JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Effect of Selenium-Enriched Probiotics on Laying Performance, Egg Quality, Egg Selenium Content, and Egg Glutathione Peroxidase Activity

Cuiling Pan,[†] Yuxin Zhao,[†] Shengfa F. Liao,[‡] Fu Chen,[†] Shunyi Qin,[†] Xianshi Wu,[†] Hong Zhou,[†] and Kehe Huang*[,†]

[†]Institute of Nutritional and Metabolic Disorders in Domestic Animals and Fowls, Nanjing Agricultural University, Nanjing 210095, China

[‡]Department of Agriculture, Alcorn State University, Alcorn State, Mississippi 39096-7500, United States

ABSTRACT: A 35-day experiment was conducted to evaluate the effect of selenium-enriched probiotics (SP) on laying performance, egg quality, egg selenium (Se) content, and egg glutathione peroxidase (GPX) activity. Five hundred 58-week-old Rohman laying hens were randomly allotted to 5 dietary treatments of 100 each. Each treatment had 5 replicates, and each replicate had 5 cages with 4 hens per cage. The SP was supplemented to a corn-soybean-meal basal diet at 3 different levels that supplied total Se at 0.2, 0.5, and 1.0 mg/kg. The basal diet served as a blank control, while the basal diet with supplemental probiotics served as a probiotics control. The results showed that dietary SP supplementation not only increased ($p < 0.05$) the rate of egg laying, day egg weight, mean egg weight, egg Se content, and egg GPX activity but also decreased ($p < 0.05$) the feed:egg ratio and egg cholesterol content. The egg Se content was gradually increased ($p < 0.05$) along with the increasing level of dietary Se. The SP supplementation also slowed down ($p < 0.05$) the drop of Haugh units (HU) of eggs stored at room temperature. The egg GPX activity had a positive correlation ($p < 0.01$) with egg Se content and a negative correlation ($p < 0.01$) with egg HU drop. These results suggested that Se contents, GPX activity, and HU of eggs were affected by the dietary Se level, whereas the egg-laying performance and egg cholesterol content were affected by the dietary probiotics. It was concluded that this SP is an effective feed additive that combines the organic Se benefit for hen and human health with the probiotics benefit for laying hen production performance. It was also suggested that the eggs from hens fed this SP can serve as a nutraceutical food with high Se and low cholesterol contents for both healthy people and patients with hyperlipidemia, fatty liver, or cardiovascular disease.

KEYWORDS: selenium-enriched probiotics, egg Haugh unit, cholesterol, glutathione peroxidase, laying hen performance

INTRODUCTION

Selenium (Se) is an essential trace element for animal and human health. As integral parts of several metabolic enzymes including glutathione peroxidase (GPX)[1] and type I iodotyronine deiodinase,[2] Se plays important roles in human antioxidant defense, thyroid function, reproduction,[3] and immune function.[4] Se deficiency may contribute to cardiovascular disease,[5] cancer,[6] asthma,[7] diabetes[8] and hypothyroidism[9] in humans, white muscle disease in sheep,[10] and exudative diathesis,[11] pancreatic fibrosis or atrophy[12] in chicks.

In China, the low level of soil Se in some geographical regions resulted in endemic prevalence of Keshan and Kaschin−Beck diseases in humans,[13,14] especially in 70−80-year-olds. So, it is essentially important to supply Se to human food to prevent or cure Se-deficiency associated diseases.

In recent years, numerous studies indicated that organic Se is more bioavailable and has higher tissue accumulation rate than inorganic Se, and the increased level of Se in animal body from organic source can persist for a longer period of time.[15,16] More importantly, organic Se is usually less toxic and has a lower environmental pollution issue than inorganic Se.[17,18] Thus, renewed interests exist in the use of organic Se to feed animals and humans.

Selenium-enriched yeast (SY, e.g., Sel-Plex) is a well-known source of organic Se. Numerous applied studies on SY have been conducted in broilers and laying hens, and the results have shown that dietary supplementation of SY resulted in better production efficiencies.[19−23] A common yeast species, *Saccharomyces cerevisiae*, in SY acts as a Se biotransformation vector,[24] and this strain of yeast, as well as some others (e.g., *Saccharomyces boulardii* and *Candida utilis*), can also serve as probiotics.[25−27] It was reported that oral administration of probiotics can protect animals against enteric infections,[28] maintain intestinal microbiota balance,[29] prevent and treat certain diarrheal diseases,[30] improve growth, enhance immune response, reduce serum cholesterol,[31] and inhibit cancer cells.[32]

Besides these yeast strains, there may be other microorganisms that also have the ability to transform inorganic Se to organic forms, and it would be economically beneficial if we could develop a new product that exerts dual effects of organic Se and probiotics at the same time. There are some reports about lactic acid bacteria (e.g., genus *Lactobacillus*) that have the ability to synthesize biomolecules containing Se.[33] To develop new Se-enriched probiotics (SP), our laboratory utilized several strains of

Received: May 20, 2011
Accepted: September 26, 2011
Revised: September 20, 2011
Published: September 26, 2011

dx.doi.org/10.1021/jf202014k | *J. Agric. Food Chem.* 2011, 59, 11424–11431

Table 1. Ingredient Composition and Calculated Nutrient Levels of the Basal Diet (on Dry Matter Basis)

ingredients	composition (%)	nutrient	levels
corn	65.0	ME,[c] MJ/kg	11.57
soybean meal	9.0	crude protein, %	15.6
bran	2.0	crude fiber, %	3.5
clover meal	5.6	lysine, %	0.77
fishmeal (55.5% CP)	8.0	methionine, %	0.32
bone powder	1.0	cystine, %	0.23
salt	0.35	calcium, %	3.56
vitamins[a]	0.05	phosphorus, %	0.75
trace minerals[b]	1.0	selenium, mg/kg	0.151
limestone	8.0		

[a] The vitamins provided per kg of diet: vitamin A (retinol acetate), 4.13 mg; vitamin D_3, 75.00 mg; vitamin E (DL-α-tocopherol acetate), 20.00 mg; menadione, 2.00 mg; vitamin B_{12}, 0.015 mg; thiamin, 1.50 mg; riboflavin, 4.50 mg; biotin, 0.10 mg; folacin, 0.50 mg; niacin, 20.00 mg; pantothenic acid, 10.00 mg; and pyridoxine, 3.00 mg. [b] The trace minerals provided per kg of diet: manganese, 60.0 mg; zinc, 50.0 mg; iron, 55.00 mg; copper, 5.0 mg; and iodine, 0.5 mg. [c] ME: metabolizable energy.

probiotics (e.g., *Lactobacillus acidophilus* and *C. utilis*) that can efficiently transform and enrich organic Se from an inorganic source (i.e., sodium selenite, SS). As a new organic source of Se, this SP product was granted a patent in China in the year 2006 (number ZL 2005 1 0040990.2).

A few studies have been conducted in this laboratory which concluded that this SP product can strongly antagonize pathogenic *Escherichia coli*, increase blood Se level, fortify immunity, enhance antioxidant status, improve the internal environment of intestinal tract, and lower mortality in mice and canine.[27,34] However, no applied research of SP in poultry has been conducted. The purpose of this study is to investigate the effect of dietary SP supplementation on hen's laying performance, egg quality, egg Se content, and egg GPX activity, with a hope to produce nutraceutical eggs for improving human health.

MATERIALS AND METHODS

Selenium Source and Chemical Reagents. The SP product used in this study is the aforementioned probiotics product developed by this laboratory. Its total Se content is 5.0 mg/L, with over 90% being organic Se and over 75% being selenomethionine. The probiotics microorganisms in this product include *L. acidophilus* (LA) and *C. utilis* (CU), and the colony forming units (CFU) of LA and CU are 10^{11}/mL and 10^9/mL, respectively.

The total cholesterol (TC) assay kit was purchased from Biosino Biotechnology & Science Inc. (Beijing, China), and the GPX assay kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All reagents used for egg yolk preparation and Se determination were of analytical or higher grade. Se stock standard solution of sodium selenite [GBW(E)080215] and the certified Se reference material, pork liver [GBW 08551], were provided by the National Research Center for Standard Materials (Beijing, China) and the Food Detection Science Institute of the Ministry of Commerce (Beijing, China), respectively. The water used in the chemical analyses was of ultrapure grade (resistance 18 MΩ/cm).

Animals and Dietary Treatments. Five hundred 58-week-old Rohman laying hens (egg rate: 86% ±) housed in wire cages were randomly allotted to 5 dietary treatments of 100 each. Each treatment was replicated five times (n = 5), and each replicate had 5 cages with 4 hens per cage. Each cage was equipped with one nest, one perch, and two fresh water nipples. Chipboards were inserted into the feeders between individual cages to avoid between-cage contamination. A corn-soybean-meal diet was formulated as a basal diet in accordance with NRC (1994) recommendations[35] to meet the nutrient requirements for laying hens (Table 1).

Table 2. Selenium Levels in Diets of Five Treatments (mg/kg)[a]

		Se level	
treatment	diet	supplemental	final total
1	basal diet	0.0	0.151
2	basal diet + probiotics	0.0	0.151
3	basal diet + SP	0.2	0.351
4	basal diet + SP	0.5	0.651
5	basal diet + SP	1.0	1.151

[a] Presented Se levels for the SP product (supplemental Se) and the basal diet are the chemically analyzed values, while for the final total Se levels of the treatment diets (except for treatment 1) they are the calculated values.

Selenium-enriched probiotics were gradually mixed into the basal diet at three different levels that supply total Se at rates of 0.2, 0.5, and 1.0 mg/kg (on a dry matter basis). Briefly, the stock volumes of 40, 100, and 200 mL of SP liquid were diluted to three 250 mL working solutions and, then, respectively sprinkled onto 1 kg of basal diet that had been evenly spread out on an appropriate salver. The mixture was stirred synchronously and blended continually to prevent conglobating. During the whole animal trial period (35 days), the corresponding diet for each treatment was prepared 2 times per day. The activity of the SP product (i.e., the number of probiotics) was examined before and at the end of the animal trial, and no significant decrease in the activity was found.

The basal diet contains 0.151 mg/kg of total Se (dry matter basis), and the final Se levels for the 5 treatment diets are listed in Table 2. The basal diet without adding SP and probiotics served as a blank control (treatment 1), while the basal diet with added probiotics served as a probiotics control (treatment 2) whose probiotics level was equal to that of the treatment 5 diet, which contains the highest level of probiotics. All hens were fed the same basal diet for 2 weeks prior to the application of experimental treatments. Feed and water were provided *ad libitum* to all the birds throughout the 35-day experiment period.

Data Recording, Sample Collection and Preparation. During the experiment period, the egg mass, the number of shell-less and cracked eggs, the feed intakes, and the egg weights were recorded daily on an individual cage basis. The rate of hen-day egg production, rate of shell-less and cracked eggs, mean weight of eggs, and the ratio of feed:egg then were calculated. At the end of the experiment, six eggs per treatment replicate were collected. One third of sample eggs were depurated and analyzed immediately for egg quality and egg GPX activity; another one-third were depurated and stored at 4 °C for analysis of egg Se content and egg cholesterol level. The remaining one-third were stored at room temperature (27−29 °C, 54−57% humidity) for 6 days before analysis of egg Haugh units (HU) and GPX activity. Egg quality parameters included egg shape index, eggshell strength, eggshell thickness, egg HU, egg yolk color, and egg yolk weight. The preparation steps for determination of egg Se content and egg GPX activity were described previously by this laboratory.[36] For cholesterol analysis each egg was prepared according to the method described by Salma et al.[37] and Elkin et al.[38] Briefly, each egg was broken and weighed. The yolk was separated and weighed, from which 1 g was homogenized with 15 mL of chloroform−methanol mixture (2:1, by volume), sonicated, and filtered by using a 10 mL syringe attached 33 mm filter unit with 0.45 μm pore size membrane. The obtained samples were then stored at −30 °C before analysis.

Table 3. Rates of Day Egg Production of the Hens (%)[a]

treatment	week 1	week 3	week 5	whole trial period[b]
1	84.35 ± 2.93 a	83.81 ± 1.08 a	83.25 ± 2.41 a	83.93 ± 2.58 a
2	84.82 ± 2.44 a	86.62 ± 1.89 b	86.51 ± 2.77 b	85.89 ± 2.35 b
3	84.47 ± 2.38 a	86.73 ± 4.03 b	86.89 ± 1.16 b	85.92 ± 2.57 b
4	85.06 ± 4.21 a	86.64 ± 2.18 b	86.67 ± 5.06 b	86.03 ± 4.03 b
5	85.16 ± 3.21 a	86.67 ± 3.08 b	86.71 ± 2.68 b	86.21 ± 2.99 b

[a] The data (mean ± SE, $n = 5$) within a column that have different letters (a, b) differ ($p \leq 0.05$). [b] The means were calculated for the whole 5-week experiment period.

Sample Analyses. Egg HU (determined by the weight and the effective height of albumen of each egg), egg yolk color (determined by the Roche Yolk color fan calibration), and egg yolk weight (after discarding egg shell and albumen) were directly measured by EMT-5200 Egg Multi Tester (Robotmation Co., Ltd., Tokyo, Japan). The eggshell strength in terms of destruction strength was measured by using EFG-0502 Eggshell Force Gauge (Robotmation Co., Ltd., Tokyo, Japan). The egg shape index and eggshell thickness were calculated with the values measured by a vernier caliper. The egg shape index is equal to the ratio of egg vertical length to egg across length. The eggshell thickness is the mean value of the blunt, the cusp, and the middle thickness of eggshell without inner shell membrane.

The egg Se content assay was performed following the method of Pan et al.[36] using an AF-610A atomic fluorescence spectrometer (Ruili Analysis Instrument Co., Beijing, China), whereas the egg TC content and GPX activity were determined by the Slectea-E-Plus Automated Biochemical Analyzer (The ELITech Group, Vital Scientific B.V., The Netherlands) using the two respective commercial kits (as aforementioned) following the manufacturers' instructions.

Briefly, the TC content assay was based on the measurement of hydrogen peroxide, which reacted with 4-aminoantipyrine and 3,5-dichloro-2-hydroxybenzenesulfonic acid sodium salt via cholesterol oxidase and peroxidase to form a flammulated compound. The absorbency value of this compound was determined at 500−600 nm wavelength, and the TC content was then calculated by the Analyzer. The GPX activity assay was based on the oxidation of glutathione (GSH) by hydrogen peroxide via GSH peroxidase. The reduced GSH was tested using hydrogen peroxide as a substrate. A nonenzyme reaction of reduced GSH and hydrogen peroxide was subtracted. GSH was then monitored by 5,5-dithiobis(2-nitrobenzoic acid) at 412 nm wavelengh. One unit (U) of GPX activity was defined as the milligrams of protein that can reduce GSH concentration by 1 μmol/L in one minute subtracting the nonenzymatic reaction.

Statistical Analysis. All the egg-laying performance, egg quality, and Se-associated data were analyzed with one-way ANOVA model using SPSS computer program (version 13.0) for windows. Final data were expressed as mean ± standard error (SE). To determine the differences between the means, multiple comparisons were conducted using the least significant difference (LSD) test. The mean difference was considered significant when $p \leq 0.05$. Bivariate regression analyses between egg GPX activity and egg Se level and between egg HU drop and egg GPX activity were both examined using the SPSS program. The coefficients of the regression parameters were considered significant when $p \leq 0.01$.

RESULTS AND DISCUSSION

Laying Production Performance. As shown in Table 3, the rates of hen-day egg production among 5 treatments did not differ in week 1. However, in week 3, in week 5, or across the whole experiment period, supplementation of probiotics or SP vs the blank control significantly increased ($p < 0.05$) the rates of hen-day egg production. Comparing to the probiotics control (treatment 2), the rates of hen-day egg production in SP treatment groups were not significantly increased ($p > 0.05$) in week 3, in week 5, or across the whole experiment period.

As shown in Table 4, compared to the blank control, supplementation of probiotics or SP significantly increased ($p < 0.05$) the day egg weight and the mean egg weight (except for treatment 3), significantly decreased ($p < 0.05$) the feed:egg ratio, and numerically decreased ($p > 0.05$) the rates of shell-less and broken eggs. However, there were no differences among treatments 2 to 5 (probiotics and SP supplementation groups) for all these parameters.

In short, the rates of day egg production of the hens from the 3-week to the end of the 5-week period (Table 3), the day egg weight, the mean egg weight, and the feed:egg ratio (Table 4) were significantly affected ($p < 0.05$) by supplementation of probiotics only or by SP, but not differentially affected by the level of Se supplementation. The daily feed intake of hens and the rate of shell-less and broken eggs were not affected ($p > 0.05$) by the supplementation of probiotics or SP.

These results suggested that the laying performance was mainly affected by the probiotics in SP but not the Se in SP. The similar results of no effect of organic or inorganic Se supplementation on laying performance were also reported by Utterback et al.,[21] Payne et al.,[22] and Bennett et al.[39] Some studies reported that the probiotics containing *Lactobacillus* affected production performance of laying hens and other birds. Panda et al.[40] reported that supplementation of probiotics significantly increased ($p < 0.05$) the egg production, shell weight, and shell thickness. Kurtoglu et al.[41] reported that supplementation of probiotics significantly increased ($p < 0.05$) egg production and feed conversion ratio, and decreased ($p < 0.05$) the number of damaged eggs. Yörük et al.[42] reported that supplementation of probiotics during the late laying stage increased egg production, reduced mortality, and improved feed conversion efficiency. Torres-Rodriguez et al.[43] reported that feeding *Lactobacillus* spp. based probiotics appeared to increase the market weight of commercial turkey and reduce the cost of turkey production. Based on the results of this study as well as the aforementioned studies, it can be concluded that the effect of SP supplementation on the laying performance was due to the contained probiotics in SP. The speculated mechanisms by which probiotics improve laying performance may be attributed to the balance of the intestinal flora by means of competitive antagonism and exclusion, and the enhancement of digestion and utilization of dietary nutrients via increasing host digestive enzyme activities and decreasing bacterial enzyme activities.[29,44,45]

Egg Selenium Content. As shown in Table 5, the Se contents in the eggs from hens fed SP were significantly higher ($p < 0.05$) than those in eggs from hens fed a basal or probiotics diet. Egg Se content was also gradually increased ($p < 0.05$) with the increase of supplemental Se level. These results indicated that the supplementation of SP in laying hen diets is very effective in increasing egg Se content. Also hens fed the diets with higher Se from SP can produce eggs with higher Se contents. These results are similar to those reported by others. Payne et al.[22] and Bennett et al.[39] reported that egg Se content was linearly increased ($p < 0.05$) as dietary Se level increased. Leeson et al.[23] reported that Se contents in eggs were greater ($p < 0.01$ or 0.05) from hens fed greater concentrations of Se. Pappas et al.[46] reported that a high-Se diet elevated the Se content of the hen eggs by 7.1-fold.

Table 4. Production Performance of the Laying Hens[a]

treatment	hen day feed intake (g)	day egg wt (g)	feed:egg ratio	mean egg wt (g)	shell-less and broken egg rate (%)
1	119.96 ± 1.98 a	54.07 ± 1.66 a	2.219 ± 0.030 b	64.44 ± 1.48 a	1.612 ± 0.175 a
2	121.16 ± 2.29 a	55.92 ± 1.88 b	2.167 ± 0.032 a	65.11 ± 1.23 c	1.495 ± 0.216 a
3	120.01 ± 3.25 a	55.65 ± 1.73 b	2.156 ± 0.053 a	64.77 ± 1.12 ac	1.550 ± 0.238 a
4	121.38 ± 2.13 a	56.19 ± 3.18 b	2.160 ± 0.034 a	65.29 ± 1.32 c	1.423 ± 0.160 a
5	121.59 ± 2.73 a	56.27 ± 2.14 b	2.161 ± 0.039 a	65.27 ± 1.23 c	1.475 ± 0.196 a

[a] The data (mean ± SE, $n = 5$) was calculated for the whole experiment period. Means within a column that have different letters (a, b, c) differ ($p \leq 0.05$).

Table 5. Selenium Contents of the Eggs (on a Wet Weight Basis)[a]

treatment	supplemental Se level (mg/kg)	egg Se content (mg/kg)[b]
1	0	0.189 ± 0.006 a
2	0	0.193 ± 0.005 a
3	0.2	0.358 ± 0.008 b
4	0.5	0.458 ± 0.011 c
5	1.0	0.582 ± 0.024 d

[a] The egg Se content for each treatment is the mean ± SE of five replicates ($n = 5$) with two eggs being analyzed and averaged for each replicate. [b] Means within a column having different letters (a, b, c, d) differ ($p \leq 0.05$).

Nevertheless, the probiotics control diet in this study yielded an egg Se content that almost equals that from the basal diet (Table 5), which suggests that egg Se content was influenced by the dietary Se content from SP but not the dietary probiotics. Higher Se content in egg is beneficial for preserving egg freshness,[47,48] and, more importantly, higher egg Se content may provide a measure to prevent or treat human diseases caused by low dietary Se supply.[13,14]

Eggshell Characteristics and Egg Haugh Units. There were no differences in either egg shape index, egg yolk color, or egg yolk weight among the five treatments (data not shown). The eggshell strength and thickness had numerical increases with SP or probiotics supplementation, but the increases ($p > 0.05$) were not statistically significant (Table 6).

The HU of fresh eggs did not differ among treatments (Table 6). After storage at room temperature for 6 days, the HU of eggs in all treatments (except for treatment 5) were significantly decreased ($p < 0.05$), but the extent of HU drop in SP supplementation groups was smaller ($p < 0.05$) than those in the basal diet and probiotics control groups. After this 6-day storage, the HU of eggs from the SP supplementation groups, but not the probiotics group, were higher ($p < 0.05$) than those from the blank control group. In addition, the HU of eggs were gradually increased with the increased levels of Se, which means the HU drop was gradually slowed with the increasing level of Se. These results indicated that dietary supplementation of SP can slow the drop of HU and extend the fresh time of the eggs stored at room temperature. At the same time, these results also suggested that the egg HU was mostly influenced by Se but not probiotics, and there might be a positive correlation between egg HU and dietary Se level.

It was well-known that HU is an important index of egg albumen quality and egg freshness. As storage time increases, the HU value drops because of the proteolysis of dense protein to rare protein. Therefore, how to maintain egg HU during storage attracted much attention. There have been different reports about the effect of dietary Se supplementation on egg HU. Arnold et al.[49] reported that adding 0.4 mg/kg of Se from SS to diets did not change egg HU. Patton[50] also reported that adding 0.3 mg/kg of Se from SS or SY did not affect the HU of fresh eggs and eggs stored for 21 or 42 days. However, Payne et al.[22] reported that the HU of eggs from hens fed SS were significantly higher than from the blank control after storage at 22 °C for 3 days, although the HU of eggs from the group fed SY were close to that in the blank control. But Wakebe[47] reported that SY supplementation can slow down the albumen degradation and maintain albumen quality during storage. Pappas et al.[48] reported that high dietary SY treatments led to greater albumen HU compared to the low SY treatments, and that after storage the egg quality was affected by dietary Se contents. Apparently, our results were similar to those of Wakebe[47] and Pappas et al.[48] As to the discrepancy among some of those results, there may be two major reasons as they explained: (1) it might be related to the contents of dietary and/or egg Se that exerts antioxidant effect by GPX; (2) it might be related to the temperature and humidity of the storage environment and/or the time of storage. However, more defined reasons must be further investigated.

Egg Cholesterol Content. As shown in Table 7, the weights of egg yolks were not different among all the treatments. The total cholesterol contents of egg yolks or of whole eggs from hens fed SP or probiotics diets were significantly lower ($p < 0.05$) than those of the blank control; however, there were no differences among hens fed SP and probiotics diets. These results indicated that dietary SP or probiotics supplementation can produce low-cholesterol eggs and that the egg cholesterol level may be influenced by the probiotics in SP but not the Se in SP.

Little is known about the mechanism by which probiotics reduce the cholesterol contents in eggs, although there are some studies which reported that certain dietary probiotics supplementation reduced egg cholesterol contents.[40,41,51,52] Panda et al.[40] and Kurtoglu et al.[41] reported that probiotics supplementation to hen diets at 100 or 200 mg/kg and 250, 500, or 750 mg/kg, respectively, decreased the egg yolk cholesterol. Mohan et al.[51] reported that the total egg cholesterol was reduced by probiotics supplementation at the overall level of 100 or 150 mg/kg. Haddadin et al.[52] reported that when *L. acidophilus* was added to hen diets at levels up to 4 million viable cells per gram of feed, the cholesterol level in yolks was decreased by 18.8%. As to the mechanism of the decrease, current hypotheses include the assimilation of cholesterol by probiotics[53] to incorporate it into the cellular membranes during probiotics growth or to convert it into coprostanol,[54] the binding of cholesterol to the probiotics cell walls,[55] the enzymatic deconjugation of bile acids by the bile-salt hydrolase of probiotics,[56] and the production of

Table 6. Eggshell Characteristics and Haugh Units of the Eggs[a]

			egg Haugh units	
treatment	eggshell strength (kg/cm^2)	eggshell thickness (mm)	fresh	after 6 days
1	3.445 ± 0.168	0.375 ± 0.018	85.16 ± 1.634 d	72.36 ± 1.416 a
2	3.488 ± 0.161	0.381 ± 0.023	84.95 ± 1.395 d	72.09 ± 1.568 a
3	3.506 ± 0.157	0.380 ± 0.016	85.00 ± 1.594 d	76.91 ± 1.474 b
4	3.474 ± 0.165	0.378 ± 0.017	85.23 ± 1.477 d	80.25 ± 1.940 bc
5	3.512 ± 0.160	0.385 ± 0.021	86.80 ± 1.864 cd	83.30 ± 1.643 c

[a] Data (mean ± SE) for each treatment were calculated from five replicates (n = 5) with two eggs being analyzed and averaged for each replicate. Means within a column having different letters (a, b c, d) differ ($p \leq 0.05$).

Table 7. The Contents of Total Cholesterol of the Eggs[a]

treatment	egg yolk wt (g)	egg yolk cholesterol (mg/g)	whole egg cholesterol (mg/egg)
1	17.10 ± 0.66 a	14.93 ± 0.83 a	255.30 ± 14.20 a
2	16.95 ± 0.54 a	11.87 ± 0.87 b	201.20 ± 14.72 b
3	16.97 ± 0.62 a	11.75 ± 0.80 b	199.40 ± 13.52 b
4	17.16 ± 0.58 a	11.53 ± 0.58 b	197.86 ± 9.99 b
5	17.08 ± 0.61 a	11.68 ± 0.68 b	199.48 ± 11.63 b

[a] Data (mean ± SE) for each treatment were calculated from 5 replicates (n = 5) with two eggs being analyzed and averaged for each replicate. Means within a column having different letters (a, b) differ ($p \leq 0.05$).

short-chain fatty acids upon the fermentation in the presence of prebiotics.[57]

In this study, the probiotics in SP include LA and CU. From the results, we cannot separate the performance between LA and CU. However, we speculate that LA may play a more important role in reducing egg cholesterol level because some studies reported that CU acts as a biotransformation processor or factory.[58,59] It may also be because of the result of the reduction of total cholesterol level in animal blood resulting from animal consumption of LA through diets.

Egg GPX Activity. As shown in Table 8, egg GPX activity was remarkably affected ($p < 0.05$) by supplemental SP and the level of Se in SP, but not by the probiotics. Compared to the blank or probiotics control, the GPX activities of the fresh eggs or the eggs stored at room temperature for 6 days were all significantly increased ($p < 0.05$) upon dietary Se supplementation at 0.5 and 1.0 mg/kg (except for the level of 0.2 mg/kg). With the supplemental Se level increasing, the GPX activities of the fresh eggs or the eggs stored at room temperature for 6 days were also gradually increased ($p < 0.05$) accordingly. After 6 days of storage at room temperature, the egg GPX activities of treatments 1 to 5 dropped by 5.57%, 4.68%, 2.94%, 2.91%, and 2.50%, respectively, although these drops were not statistically significant. These results show that SP supplementation can increase the GPX activity of eggs, and the extent of increase might be positively correlated to the dietary Se level. These results suggest that the egg GPX activity was affected by Se in SP but not probiotics, and that supplementation of SP may slow the drop of GPX activity in eggs after storage at room temperature for several days.

These results of this study were in agreement with the finding of Wakebe[47] that SY supplementation to hen diets improved the GPX activity of eggs. Surai's research[19] showed that SY supplementation to hen diets increased ($p < 0.05$) the GPX activities in liver and plasma of 1-day-old chicken through yolk embryo, because these activities rightly reflected the GPX activities in yolk embryos. However, the activities in liver and plasma did not continually increase along with the continual increase of supplemental Se level. The disagreement between Surai's results[19] and this study's is probably because the supplemental level of Se (0.4 mg/kg) used in Surai's research was lower than what this study used (0.5 or 1.0 mg/kg).

There are also other similar studies about the effect of Se supplementation to broiler or hen's diets on tissue GPX activities. In 2003, Mahmoud[20] discovered that adding 0.46 mg/kg of Se in the form of SY to the diet for newly hatched broilers remarkably increased ($p < 0.05$) the liver GPX activity. In 2005, Payne et al.[60] reported that pGPX3 (plasma glutathione peroxidase) activity was greater in broilers fed diets with 0.30 mg/kg of Se from SY or SS than in those fed the basal corn-soybean-meal diet without Se supplementation, and that the pGPX3 remained greater in birds previously fed a diet with supplemental SY than in those fed supplemental SS. In 2007, Yoon et al.[15] reported that when Se from SY or SS was supplemented to broiler diets at levels of 0.1, 0.2, or 0.3 mg/kg, blood GPX activities increased ($p < 0.05$) as the concentration of Se in diets increased. In 2008, Leeson et al.[23] reported that liver GPX activity was greater ($p < 0.01$) in hens fed selenite or Se yeast. These results indicate that either organic or inorganic Se supplemented to broiler or hen's diets can result in greater tissue GPX activities, which suggests that there is a positive relationship between dietary Se level and tissue GPX activity.

Relationship between Egg GPX Activity and Egg Se or HU. As is known, HU of eggs can gradually drop with storage time, which is also shown in Table 6 of this study, and egg nutrients can be wasted and eventually the egg will be deteriorated. As an essential element, Se is located in the active center of GPX for this enzyme to perform antioxidant function. Also, it is known that GPX can prevent cell from free radical damnification. Now, whether or not GPX can influence egg HU value during the time of storage is a rational question.

Bivariate regression analyses of the data (Tables 8 and 5; Tables 6 and 8) showed that there was a positive quadratic correlation ($r^2 = 0.993$, $p < 0.01$) between egg GPX activity and egg Se content (Figure 1), which means that the higher the egg Se content, the higher the egg GPX activity. Furthermore, there was a negative quadratic correlation ($r^2 = 0.988$, $p < 0.01$) between the drop of egg HU value and GPX activity (Figure 2), which means that the higher the GPX activity, the lower the drop of egg HU value. These analyses suggest that the dietary Se plays an important role in prolonging the egg freshness and maintaining the egg quality during storage. A suggested mechanism for this is that a certain amount of dietary Se is gradually transferred to the eggs and, then, the egg GPX activity is improved. A higher GPX

Table 8. The Glutathione Peroxidase Activities of the Eggs[a]

treatment	supplemental Se level (mg/kg)	egg GPX act. (U) fresh	egg GPX act. (U) after 6 days
1	0	22.96 ± 0.726 a	21.68 ± 0.969 a
2	0	23.29 ± 0.751 a	22.20 ± 0.787 a
3	0.2	24.12 ± 0.818 ab	23.41 ± 0.546 ab
4	0.5	26.49 ± 0.856 c	25.72 ± 0.615 c
5	1.0	28.82 ± 0.626 d	28.10 ± 0.701 d

[a] Data (mean ± SE) for each treatment were calculated from 5 replicates (n = 5) with two eggs being analyzed and averaged for each replicate. Means within a column having different letters (a, b, c, d) differ ($p \leq 0.05$).

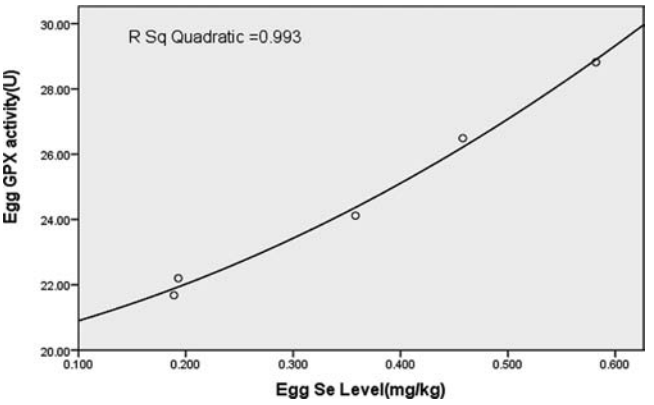

Figure 1. The relationship between GPX activities and Se contents of the eggs.

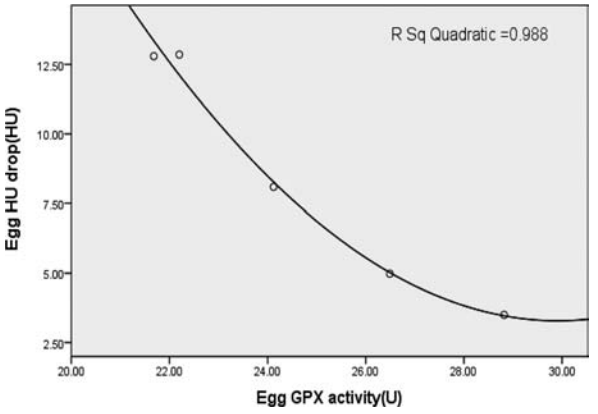

Figure 2. The relationship between the HU drop and GPX activities of the eggs.

activity could protect eggs from free radical oxidation, eggshell deterioration, and egg content liquidation. Therefore, the egg quality degradation would be slowed down and the eggs' shelf life extended.

General Discussion. As an essential element for human health, Se comes mainly from food. In China, about 72% of surface soil is low in Se and the Se contents in a variety of foods are therefore low. Thus, typical human diets do not provide enough Se to meet the recommendation levels, and it is necessary to supplement human diets with Se, especially for those who live in the low Se region, such as the low Se belt from the northeast to the southwest of China. As is seen in this study, the eggs produced from the hens fed the SP supplemented diets are rich in Se and low in cholesterol. Therefore, this type of egg could be used as a nutraceutical food for people who live in the low Se region or for senior or middle-aged people, especially those having hyperlipidemia and/or cardiovascular disorders. Providing Se-enriched eggs is a convenient way to correct the Se deficiency in human diets.

As a cofactor for signaling molecules, a precursor for steroid hormones, and an essential membrane component,[61] cholesterol is an indispensable nutrient for human health. An appropriate level of body cholesterol is beneficial for juvenile brain growth, learning and memory ability,[62] immunity,[63] and other physiological functions. However, excess blood cholesterol could lead to hyperlipidemia and further to atherosclerosis,[64] coronary disease, and other complications.[65,66] Therefore, low cholesterol foods are beneficial in terms of health of senior and middle-aged people and also of the people with hyperlipidemia or cardiovascular diseases.

Eggs are rich in nutrients, convenient for eating, and ready for digestion and absorption. Consequently, many consumers use eggs as one of the primary supplies of dietary proteins without knowing that eggs are usually rich in cholesterol. Spence et al.[67] reported that dietary cholesterol (when it is high) is harmful to the arteries and suggested that patients at risk of cardiovascular disease should limit their intake of cholesterol. However, the eggs from the dietary SP treated hens as in this study are low in cholesterol and, therefore, can be used to satisfy people's special needs.

Based on the present study, a 60−65 g egg from a SP-fed hen will provide approximately 27−30 μg of Se, and the cholesterol content in one egg is about 198−199 mg and about 21.9−22.5% lower ($p < 0.05$) than an egg from the control group. A common 60−65 g egg from the market contains only 11−12 μg of Se but 253−257 mg of cholesterol. Obviously, the eggs produced with our SP-treated diet had higher Se but lower cholesterol contents than the common eggs.

The Nutrition Council of China published the Referenced Dietary Nutrient Intake of Chinese in 2000. The adequate intake of Se for people over 14 years old is 50 μg/day, and the maximal intakes for youths (14−18 years old) and those over 18 years old are 360 and 400 μg/day, respectively.[68] Eating 2.0 eggs/day as produced in this study would not only provide enough Se for people over 14 years old but also minimize the risk of hyperlipidemia or fatty liver caused by high cholesterol intake. The currently recommended upper limit of dietary cholesterol (DC) is different between healthy and unhealthy people. In Canada, the recommended DC intake for persons with diabetes or hyperlipidemia should be limited to 200 mg/day,[67] whereas less than 300 mg/day is recommended for healthy persons in the United States.[69] Obviously, it is safe for healthy persons to consume 1.5 eggs/day as yielded in this study, and for persons with diabetes or hyperlipidemia to consume 1.0 egg/day.

In conclusion, this study demonstrated that the SP product used in this study can serve as an effective feed additive that combines the organic Se benefit for hen and human health with the probiotics benefit for hen production performance. Dietary SP supplementation can increase laying rate of hens, egg weight, egg Se content, and egg GPX activity, whereas it can decrease the feed:egg ratio and the egg total cholesterol content. The SP

supplementation also slows down the HU drop of eggs stored at room temperature. The egg GPX activity has a positive correlation with egg Se content, and a negative correlation with the drop of egg HU value. Hens fed a SP supplemented diet can lay eggs rich in Se, low in cholesterol, and long in shelf life. This type of egg should be a valuable source of nutrients, especially of Se, for not only healthy humans but also, maybe more importantly, for people with hyperlipidemia, fatty liver, or cardiovascular disease.

AUTHOR INFORMATION

Corresponding Author

*College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, Jiangsu Province, China. Tel: +86-25-84395507. Fax: +86-25-84398669. E-mail: khhuang@njau.edu.cn.

Funding Sources

This research was supported by the National Natural Science Foundation of China (Grants: 30871892, 31011130155) and the fund from the Priority Academic Program Development of Jiangsu Institution of Higher Education.

ACKNOWLEDGMENT

We thank Dr. Shanhai Pan for his joint effort in manuscript preparation. We also thank Dr. Yinfei Tan for help in revision of the manuscript.

REFERENCES

(1) Rotruck, J. T.; Pope, A. L.; Ganther, H. E.; Swanson, A. B.; Hafeman, D. G.; Hoekstra, W. G. Selenium: biochemical role as a component of glutathione peroxidase. *Science* **1973**, *179* (73), 588–590.

(2) Berry, M. J.; Banu, L.; Larsen, P. R. Type I iodothyronine deiodinase is a selenocysteine-containing enzyme. *Nature* **1991**, *349* (6308), 438–440.

(3) Rayman, M. P. The importance of selenium to human health. *Lancet* **2000**, *365* (9225), 233–241.

(4) McKenzie, R. C.; Rafferty, T. S.; Beckett, G. J. Selenium: an essential element for immune function. *Immunol. Today* **1998**, *19* (8), 342–345.

(5) Yoshizawa, K.; Ascherio, A.; Morris, J. S.; Stampfer, M. J.; Giovannucci, E.; Baskett, C. K. Prospective study of selenium levels in toenails and risk of coronary heart disease in men. *Am. J. Epidemiol.* **2003**, *158* (9), 852–860.

(6) Rayman, M. P. Selenium in cancer prevention: a review of the evidence and mechanism of action. *Proc. Nutr. Soc.* **2005**, *64* (4), 527–542.

(7) Hoffmann, P. R. Selenium and asthma: a complex relationship. *Allergy* **2008**, *63* (7), 854–856.

(8) Faure, P. Protective effects of antioxidant micronutrients (vitamin E, zinc and selenium) in type 2 diabetes mellitus. *Clin. Chem. Lab. Med.* **2003**, *41* (8), 995–998.

(9) Reid, G. M.; Territ, H. Sudden Infant Death Syndrome and Placental Disorders: the Thyroid- selenium Link. *Med. Hypotheses* **1997**, *48* (4), 317–324.

(10) Whanger, P. D.; Weswig, P. H.; Schmitz, J. A.; Oldfield, J. E. Effects of selenium and vitamin E on blood selenium levels, tissue glutathione peroxidase activities and white muscle disease in sheep fed purified or hay diets. *J. Nutr.* **1977**, *107* (7), 1298–1307.

(11) Noguchi, T.; Cantor, A. H.; Scott, M. L. Mode of action of selenium and vitamin E in prevention of exudative diathesis in chicks. *J. Nutr.* **1973**, *103* (10), 1502–1511.

(12) Whitacre, M. E.; Combs, G. F., Jr. Selenium and mitochondrial integrity in the pancreas of the chick. *J. Nutr.* **1983**, *113* (10), 1972–83.

(13) The Research Group of Keshan Disease of Chinese Academy of Medical Sciences. Research on the relation between selenium and Keshan Disease. *Acta Acad. Med. Sin* . **1979**, *1* (1), 75–80.

(14) Research Team of Environment and Endemic Diseases. Studies on low selenium belt in china and pathogeny of Keshan and Kaschinbeck diseases. *Environ. Sci.* **1986**, 7 (4), 89–93.

(15) Yoon, I.; Werner, T. M.; Butler, J. M. Effect of source and concentration of selenium on growth performance and selenium retention in broiler chickens. *Poult. Sci.* **2007**, *86* (4), 727–730.

(16) Reeves, P. G.; Leary, P. D.; Gregoire, B. R.; Finley, J. W.; Lindlauf, J. E.; Johnson, L. K. Selenium bioavailability from buck-wheat bran in rats fed a modified AIN-93G torula yeast-based diet. *J. Nutr.* **2005**, *135* (11), 2627–2633.

(17) Kim, Y. Y.; Mahan, D. C. Comparative effects of high dietary levels of organic and inorganic selenium on selenium toxicity of growing-finishing pigs. *J. Anim. Sci.* **2001**, *79* (4), 942–948.

(18) Hoffman, D. J. Role of selenium toxicity and oxidative stress in aquatic birds. *Aquat. Toxicol.* **2002**, *57* (1/2), 112–126.

(19) Surai, P. F. Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. *Br. Poult. Sci.* **2000**, *41* (2), 235–243.

(20) Mahmoud, K. Z.; Edens, F. W. Influence of selenium sources on age-related and mild heat stress related changes of blood and liver glutathione redox cycle in broiler chickens (*Gallus domesticus*). *Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.* **2003**, *136* (4), 921–934.

(21) Utterback, P. L.; Parsons, C. M.; Yoon, I. Effect of supplementing selenium yeast in diets of laying hens on egg selenium content. *Poult. Sci.* **2005**, *84* (12), 1900–1901.

(22) Payne, R. L.; Lavergne, T. K.; Southern, L. L. Effect of inorganic versus organic selenium on hen production and egg selenium concentration. *Poult. Sci.* **2005**, *84* (2), 232–237.

(23) Leeson, S.; Namkung, H.; Caston, L.; Durosoy, S.; Schlegel, P. Comparison of selenium levels and sources and dietary fat quality in diets for broiler breeders and layer hens. *Poult. Sci.* **2008**, *87* (12), 2605–2612.

(24) Suhajda, A.; Hegóczki, J.; Janzsó, B.; Pais, I.; Vereczkey, G. Preparation of selenium yeasts: I. Preparation of selenium enriched yeasts *Saccharomyces cerevisiae*. *J. Trace Elem. Med. Biol.* **2000**, *14* (1), 43–47.

(25) Agarwal, N.; Kamra, D. N.; Chaudhary, L. C.; Sahoo, A.; Pathak, N. N. Selection of *Saccharomyces cerevisiae* strains for use as a microbial feed additive. *Lett. Appl. Microbiol.* **2000**, *31* (4), 270–273.

(26) Czerucka, D.; Piche, T.; Rampal, P. Yeast as probiotics-*Saccharomyces boulardii*. *Aliment. Pharmacol. Ther.* **2007**, *26* (6), 767–778.

(27) Yang, J.; Hang, K.; Qin, S.; Wu, X.; Zhao, Z.; Chen, F. Antibacterial action of selenium-enriched probiotics against pathogenic *Escherichia coli*. *Dig. Dis. Sci.* **2009**, *54* (2), 246–254.

(28) Reid, G.; Burton, J. Use of Lactobacillus to prevent infection by pathogenic bacteria. *Microbes Infect.* **2002**, *4* (3), 319–324.

(29) Elmer, G. W. Probiotics: "living drugs". *Am. J. Health. Syst. Pharm.* **2001**, *58* (12), 1101–1109.

(30) Szajewska, H.; Skorka, A.; Ruszczyński, M.; Gieruszczak-Białek, D. Meta-analysis: Lactobacillus GG for treating acute diarrhea in children. *Aliment. Pharmacol. Ther.* **2007**, *25* (8), 871–881.

(31) Lin, D. C. Probiotics as functional foods. *Nutr. Clin. Pract.* **2003**, *18* (6), 497–506.

(32) Brown, A. C.; Valiere, A. Probiotics and medical nutrition therapy. *Nutr. Clin. Care* **2004**, *7* (2), 56–68.

(33) Calomme, M.; Hu, J.; Van den Branden, K.; Vanden Berghe, D. A. Seleno-lactobacillus. An organic selenium source. *Biol. Trace Elem. Res.* **1995**, *47* (1−3), 379–383.

(34) Ren, Z.; Zhao, Z.; Wang, Y.; Huang, K. Preparation of selenium/zinc-enriched probiotics and their effect on blood selenium and zinc concentrations, antioxidant capacities, and intestinal microflora in canine. *Biol. Trace Elem. Res.* **2011**, *141* (1−3), 170–183.

(35) NRC (National Research Council). *Nutrient Requirements of Poultry*, 9th revised ed.; National Academy Press: Washington, DC, 1994.

(36) Pan, C.; Huang, K.; Zhao, Y.; Qin, S.; Chen, F.; Hu, Q. Effect of selenium source and level in hen's diet on tissue selenium deposition and egg selenium concentrations. *J. Agric. Food Chem.* **2007**, *55* (3), 1027–1032.

(37) Salma, U.; Miah, A. G.; Tareq, K. M.; Maki, T.; Tsujii, H. Effect of dietary Rhodobacter capsulatus on egg-yolk cholesterol and laying hen performance. *Poult. Sci.* **2007**, *86* (4), 714–719.

(38) Elkin, R. G.; Rogler, J. C. Reduction of the cholesterol content of eggs by the oral administration of lovastatin to laying hens. *J. Agric. Food Chem.* **1990**, *38* (8), 1635–1641.

(39) Bennett, D. C.; Cheng, K. M. Selenium enrichment of table eggs. *Poult. Sci.* **2010**, *89* (10), 2166–2172.

(40) Panda, A. K.; Reddy, M. R.; Rama Rao, S. V.; Praharaj, N. K. Production performance, serum/yolk cholesterol and immune competence of White Leghorn layers as influenced by dietary supplementation with probiotic. *Trop. Anim. Health Prod.* **2003**, *35* (1), 85–94.

(41) Kurtoglu, V.; Kurtoglu, F.; Seker, E.; Coskun, B.; Balevi, T.; Polat, E. S. Effect of probiotic supplementation on laying hen diets on yield performance and serum and egg yolk cholesterol. *Food Addit. Contam.* **2004**, *21* (9), 817–823.

(42) Yörük, M. A.; Gül, M.; Hayirli, A.; Macit, M. The effects of supplementation of humate and probiotic on egg production and quality parameters during the late laying period in hens. *Poult. Sci.* **2004**, *83* (1), 84–88.

(43) Torres-Rodriguez, A.; Donoghue, A. M.; Donoghue, D. J.; Barton, J. T.; Tellez, G.; Hargis, B. M. Performance and condemnation rate analysis of commercial turkey flocks treated with a Lactobacillus spp.-based probiotic. *Poult. Sci.* **2007**, *86* (3), 444–446.

(44) Yeo, J.; Kim, K. I. Effect of feeding diets containing an antibiotic, a probiotic, or yucca extract on growth and intestinal urease activity in broiler chicks. *Poult. Sci.* **1997**, *76* (2), 381–385.

(45) Lutful Kabir, S. M. The role of probiotics in the poultry industry. *Int. J. Mol. Sci.* **2009**, *10* (8), 3531–3546.

(46) Pappas, A. C.; Karadas, F.; Surai, P. F.; Speake, B. K. The selenium intake of the female chicken influences the selenium status of her progeny. *Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.* **2005**, *142* (4), 465–474.

(47) Wakebe, M. Feed for chicken and for hen. Japanese Patent Office, Application number 08-179629, Patent number 10-023864. Jan 27, 1998. Applicant: Fujisawa Pharmaceut Co., Ltd.

(48) Pappas, A. C.; Acamovic, T.; Sparks, N. H.; Surai, P. F.; McDevitt, R. M. Effects of supplementing broiler breeder diets with organic selenium and polyunsaturated fatty acids on egg quality during storage. *Poult. Sci.* **2005**, *84* (6), 865–874.

(49) Arnold, R. L.; Olson, O. E.; Carlson, C. W. Dietary selenium and arsenic additions and their effects on tissue and egg selenium. *Poult. Sci.* **1973**, *52* (3), 847–854.

(50) Patton, N. D. Organic selenium in the nutrition of laying hens: Effects on egg selenium content, egg quality and transfer to developing chick embryos. Ph.D. Diss., University of Kentucky: Lexington, KY, 2000.

(51) Mohan, B.; Kadirvel, R.; Bhaskaran, M.; Natarajan, A. Effect of probiotic supplementation on serum/yolk cholesterol and on egg shell thickness in layers. *Br. Poult. Sci.* **1995**, *36* (5), 799–803.

(52) Haddadin, M. S.; Abdulrahim, S. M.; Hashlamoun, E. A.; Robinson, R. K. The effect of *Lactobacillus acidophilus* on the production and chemical composition of hen's eggs. *Poult. Sci.* **1996**, *75* (4), 491–494.

(53) Pereira, D. I; Gibson, G. R. Cholesterol assimilation by lactic acid bacteria and bifidobacteria isolated from the human gut. *Appl. Environ. Microbiol.* **2002**, *68* (9), 4689–4693.

(54) Lye, H. S.; Rusul, G.; Liong, M. T. Removal of cholesterol by lactobacilli via incorporation and conversion to coprostanol. *J. Dairy. Sci.* **2010**, *93* (4), 1383–1392.

(55) Liong, M. T.; Shah, N. P. Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. *J. Dairy Sci.* **2005**, *88* (1), 55–66.

(56) Lambert, J. M.; Bongers, R. S.; de Vos, W. M.; Kleerebezem, M. Functional analysis of four bile salt hydrolase and penicillin acylase family members in *Lactobacillus plantarum* WCFS1. *Appl. Environ. Microbiol.* **2008**, *74* (15), 4719–4726.

(57) De Preter, V.; Vanhoutte, T.; Huys, G.; Swings, J.; De Vuyst, L.; Rutgeerts, P.; Verbeke, K. Effects of *Lactobacillus casei* Shirota, *Bifidobacterium breve*, and oligofructose-enriched inulin on colonic nitrogen-protein metabolism in healthy humans. *Am. J. Physiol.* **2007**, *292* (1), G358–G368.

(58) Shimada, H.; Kondo, K.; Fraser, P. D.; Miura, Y.; Saito, T.; Misawa, N. Increased carotenoid production by the food yeast *Candida utilis* through metabolic engineering of the isoprenoid pathway. *Appl. Environ. Microbiol.* **1998**, *64* (7), 2676–280.

(59) Ikushima, S.; Fujii, T.; Kobayashi, O.; Yoshida, S.; Yoshida, A. Genetic engineering of *Candida utilis* yeast for efficient production of L-lactic acid. *Biosci., Biotechnol., Biochem.* **2009**, *73* (8), 1818–1824.

(60) Payne, R. L.; Southern, L. L. Changes in glutathione peroxidase and tissue selenium concentrations of broiler after consuming a diet adequate in selenium. *Poult. Sci.* **2005**, *84* (8), 1268–1276.

(61) Pfrieger, F. W. Role of cholesterol in synapse formation and function. *Biochim. Biophys. Acta* **2003**, *1610* (2), 271–280.

(62) Schreurs, B. G. The effects of cholesterol on learning and memory. *Neurosci. Biobehav. Rev.* **2010**, *34* (8), 1366–1379.

(63) Nguyen, D. H.; Espinoza, J. C.; Taub, D. D. Cellular cholesterol enrichment impairs T cell activation and chemotaxis. *Mech. Ageing. Dev.* **2004**, *125* (9), 641–650.

(64) Havel, R. J. Biology of cholesterol, lipoproteins and atherosclerosis. *Clin. Exp. Hypertens., Part A* **1989**, *11* (5−6), 887–900.

(65) Xu, J.; Eilat-Adar, S.; Loria, C.; Goldbourt, U.; Howard, B. V.; Fabsitz, R. R.; Zephier, E. M.; Mattil, C.; Lee, E. T. Dietary fat intake and risk of coronary heart disease: the strong heart study. *Am. J. Clin. Nutr.* **2006**, *84* (4), 894–902.

(66) Houston, D. K.; Ding, J.; Lee, J. S.; Garcia, M.; Kanaya, A. M.; Tylavsky, F. A.; Newman, A. B.; Visser, M.; Kritchevsky, S. B. Dietary fat and cholesterol and risk of cardiovascular disease in older adults: the health ABC study. *Nutr. Metab. Cardiovasc. Dis.* **2011**, *21* (6), 430–437.

(67) Spence, J. D.; Jenkins, D. J.; Davignon, J. Dietary cholesterol and egg yolks: not for patients at risk of vascular disease. *Can. J. Cardiol.* **2010**, *26* (9), e336–339.

(68) Nutrition Council of China. *Acta Nutr. Sin.* **2001**, *23*, 193–196.

(69) Fernandez, M. L.; Calle, M. Revisiting dietary cholesterol recommendations: does the evidence support a limit of 300 mg/d? *Curr. Atheroscler. Rep.* **2010**, *12* (6), 377–383.