



## The Effects of *Rhodobacter capsulatus* KCTC-2583 on Cholesterol Metabolism, Egg Production and Quality Parameters during the Late Laying Periods in Hens

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**ABSTRACT:** An experiment was conducted to investigate the effects of dietary supplementation of *Rhodobacter capsulatus* KCTC-2583 on egg-yolk and serum cholesterol, egg production and quality parameters during the late laying periods in hens. A total of 160 Hy-Line Brown layers (54 wk-old) were randomly allotted to 4 treatment groups on the basis of laying performance. Each treatment had 4 replicates with 10 birds each (40 birds per treatment). Two hens were confined individually with cage size 35×35×40 cm and each 10 birds (5 cages) shared a common feed trough between them forming one experimental unit. Dietary treatments were; basal diet supplemented with 0 (control), 0.05, 0.10 and 0.15% *R. capsulatus* KCTC-2583. Experimental diets were fed in meal form for 56 d. Dietary supplementation of increasing levels of *R. capsulatus* KCTC-2583 reduced (linear,  $p < 0.05$ ) egg-yolk cholesterol and triglycerides (d 28, 42 and 56) concentrations. Also, serum cholesterol and triglycerides (d 21, 42 and 56) concentrations were linearly reduced ( $p < 0.05$ ) with increasing dietary *R. capsulatus* KCTC-2583. Laying hens fed a diet supplemented with increasing levels of *R. capsulatus* KCTC-2583 had increased (linear;  $p < 0.05$ ) overall egg production, egg weight, egg mass and feed efficiency. However, dietary treatments had no effect (linear or quadratic;  $p > 0.05$ ) on feed intake of laying hens. At d 28 and 56, breaking strength and yolk colour of eggs were linearly improved ( $p < 0.05$ ) in laying hens fed dietary increasing levels of *R. capsulatus* KCTC-2583. Dietary treatment had no effects (linear or quadratic;  $p > 0.05$ ) on albumin height, shell thickness and shell weight at any period of experiment. These results indicate that dietary supplementation of *R. capsulatus* KCTC-2583 has the potential to improve the laying hen performance and lead to the development of low cholesterol eggs during late laying period in Hy-Line Brown hens. (**Key Words:** Cholesterol, Egg Quality, Laying Hens, Performance, *Rhodobacter capsulatus* KCTC-2583)

### INTRODUCTION

With growing concern over the relationship between diet and health, there is an increasing emphasis on modification of fat and cholesterol content of edible animals and poultry products. Chicken eggs are an excellent source of the many essential nutrients, like high quality protein,

calcium, phosphorus, folic acid,  $\alpha$ -tocopherol and other B vitamins. However, chicken eggs yolk is rich source of cholesterol (213 mg/egg; USDA, 1991), which limit the consumption of eggs by health-conscious consumers to avoid hypercholesterolemia and heart related problems. Therefore, the research goals are directed towards the production of low cholesterol eggs. During past few decades, attempts to lowering egg-yolk cholesterol have centered mostly on genetic selection, pharmacological intervention or alteration of the laying hen diets with various nutrients like probiotics, nutraceuticals or non-nutritive factors. However, genetic selection had limited cholesterol reducing effect (Hollands et al., 1980; Ansah et al., 1985) and use of pharmaceuticals causes serious problem like drug residues in eggs (Salyers et al., 2004; Mathur and Singh, 2005). Therefore there is urgent need for developing the feed additives which can reduce the egg cholesterol without affecting the human health.

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Previous studies on dietary supplementation of chromium (Uyanik et al., 2002), copper (Balevi and Coskun, 2004), garlic paste (Chowdhury et al., 2002; Mottaghtalab and Taraz, 2002), omega-3 fatty acid (Lewis et al., 2000), tamarind (Chowdhury et al., 2005) had reported reduction in egg cholesterol or triglyceride concentrations. The dietary supplementation of the photosynthetic bacteria, a *R. capsulatus* also resulted in a marked reduction of egg-yolk or broiler meat cholesterol and triglyceride concentrations (Salma et al., 2007a;b). Tsujii et al. (2007) observed marked reduction in serum cholesterol in rat fed diets supplemented with *R. capsulatus*. Numerous studies have been conducted to determine the effects of various feed additives including *R. capsulatus* on performance and egg cholesterol during early laying period in hens. To our knowledge, the effect of *R. capsulatus* during late laying period has not been tested. Therefore, the objectives of present study were to investigate the effects of dietary supplementation of *R. capsulatus* KCTC-2583 on egg production, quality parameters and egg-yolk cholesterol during the late laying periods in Hy-Line Brown hens.

## MATERIAL AND METHODS

The protocol for this experiment was approved and birds were cared according to the guidelines of the Institutional Animal Care and Use Committee of Kangwon National University, Chuncheon, Korea.

### Culture and preparation of *Rhodobacter capsulatus* KCTC-2583

*Rhodobacter capsulatus* KCTC-2583 (Korean Collection for Type Culture-2583) used in the present study was obtained from the Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea. The *R. capsulatus* KCTC-2583 cells were grown in outdoor culture under natural illuminations for 96 h. Briefly, cells of *R. capsulatus* KCTC-2583 were collected by centrifugation at 10,000×g for 10 min. The residual cell mass were mixed with corn meal (1:5) and dried using a forced-air drying oven at 60°C and stored at 4°C. The viable cell count of *R. capsulatus* KCTC-2583 was 10<sup>8</sup> cfu/g.

### Birds, diets and management

A total of 160 Hy-Line Brown layers (54 wk-old) were randomly allotted to 4 treatment groups on the basis of laying performance. Each treatment had 4 replicates with 10 birds each (40 birds per treatment). Two hens were confined individually with cage size 35×35×40 cm and each 10 birds (5 cages) shared a common feed trough between them forming one experimental unit. Dietary treatments were; basal diet supplemented with 0 (control), 0.05, 0.10 and

0.15% *R. capsulatus* KCTC-2583. All the birds were fed isocaloric and isoprotineous diet in mash form for 56 d. *R. capsulatus* KCTC-2583 product were added to basal diet by equally replacing corn. All the nutrients met or exceeded the nutrient requirements as recommended by NRC (1994) as shown in Table 1. The birds were provided daily *ad libitum* feed and clean drinking water during 56 d feeding period. Laying hens were exposed to a 16-h incandescent light period.

### Sampling and measurement

The laying hens were weighed at beginning and on d 28 and 56 of experimental feeding. Daily egg production and egg weight per treatment group was recorded to determine the hen day egg production and the egg mass production

**Table 1.** Ingredient and chemical composition of basal diet (as-fed basis)<sup>1</sup>

Items	Basal diet
Ingredients (%)	
Corn	48.13
Wheat	5.00
Soybean meal (45%)	20.30
Rape seed meal	2.00
Corn gluten meal	5.90
Distillers dried grains with solubles	3.00
Rice bran	3.00
Animal fat	1.62
Choline chloride (50%)	0.06
L-Lysine (24%)	0.09
Methionine hydroxyl analog (88%)	0.08
Limestone	9.53
Dicalcium phosphate	0.76
Salt	0.23
NaHCO <sub>3</sub>	0.10
Vitamin premix <sup>2</sup>	0.10
Mineral premix <sup>3</sup>	0.10
Chemical composition (%)	
ME (kcal/kg)	2,750
DM	91.23
CP	16.60
Ash	5.09
Ca	4.02
Available P	0.30
Lysine	0.89
Met+cys	0.74
Cholesterol	0.015

<sup>1</sup> Dietary treatments were; basal diet supplemented with 0 (control), 0.05, 0.10 and 0.15% *Rhodobacter capsulatus* KCTC-2583.

<sup>2</sup> Supplied per kilogram of diet: 9,000 IU vitamin A, 1,800 IU vitamin D<sub>3</sub>, 30 IU vitamin E, 1.5 mg vitamin K<sub>3</sub>, 1.5 mg vitamin B<sub>1</sub>, 5 mg vitamin B<sub>2</sub>, 4 mg vitamin B<sub>6</sub>, 0.025 mg vitamin B<sub>12</sub>, 15 mg pantothenic acid, 35 mg niacin, 0.15 mg biotin, 0.65 mg folic acid, 12 mg antioxidant.

<sup>3</sup> Supplied per kilogram of diet: 45 mg Fe as ferrous sulfate, 0.25 mg Co as cobalt sulfate, 50 mg Cu as copper sulfate, 15 mg Mn as manganous oxide, 25 mg Zn as zinc oxide, 0.35 mg I as potassium iodide and 0.13 mg Se as sodium selenite.

(g/d/hen). Feed intake was recorded weekly and feed conversion efficiency (feed intake:egg mass) was calculated during 56 d feeding period. Eggs from each group were collected at beginning and at 2 wks interval during feeding period for measuring egg quality (egg weight, shell weight, shell thickness, yolk colour, and Haugh unit) and for analysis of yolk cholesterol and triglyceride. Egg shell breaking strength ( $\text{kg/m}^2$ ) was measured by using texture analyzer (FHK Fujihira Industry co. LTD, Japan). The egg was laid in vertical position on pan of texture analyzer was squeezed and the shell hardness was measured. Yolk colour was evaluated by using colorimetric fan (Roche) and scored according to their intensity. Egg shell weight was measured using electric weighing balance.

At the beginning and on d 21, 42 and 56 of experimental feeding, a 5 ml blood sample was collected by bronchial wing vein puncture from 2 randomly selected hens in each group using sterilized syringes and needles. After 90 min standing at room temperature, serum was isolated by centrifugation at  $3,000\times g$  for 15 min. Separated serum samples were stored at  $-80^\circ\text{C}$  and later analysed for concentrations of total cholesterol and triglycerides.

#### Extraction of yolk and diet lipid

One gram of egg-yolk was placed into a centrifuge tube and mixed with 15 ml of chloroform:methanol (2:1 v/v), sonicated and filtered as described by Elkin and Rogler, (1990). Dietary lipid were extracted by same procedure using 5-g feed sample with 40 ml of chloroform:methanol.

#### Chemical and enzymatic analysis

Experimental diet was analysed in triplicate for DM (Method 930.15), CP (Method 990.03), ash (Method

942.05), Ca and P (Method 985.01) using AOAC (2007). Gross energy of experimental diet was measured by a bomb calorimeter (Model 1216, Parr Instrument Co., Moline, IL, USA). Total cholesterol and triglycerides in the serum and egg-yolk were determined enzymatically using commercial reagent kits (ASAN Total Cholesterol, AM 202-K and ASAN TG-S, AM 157S-K, respectively, ASAN Pharm. Co. Ltd., Geonggi-do, Korea).

#### Statistical analysis

The data generated in present study was subjected to statistical analysis using GLM procedure of SAS (SAS Inst. Inc., Cary, NC, USA) in a randomized complete block design. The orthogonal polynomials were used to evaluate the linear and quadratic effects of dietary *R. capsulatus* KCTC-2583 levels (0, 0.25, 0.50 and 0.75%). For analysis of egg and serum cholesterol and triglycerides, individual egg and hen were used as experimental unit respectively. For analysis of production performance parameters, a replicate of 10 birds was used as experimental unit. Probability values of less than 0.05 ( $p < 0.05$ ) were considered significant.

## RESULTS

#### Egg yolk cholesterol and triglycerides

Dietary supplementation of increasing level of *R. capsulatus* KCTC-2583 reduced (linear,  $p < 0.05$ ; Table 2) eggs cholesterol and triglycerides (d 28, 42 and 56) concentrations. At d 56, reduction of egg-yolk cholesterol were 9.33, 13.56 and 19.29% in laying hen fed diet supplemented with 0.05, 0.10 and 0.15% *R. capsulatus* KCTC-2583, respectively, as compared to laying hens fed

**Table 2.** Effects of dietary supplementation of *Rhodobacter capsulatus* KCTC-2583 on egg-yolk cholesterol and triglyceride concentrations in laying hen<sup>1</sup>

	<i>R. capsulatus</i> KCTC-2583 (%)				SEM	p-value <sup>2</sup>	
	0 (Control)	0.05	0.10	0.15		Linear	Quadratic
Yolk cholesterol (mg/g)							
d 0	15.78	15.85	15.75	15.67	0.12	0.725	0.774
d 14	15.38	14.91	14.77	14.65	0.18	0.183	0.762
d 28	15.29	14.34	14.13	13.85	0.21	0.013	0.349
d 42	15.36	14.00	13.68	12.99	0.34	0.013	0.563
d 56	15.55	14.10	13.44	12.56	0.35	0.001	0.564
% reduction (d 56)	0.0	9.33	13.56	19.29	2.18	0.001	0.564
Yolk triglyceride (mg/g)							
d 0	254.47	257.85	252.08	254.74	4.24	0.888	0.963
d 14	251.19	242.23	237.49	232.84	5.72	0.061	0.744
d 28	253.35	235.84	232.12	224.67	4.84	0.031	0.551
d 42	245.91	222.33	216.65	207.58	5.25	0.017	0.469
d 56	247.19	219.14	206.88	201.42	6.16	0.001	0.105
% reduction (d 56)	0.0	11.35	16.31	18.52	2.27	0.001	0.105

<sup>1</sup> Values are the mean of 5 eggs per treatment.

<sup>2</sup> Linear and quadratic effect of increasing dietary levels of *Rhodobacter capsulatus* KCTC-2583 (0, 0.05, 0.10 and 0.15% of basal diet).

**Table 3.** Effects of dietary supplementation of *Rhodobacter capsulatus* KCTC-2583 on serum cholesterol and triglycerides concentrations in laying hen<sup>1</sup>

	<i>R. capsulatus</i> KCTC-2583 (%)				SEM	p-value <sup>2</sup>	
	0 (Control)	0.05	0.10	0.15		Linear	Quadratic
Serum cholesterol (mmol/L)							
0 d	4.61	4.56	4.59	4.67	0.04	0.637	0.498
d 21	4.88	4.63	4.55	4.32	0.07	0.020	0.890
d 42	4.65	4.32	4.10	3.88	0.10	0.003	0.742
d 56	4.86	4.37	4.09	3.85	0.12	0.001	0.395
% reduction (d 56)	0.00	10.05	15.78	20.82	2.38	0.001	0.395
Serum triglyceride (mmol/L)							
0 d	5.24	5.20	5.13	5.17	0.05	0.311	0.519
d 21	5.28	4.98	4.84	4.62	0.09	0.020	0.645
d 42	5.22	4.81	4.55	4.39	0.15	0.001	0.169
d 56	5.31	4.61	4.38	4.19	0.19	0.001	0.198
% reduction (d 56)	0.00	13.26	17.59	21.11	2.15	0.001	0.198

<sup>1</sup> Values are the mean of 5 hens per treatment.<sup>2</sup> Linear and quadratic effect of increasing dietary levels of *R. capsulatus* KCTC-2583 (0, 0.05, 0.10 and 0.15% of basal diet).

control diet. Whereas, percent reduction in yolk triglycerides at d 56 were, 11.35, 16.31 and 18.52 in laying hen fed dietary 0.05, 0.10 and 0.15% *R. capsulatus* KCTC-2583, respectively.

#### Serum cholesterol and triglycerides

Serum cholesterol and triglycerides (d 21, 42 and 56) concentrations were linearly reduced ( $p < 0.05$ : Table 3) with increasing dietary levels of *R. capsulatus* KCTC-2583. At d

56, hens fed dietary 0.05, 0.10 or 0.15% *R. capsulatus* KCTC-2583 had 10.05, 15.78 and 20.82% reduction in serum cholesterol, respectively. Percent reduction in yolk triglycerides at d 56 were, 13.26, 17.59 and 21.11, respectively, in laying hen fed dietary 0.05, 0.10 and 0.15% *R. capsulatus* KCTC-2583.

#### Layer performance

Laying hens fed diet supplemented with increasing

**Table 4.** Effects of dietary supplementation of *Rhodobacter capsulatus* KCTC-2583 on egg production, egg weight, egg mass, feed intake and feed efficiency in laying hens<sup>1</sup>

Item	<i>R. capsulatus</i> KCTC-2583 (%)				SEM	p values <sup>2</sup>	
	0 (Control)	0.05	0.10	0.15		Linear	Quadratic
1 to 4 wk							
Egg production (%)	77.23	77.32	77.72	78.75	0.23	0.111	0.745
Egg weight (g)	60.71	62.32	62.88	63.18	0.32	0.021	0.230
Egg mass <sup>3</sup> (g/d/hen)	46.88	48.18	48.86	49.74	0.32	0.010	0.456
Feed intake (g/d/hen)	124.82	124.64	123.88	124.35	0.38	0.277	0.577
Feed efficiency <sup>4</sup>	2.66	2.59	2.54	2.50	0.02	0.019	0.477
5 to 8 wk							
Egg production (%)	76.61	77.68	78.39	79.64	0.22	0.075	0.856
Egg weight (g)	63.04	63.68	64.17	64.48	0.41	0.007	0.388
Egg mass (g/d/hen)	48.29	49.47	50.29	51.35	0.45	0.042	0.915
Feed intake (g/d/hen)	124.56	123.13	122.49	122.24	0.51	0.111	0.572
Feed efficiency	2.58	2.49	2.44	2.38	0.02	0.003	0.635
Overall (1 to 8 wk)							
Egg production (%)	76.92	77.50	78.06	79.20	0.17	0.021	0.752
Egg weight (g)	61.87	63.00	63.53	63.83	0.24	0.001	0.107
Egg mass (g/d/hen)	47.59	48.83	49.58	50.55	0.27	0.010	0.661
Feed intake (g/d/hen)	124.69	123.89	123.18	123.29	0.42	0.067	0.425
Feed efficiency	2.62	2.54	2.48	2.44	0.02	0.001	0.416

<sup>1</sup> All measurements were done as fresh basis; values are mean of 4 replicates of 10 hens each.<sup>2</sup> Linear and quadratic effect of increasing dietary levels of *R. capsulatus* KCTC-2583 (0, 0.05, 0.10 and 0.15% of basal diet).<sup>3</sup> Egg mass = (egg production × egg weight)/100. <sup>4</sup> Feed efficiency = Feed intake:egg mass (g:g).

levels of *R. capsulatus* KCTC-2583 had better (linear;  $p < 0.05$ ; Table 4) overall egg production, egg weight, egg mass and feed efficiency. However, dietary treatments had no effect (linear or quadratic;  $p > 0.05$ ) on feed intake of laying hens. Mortality was 0% in all groups during the experimental period.

### Egg quality

At d 28 and 56, breaking strength and yolk colour of eggs were linearly improved ( $p < 0.05$ ; Table 5) in laying hens fed diet supplemented with increasing levels of *R. capsulatus* KCTC-2583. Dietary treatment had no effects (linear or quadratic;  $p > 0.05$ ) on albumin height, shell thickness and shell weight at any period of experiment.

## DISCUSSION

Previous attempts to reduce the egg-yolk cholesterol were mainly focused on the inclusion of pharmaceutical drugs and other agents to laying hens diets (Nobel, 1987; Hargis, 1988). However, occurrence of drug residues in eggs and the high cost of the pharmaceuticals limit their use as cholesterol reducing agents. As a result, poultry industry must focus on alternative to pharmaceutical drugs for reducing the egg-yolk cholesterol under commercial condition. In this sense, the photosynthetic bacteria of genus *Rhodobacter* had emerged as effective feed additive

to reduce the cholesterol contents of egg-yolk (Salma et al., 2007a) and serum (Tsujii et al., 2007). In present study, we supplemented *R. capsulatus* KCTC-2583 to laying hen diets during their late laying cycle and its effects on cholesterol metabolism and laying performance were investigated.

The reduced cholesterol concentrations in serum and egg-yolk of the laying hens fed diets supplemented with increasing levels of *R. capsulatus* KCTC-2583 observed in the present study is in good agreement with data reported by the Salma et al. (2007a), who observed linear reduction of egg-yolk and serum cholesterol concentrations in hens fed diet supplemented with increasing levels (0.01, 0.02 and 0.04%) of *R. capsulatus*. Similarly, it was reported that laying hens fed diet supplemented with combination of *R. capsulatus* and karaya saponin had reduced serum (32%) and egg-yolk cholesterol (18%) concentrations (Afrose et al., 2010). Chowdury et al. (2002;2005) and Shim et al. (2004) observed reduction in egg yolk cholesterol in laying hens fed diet supplemented with garlic, tamarind and *Codonopsis lanceolata* roots, respectively. Observations made in the present study, Salma et al. (2007a) and Afrose et al. (2010) reveal that the cholesterol concentration in egg-yolk has a positive correlation with change of the cholesterol content of the serum. In present study, after 56 d feeding, there were 9.33, 13.56 and 19.29% reduction in egg-yolk cholesterol and 10.05, 15.78 and 20.82% reduction of serum cholesterol, respectively in hens fed diet

**Table 5.** Effects of dietary *Rhodobacter capsulatus* KCTC-2583 on egg quality (breaking strength, albumin height, shell thickness, yolk colour and shell weight) at different interval in laying hen<sup>1</sup>

	<i>R. capsulatus</i> KCTC-2583 (%)				SEM	p value <sup>2</sup>	
	0 (Control)	0.05	0.1	0.15		Linear	Quadratic
Breaking strength (kg/m <sup>2</sup> )							
0 d	3.78	3.74	3.73	3.75	0.11	0.571	0.605
4 wk	3.79	3.9	4.08	4.19	0.20	0.009	0.687
8 wk	3.83	4.02	4.16	4.16	0.15	0.032	0.436
Albumin height (mm)							
0 d	7.93	7.83	8.00	7.92	0.13	0.756	0.779
4 wk	8.58	8.75	8.91	8.80	0.16	0.794	0.836
8 wk	8.66	8.88	9.07	9.03	0.11	0.559	0.791
Yolk color (Roche color fan scale)							
0 d	7.4	7.6	7.8	7.4	0.17	0.876	0.305
4 wk	7.8	8.2	8.6	8.8	0.22	0.063	0.793
8 wk	8	8.6	9.2	9.4	0.22	0.006	0.564
Shell thickness (mm)							
0 d	0.39	0.40	0.41	0.38	0.04	0.512	0.176
4 wk	0.39	0.41	0.39	0.41	0.05	0.505	0.813
8 wk	0.39	0.41	0.40	0.41	0.05	0.440	0.728
Shell weight (g)							
0 d	7.14	7.21	7.22	7.04	0.14	0.677	0.731
4 wk	7.17	7.36	7.44	7.47	0.12	0.432	0.767
8 wk	7.10	7.32	7.44	7.48	0.15	0.280	0.728

<sup>1</sup> All measurements were done as fresh basis; values are mean of 5 eggs per treatment.

<sup>2</sup> Linear and quadratic effect of increasing dietary levels of *R. capsulatus* KCTC-2583 (0, 0.05, 0.10 and 0.15% of basal diet).

supplemented with 0.05, 0.10 and 0.15% *R. capsulatus* KCTC-2583. However, in Salma et al. (2007a) study dietary supplementation of 0.04% *R. capsulatus* had 15% reduction in eggs-yolk cholesterol. The variation in the reduction in egg-yolk cholesterol in different studies might be due to variation in dose of the *R. capsulatus* and age of the hens. In present study we used hens in their late laying cycles (54 wk), whereas Samla et al. (2007a) and Afrose et al. (2010) used hens in early laying cycle (20 to 23 wk).

In this study, after 56 d of experimental feeding, hens fed diet supplemented with 0.05, 0.10 and 0.15% *R. capsulatus* KCTC-2583 had reduced serum (13.26, 17.59 and 21.11%, respectively) and egg-yolk triglycerides (11.35, 16.36 and 18.52%, respectively) concentrations than hens fed diet without *R. capsulatus* KCTC-2583. Present findings are consistent with data reported by Salma et al. (2007a), who observed reduction in serum and egg-yolk triglycerides in laying hens fed diet supplemented with increasing levels (0.01, 0.02 and 0.04) of *R. capsulatus* during their early laying period. Similarly, Tsujii et al. (2007) reported reduced serum triglycerides concentration in rats fed diet supplemented with *R. capsulatus*. Lee et al. (1990) also reported similar results in rats fed diet supplemented with *Rhodopseudomonas palustris*.

Many pathways and factors have been reported to contribute to the hypocholesterolemic and other beneficiary effects of *R. capsulatus*, which were not clearly understood. Kobayashi and Kurata (1978) reported that *R. capsulatus* contains 4.2% carotenoids, which are known as hypocholesterolemic agent. Hypocholesterolemic effects in the present study might be due to carotenoids content of the *R. capsulatus* KCTC-2583. Some of the previous studies reported reduced serum cholesterol concentrations in rats fed carotenoid-rich diet (Amen and Lachance, 1974; Yeum and Rusell, 2002). In addition, it has reported that *R. capsulatus* contains many known and unknown factors, which might be associated with the improved performance and hypocholesterolemic effects (Salma et al., 2007a). A *R. capsulatus* are rich source of minerals (Mg, Mn, Fe, and Cu etc), amino acids (arginine, glycine and lysine), vitamins (B2, B6, folic acid, C, E) and most of them were demonstrated to be cholesterol lowering effects (Vahouny et al., 1984; Ouchi et al., 1990; Phonpanichrasamee et al., 1990; Bakalli et al., 1995). A *Rhodobacter* may change the enzymes, which are associated in regulating the cholesterol synthesis, oxidation or elimination for lowering the cholesterol in laying hens, which might be responsible for hypocholesterolemic effect of *R. capsulatus* KCTC-2583 used in the present study.

In the present study, dietary supplementation of increasing levels of *R. capsulatus* KCTC-2583 had linear improvement in overall egg production, egg weight, egg mass and had better feed efficiency. In contrast to the

present results, Salma et al. (2007a) reported no effects of dietary increasing levels of *R. capsulatus* on egg production, egg weight, egg mass and feed efficiency. This variation in results might be variation in age of hens, strain of *R. capsulatus* and dose of dietary supplementation. The levels of *R. capsulatus* KCTC-2583 used in our study (0, 0.05, 0.10 and 0.15%) were much greater than the levels of *R. capsulatus* (0, 0.01, 0.02 and 0.04) used by Salma et al. (2007a). In this study, dietary treatments had no effect on feed intake, which is in good agreement with data reported by Salma et al. (2007a), who also observed no effect of dietary *R. capsulatus* on feed intake of laying hens. Improved laying performance in present study might be due to cumulative effect of probiotics like action of *R. capsulatus* KCTC-2583, including improvement in nutrient digestion (Choi et al., 2011; Sen et al., 2011; Kim et al., 2012), maintenance of beneficial microbial population (Fuller, 1989; Sen et al., 2012) or alteration of bacterial metabolism (Jin et al., 1997; Choi et al., 2011). Increased feed efficiency in our study is due to increased egg production without affecting the feed intake.

In this study, supplementation of *R. capsulatus* KCTC-2583 to laying hen diet during the late laying period improved the yolk color and the breaking strength of the eggs. The present findings are in good agreement with results of Salma et al. (2007a), who observed improvement in egg yolk color in laying hens fed increasing levels of the *R. capsulatus*. The improved yolk color in present study and Salma et al. (2007a) report might be due to high carotenoids content of the *R. capsulatus*. Previous study with dietary supplementation of external carotenoids reported improved carotenoids content and pigmentation of eggs in quail (Karadas et al., 2006).

In conclusion, results obtained in the present study indicated that dietary supplementation of *R. capsulatus* KCTC-2583 had potential to improve the laying hen performance and lead to the development of low cholesterol eggs during late laying period in Hy-Line Brown hens. However, further studies are needed to identify the exact mechanism of hypocholesterolemic effect of *R. capsulatus* KCTC-2583.

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