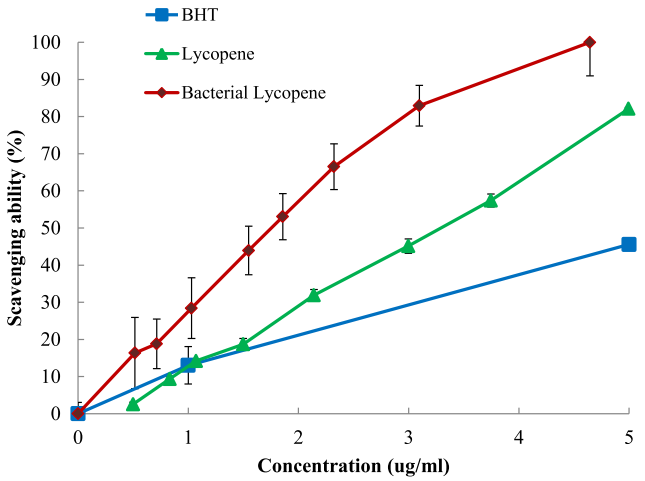

FIG. 1. DPPH free radicals scavenging capacity of bacterial lycopene (BL), lycopene and BHT. Each value represent Mean ± SD (n = 5).

Antioxidant activity assay The free radical scavenging activity was measured according to the reported method (18). In short, 0.1 mM solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in ethanol was prepared and added to the samples at different concentrations. After 30 min, the absorbance was measured at 517 nm and compared against the control, butylated hydroxytoluene (BHT). A lower absorbance in the reaction mixture indicated a higher level of free radical scavenging activity; it was calculated using the following equation:

$$\text{DPPH scavenging ability}\,(\%) = 100 - [(A0 - A1/A0) \times 100] \quad (1)$$

where A0 is the absorbance of the control reaction and A1 is the absorbance in the presence of bacterial lycopene (BL) and CL.

The reductive capability of BL and CL was quantified using Oyaizu's method (19). Briefly, BL and CL were mixed with phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (1.0%). The mixture was incubated at 50°C for 30 min, and the reaction terminated by adding a portion of trichloroacetic acid (10.0%). The upper layer of the solution was mixed with $FeCl_3$ (0.1%), and the absorbance was measured at 700 nm in a spectrophotometer, using ascorbic acid as a control. Increased absorbance of the reaction mixture indicated greater reducing power.

Laying quails, management and egg sample collection A total of 70 100-day-old laying quails (*Coturnix coturnix japonica*) were randomly allocated into 7 dietary groups, each containing 10 birds, including 5 replicates per group and 2 birds per replicate. Over a 4-week period, each group, including the control group (basal diet), received a corn/soybean meal diet or the basal diet supplemented with BL, CL or CA for 6 or 18 mg/kg, respectively. CL and CA were purchased from a local company (Masterasia and Eric Biotechnology Co. Ltd., Taiwan). The birds were housed in individual wire cages with nipple drinkers. Table 1 presents the ingredients and nutrient composition of the control (basal) diet. The basal diet, a typical layer diet containing 3050 ME kcal/kg and 22.2% crude protein, was calculated to meet or slightly exceed the nutrient requirements recommended by the National Research Council (20). The proximate composition was analyzed according to the AOAC (21). The experimental protocols were approved by the Animal Care and Use Committee of National Chung Hsing University. Feed and water were provided *ad libitum*, and the light regimen was 17 h of continuous light per day. The temperature was set at 25°C and relative humidity at 60%. The body weights of the laying hens were measured at the beginning and end of this study. Daily egg production was individually recorded and the egg mass was calculated throughout the experimental period. Feed intake was recorded on a replicate basis at weekly intervals and calculated as g/day/bird. The value of the feed conversion ratio (FCR) was calculated on a weekly basis for each group. The FCR was expressed as kilograms of feed consumed per kilograms of eggs produced. Every Sunday, two eggs were collected randomly from each replicate per group (total 10 eggs) to measure egg quality during the experimental period. Weights of the egg yolk, albumin and shell were measured. Yolk index was calculated as yolk height divided by yolk diameter. The Haugh unit values were calculated using the Haugh unit formula, based on egg weight and the height of albumen as determined by an albumen height analyzer (DET 6000, Nabel Co. Ltd., Kyoto, Japan). Eggshell thickness was determined as the mean value of measurements taken at three locations on the egg (sharp end, blunt end and middle section) using a dial pipe gauge. At week 4, 10 egg yolks per group (5 replicates) were separated from the albumen, 10 mg samples of yolks were weighed in the tube. The yolk lipids were extracted with isopropanol, vortexed for 5 min and then centrifuged at 2500 ×*g* for 20 min. Yolk triglyceride and cholesterol concentrations (mg/g yolk or mg/yolk) were determined in the filtered samples by ultraviolet spectrophotometer using commercial kits purchased from the Randox Laboratories Co., Ltd. (Antrim, UK) and calculated according to the methods previously described (22,23).

TABLE 1. Ingredients and nutrient composition of the experimental diet.[a]

Ingredients	%
Corn	42.31
Full-fat soybean meal	11.35
Corn gluten meal	5.00
De-hulled soybean meal	23.20
Fish meal (67% of CP)	1.25
Meat and bone meal (50% of CP)	3.00
Corn DDGS	3.20
Oyster shell meal	1.00
Limestone	6.70
Calcium phosphate (21%)	1.25
Salt	0.20
L-Lysine	0.20
DL-Methionine	0.25
Threonine	0.03
Choline chloride	0.06
Vitamin-mineral premix[b]	1.00
Total	100.0
Calculated value	
ME (kcal/kg)	3050.0
Crude protein	22.2
Ether extract	5.04
Ash content	11.68
Calcium	3.63
Phosphate	0.81
L-Lysine	1.32
DL-Methionine	0.66
Cystine	0.36
Threonine	0.92

[a] The control group was fed the basal diet (corn-soybean meal). Other groups were added with 6 or 18 mg/kg dried bacterial lycopene, commercial lycopene and canthaxanthin in the basal diet, respectively.

[b] Diets contained per kilogram of diet: vitamin A, 8000 IU; vitamin D, 1500 IU; riboflavin, 4 mg; cobalamin, 10 μg; vitamin E, 15 mg; vitamin K, 2 mg; choline, 500 mg; niacin, 25 mg; manganese, 60 mg; zinc, 50 mg; iron, 50 mg/kg; copper, 3 mg/kg; selenium, 0.26 mg/kg.

Blood collection for serum lipids and antioxidant enzyme determination At week 4, 5 mL of blood was collected from one layer per replicate (5 birds per group). Blood was drawn from the birds' left wing veins using a sterilized syringe and needle, and measured for total lipid, triglyceride, cholesterol and antioxidant enzyme content. Serum samples were isolated for approximately 4–5 h at room temperature after collection, and centrifuged at 3000 ×*g* for 30 min at 4°C. The samples were then stored at −4°C for up to 2 d until analysis. A spectrophotometer was used to colorimetrically assay the MDA content and the activities of glutathione peroxidase (GPx) and superoxide dismutases (SOD) (14,24). The procedures were conducted with assay kits purchased from Cayman Chemical Co., Ltd. (Ann Arbor, MI, USA). Serum samples were measured in triplicate and, with the appropriate dilutions, enzymatic activities were allowed to achieve the linear range of standard curves. Antioxidant enzyme activities were expressed as unit (U) per milliliter of serum, as described by Lee et al. (25).

Statistical analysis The data were analyzed by performing ANOVAs for completely randomized designs using the GLM procedure of the SAS software program (Statistical Analysis System, ver. 8.1, SAS Institute Inc., Cary, NC, USA). Significant statistical differences among the various treatment group means were determined using Tukey's honestly significant difference (HSD) test. The effects of the experimental diets on response variables were considered to be significant at $P < 0.05$.

RESULTS

Recombinant lycopene antioxidant capacity DPPH scavenging ability of recombinant lycopene is shown in Fig. 2. As seen, BL had 100% scavenging capacity of DPPH free radicals at a concentration of 4.65 μg/ml. The scavenging capacity of BL was higher than that of BHT and CL. When the lycopene or BHT concentration was 5.0 μg/mL, DPPH scavenging capacity dropped to 82% and 46%, respectively. The reducing power results for recombinant lycopene are outlined in Fig. S1. The results showed that BL and CL were similar to ascorbic acid and the reducing activities of BL, CL and ascorbic acid increased linearly.