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Effect of Red Mold Rice Supplements on Serum and Egg Yolk Cholesterol Levels of Laying Hens

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Monacolin K is a secondary metabolite of *Monascus* species and reduces cholesterol levels. This research focuses on the effect of adding red mold rice to hens' diet on cholesterol level in egg yolk and on cholesterol, triglyceride, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels in serum. Forty-eight Hy-line laying hens of 48 weeks of age were studied by dividing them into four groups. Except for the control group, the feed for three other groups contained 2.0, 5.0, and 8.0% red mold rice (monacolin K concentrations were 0.0145, 0.035, and 0.056%, respectively). The experiment lasted 6 weeks. During this period, egg weight and egg production were recorded every day, and cholesterol, triglyceride, HDL, and LDL in serum were measured weekly as well. The result showed that the cholesterol in eggs produced by experimental groups was lower than that of the control group (0%, 194.14 ± 8.30; 2%, 167.17 ± 4.34; 5%, 168.93 ± 9.38; 8%, 183.02 ± 7.63 mg/egg; $p < 0.05$), and the triglyceride (0%, 1494 ± 178; 2%, 1280 ± 174; 5%, 1189 ± 248; 8%, 1381 ± 218 mg/dL; $p < 0.05$) and LDL levels (0%, 36.81 ± 5.53; 2%, 32.25 ± 7.93; 5%, 30.06 ± 4.39; 8%, 28.81 ± 4.16 mg/dL; $p < 0.05$) were also significantly lowered in the experimental groups. However, the HDL level did not show significant change for either control or experimental groups (0%, 36.06 ± 3.96; 2%, 36.25 ± 5.39; 5%, 33.13 ± 3.68; 8%, 31.44 ± 4.29 mg/dL; $p > 0.05$). Besides, the addition of red mold rice also helps to inhibit production of malondialdehyde (MDA) in serum lipid oxidation (0%, 27.42 ± 0.53; 2%, 25.62 ± 0.76; 5%, 24.35 ± 0.59; 8%, 23.63 ± 0.48 μM; $p < 0.05$).

KEYWORDS: ***Monascus*; monacolin K; red mold rice; cholesterol; egg yolk; laying hens**

INTRODUCTION

The relationship between cholesterol and atherosclerosis has long been of concern. Plasma total cholesterol and low-density lipoprotein (LDL) are closely related to atherosclerosis, and excessive concentration of these two materials may lead to coronary artery disease or death (*1*, *2*). Ordinary chicken eggs provide protein, vitamins, and lipids that contain high levels of cholesterol. Thus, eggs are considered to be a high-cholesterol food. The American Heart Association recommended that cholesterol consumption for each person should be limited to 300 mg per day, and the whole egg yolk consumption should be limited to three to four per week (*3*, *4*). People are paying more attention to health and are thus lowering their consumption of high-cholesterol food. Because cholesterol in eggs accounts for >50% of daily intake, the consumption of eggs can hardly increase. Therefore, low-cholesterol eggs would not only be beneficial to the public's health but also bear business advantage.

Egg cholesterol is first biosynthesized in the liver of laying hens (*5*) and secreted into the plasma in the form of very low-density lipoproteins (VLDL) (*6*, *7*). VLDL are then transported to the ovary, where they are bound and taken up by growing chicken oocytes via receptor-mediated endocytosis (*8*). Egg cholesterol has been shown to vary with species of bird, breed, or strain, as well as age of fowl.

Egg cholesterol content can be altered by (1) genetic selection, such as upward direction method (*9*) or selection of hens that produce low-cholesterol eggs (*10*); (2) diet alteration, such as adding dietary fiber (*11*), cholesterol (*12*, *13*), saturated and polyunsaturated fatty acid (*14*), cupric sulfate pentahydrate (*15*), protein and essential amino acids (*16*), chia (*17*), or red microalga (*18*) to laying hens' feed; and (3) using hypocholesterolemic agents, such as adding probucol (*19*), dichloroacetate (*20*), atorvastatin, lovastatin, simvastatin (*21*−*24*), or tocotrienols (*25*) to laying hens' feed to regulate the egg yolk cholesterol level. Of all the methods mentioned above, lovastatin (monacolin K) showed a different result in lowering the cholesterol level in eggs.

Monascus species have been widely used in the diet and as folk remedies for a thousand years in Asia. Endo (*26*) discovered the more active methylated form of compactin known as monacolin K (lovastatin) in broths of *Monascus ruber*. Monacolin K (also known as lovastatin, mevinolin, and mevacor) is

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a secondary metabolite of *Monascus* species, which inhibits the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in cholesterol biosynthesis, that is, the conversion of HMG-CoA to mevalonate, catalyzed by HMG-CoA reductase (*27*). As a matter of fact, monacolin K produced by *Monascus* species has been used as a functional dietary supplement to reduce the cholesterol level in the human body.

The objective of this study was to investigate the effect of red mold rice supplementation at different concentrations on egg quality, laying performance, serum cholesterol, serum triglyceride, HDL, LDL, serum lipid peroxidation, and egg yolk cholesterol in Hy-line laying hens. To our knowledge, the effect of red mold rice on eggs has yet to be investigated in previous studies.

MATERIALS AND METHODS

Microorganism. The screening for red mold rice production was carried in our laboratory using the strain *Monascus purpureus* NTU803. The cultures were maintained on potato dextrose agar (PDA) slanted at 10 °C and transferred monthly.

Microorganism and Seed Cultures. The screening for red mold rice production was carried using *M. purpureus* NTU803. The cultures were maintained on PDA slanted at 10 °C and transferred monthly. Seed cultures were prepared by transferring a loopful of spore from the PDA slanted into a 500 mL Hinton flask containing 100 mL of basal medium (100 g of dextrose, 10 g of peptone, 2 g of KNO_3, 2 g of $NH_4H_2PO_4$, 0.5 g of $MgSO_4 \cdot 7H_2O$, and 0.1 g of $CaCl_2$ made up in 1000 mL of distilled water, pH 6.0). The cultures were incubated at 30 °C for 48 h at 110 rpm. After that, 5% inoculum was transferred for solid-state fermentation.

Solid-State Fermentation and Red Mold Rice Preparation. The red mold rice preparation method had been proposed in our previous studies (*28*). Long-grain rice was purchased from a local supermarket and was used as the substrate for red mold rice production under solid-state cultivation. Five hundred grams of long-grain rice was soaked in distilled water for 8 h. After that, excess water was removed with a sieve. The soaked rice was autoclaved for 20 min at 121 °C in a "koji-dish" (the koji-dish is made of wood with the dimensions 30 × 20 × 5 cm). After being cooled, the substrate was inoculated with a 5% (v/w) spore suspension of *M. purpureus* NTU803, and the inoculated substrate was cultivated at 30 °C for 10 days. The crushed and dried substrate with the mold, called red mold rice, was used as a supplement to the basal diet.

Experimental Design. A feeding trial was conducted during the summer on hens of 48−54 weeks of age. Forty-eight Single-Comb White Leghorn (SCWL) layers hens (Hy-line W36) were divided at radom into four treatment groups with 12 birds each and were placed on one of the following four dietary treatments: basal diet or basal diet supplemented with red mold rice at a content rate of 2.0, 5.0, or 8.0% (monacolin K concentration equivalent to 0.014, 0.035, or 0.056%, respectively). The composition of the basal diet is shown in **Table 1**. The hens were housed at random in cages with two birds per cage. Ad libitum food and water were provided throughout the experimental period.

Performance Variables and Egg Quality. Body weights of the laying hens were recorded at the beginning and at the end of the study to determine the weight changes. Feed consumption was measured weekly. The number of eggs and egg weights were recorded daily. Egg quality measurements were conducted weekly using all of the eggs laid during 1 day from all treatments groups. Egg quality was expressed in terms of eggshell weight, eggshell thickness, specific gravity, yolk weight, and yolk color. Eggshell samples were taken from three locations (air cell, equator, and sharp end) using a micrometer gauge (Mitutoyo, Japan) to determine eggshell thickness. Yolk color (1, light yellow; 15, orange) was measured by using the Roche color fan. Specific gravity of eggs was determined by using the saline flotation method proposed by Hamilton et al. (*29*). Salt solutions were prepared in incremental concentration of 0.005 in the range from 1.065 to 1.110.

Table 1. Composition and Calculated Analysis of Basal Diets

ingredient	
corn, %	56.8
soybean meal, %	12.0
full fat soybean meal, %	10.0
meat and bone meal, %	3.0
fish meal, %	1.0
corn gluten meal	3.0
wheat bran, %	3.0
limestone, %	6.5
oyster	2.0
oil, %	1.0
dicalcium phosphate, %	0.3
sodium chloride, %	0.4
vitamin[a] and mineral[b] premix, %	1.0
total	100.0
calculated and determined analysis	
crude protein, %	17.0
fat, %	4.5
metabolizable energy, kcal/kg	2800.0
total phosphate, %	0.56
calcium, %	4.2
lysine	0.9
methionine	0.45
glycine, %	0.77

[a] Vitamin premix supplied the following per kilogram of basal diet: vitamin A, 12600 IU; vitamin D_3, 3000 ICU; vitamin E, 18 IU; vitamin K, 3.6 mg; riboflavin, 6.6 mg; niacin, 40.8 mg; pantothenic acid, 14.4 mg; vitamin B_{12}, 0.012 mg; folic acid, 0.6 mg; pyridoxine, 1.2 mg. [b] Mineral premix supplied the following per kilogram of basal diet: Mn, 31 mg; Cu, 7.1 mg; Zn, 44.8 mg; Fe, 20 mg.

Eggshell strength was determined by using the puncture method suggested by Carter (texture analyzer, Stable Micro System TA-XT2i) (*30*).

Egg Preparation for Cholesterol Analyses. Eggs were broken and the yolks separated from the albumen and rolled across a moistened paper towel to remove the chalazae and any adhering albumen. Three yolk composites from each treatment were prepared by separating the yolk material from the vitelline membrane and blending gently by hand to prevent incorporation of air bubbles into the sample. About 0.2 g of pooled yolk samples (three eggs per pool) were analyzed for cholesterol concentration by using the spectrophotometric method proposed by Rudel and Morris (*31*).

Serum Preparation for Cholesterol, Triglyceride, HDL, and LDL Analyses. Blood samples of ∼2 mL were collected from the vein on the brachial wing of individual birds, and the cholesterol, triglyceride, LDL, and HDL concentrations were determined using a commerical diagnostic kit provided by Cobas Mira (Roche Diagnostic Systems Inc., Montclair, NJ).

Serum Lipid Peroxidation (TBARS Assay). Thiobarbituric acid-reactive substances (TBARS) were monitored according to the procedure previously described (*32*). Briefly, 500 μL of serum was well mixed with 3 mL of 5% trichloroacetic acid and 1 mL of freshly prepared 60 mmol/L thiobarbituric acid (TBA). After incubation at 80 °C for 90 min, the samples were cooled at room temperature and then centrifuged at 1000*g* for 15 min at 4 °C. The supernatant absorbance was read at 535 nm.

Statistical Analyses. All data obtained in the experiment were subjected to analysis of variance (ANOVA); if appropriate ($p < 0.05$), post-hoc analyses were carried out using Duncan's test for multiple comparisons. Statements of statistical significance were based on $p < 0.05$. These analyses were accomplished by using statistical analyses configured for a computer (SPSS, release 9.0, SPSS, Inc).

RESULTS

Analysis of Monacolin K in Hens' Feed. This research divided the hens into four groups. Except for the control group, 2.0, 5.0, and 8.0% of red mold rice was added to the laying

Table 2. Effect of Red Mold Rice on Laying Hen Performance Egg Quality[a]

red mold rice (%)	monacolin K content (%)	specific gravity	shell wt (g)	shell thickness (mm)	shell strength (g/cm^2)	yolk color score
0	0	1.0855 ± 0.0032a	5.77 ± 0.13ab	0.433 ± 0.012a	4099 ± 234a	6.90 ± 0.18a
2.0	0.014	1.0889 ± 0.0022a	5.72 ± 0.20a	0.439 ± 0.009a	3878 ± 215ab	7.11 ± 0.18a
5.0	0.035	1.0876 ± 0.0030a	5.92 ± 0.07bc	0.438 ± 0.013a	3583 ± 251b	7.38 ± 0.21b
8.0	0.056	1.0875 ± 0.0044a	5.96 ± 0.13c	0.441 ± 0.006a	3698 ± 180b	8.02 ± 0.15c

[a] The duration of the experiment was 42 days. Data are presented as means ± SD. Mean values within each column with different letters are significantly different ($p < 0.05$).

Table 3. Effect of Red Mold Rice on Laying Hen Performance Body Weight, Egg Production, Egg Weight, Feed Consumption, and Feed Conversion[a]

red mold rice (%)	monacolin K content (%)	body wt change (g)	egg production (%)	egg wt (g)	yolk wt (g/egg)	yolk cholesterol (mg/egg)	feed consumption (g/hen/day)	feed conversion (kg of feed/kg of egg)
0	0	−29.0	80.93 ± 3.23a	64.86 ± 0.81a	16.49 ± 0.48a	194.14 ± 8.30a	89.84 ± 2.15a	1.726 ± 0.032a
2.0	0.014	−15.8	77.77 ± 2.72ab	61.51 ± 1.53b	15.97 ± 0.83a	167.17 ± 4.34b	91.72 ± 1.91a	1.932 ± 0.025b
5.0	0.035	± 7.5	80.31 ± 2.90ab	65.51 ± 0.90a	16.55 ± 0.83a	168.93 ± 9.38bc	94.43 ± 2.11b	1.826 ± 0.019c
8.0	0.056	−46.2	76.96 ± 1.66b	65.14 ± 1.31a	16.77 ± 0.57a	183.02 ± 7.63c	97.17 ± 1.82c	1.968 ± 0.055b

[a] The duration of the experiment was 42 days. Data are presented as means ± SD. Mean values within each column with different letters are significantly different ($p < 0.05$).

Table 4. Effect of Red Mold Rice on Laying Hen Performance Serum LDL, HDL, Triglyceride, and Cholesterol[a]

red mold rice (%)	monacolin K content (%)	mg/dL				ratios	
		LDL	HDL	triglyceride	cholesterol	HDL/cholesterol	HDL/LDL
0	0	36.81 ± 5.53a	36.06 ± 3.96a	1494 ± 178a	141.66 ± 9.50a	0.254 ± 0.013a	0.987 ± 0.079a
2.0	0.014	32.25 ± 7.93ab	36.25 ± 5.39a	1280 ± 174b	129.83 ± 13.18ab	0.278 ± 0.021b	1.146 ± 0.114b
5.0	0.035	30.06 ± 4.39b	33.13 ± 3.68a	1189 ± 248b	114.25 ± 16.43c	0.291 ± 0.015b	1.107 ± 0.053b
8.0	0.056	28.81 ± 4.16b	31.44 ± 4.29a	1381 ± 218ab	126.17 ± 19.75bc	0.250 ± 0.012a	1.093 ± 0.079b

[a] The duration of the experiment was 42 days. Data are pressented as means ± SD. Mean values within each column with different letters are significantly different ($p < 0.05$).

hens' feed, respectively. Through HPLC analysis, we found that the contents of monacolin K in the hens' feeds were 0.0140 ± 0.0003, 0.0350 ± 0.0007, and 0.0560 ± 0.0004%, respectively, and the results are shown in **Table 2**.

Egg Quality. Table 2 shows the items analyzed. These include specific gravity, shell weight, shell thickness, shell strength, and yolk color. The results showed that the content level of red mold rice had no significnt effect on specific gravity and shell thickness. Addition of 8.0% red mold rice showed a significant difference in shell weight. On the contrary, addition of 2.0% red mold rice showed no significant differnce in shell weight. Addition of 5.0% red mold rice showed the lowest shell strength and was significantly different compared to control groups. The data indicated that addition of red mold rice seems to reduce shell strength. The yolk color score showed that the darkest color occurred when 8.0% of red mold rice was incorporated into the feed, and the lightest yolk color was in the control group.

Laying Performance. The effect of red mold rice content in the hens' diet on body weight, egg production, egg weight, yolk weight, feed consumption, and feed conversion is shown in **Table 3**. The hens showed significant loss of body weight before and after the experiment (0%, 29.0 g; 2.0%, 15.8 g; 5.0%, 7.5 g; 8.0%, 46.2 g). Addition of 8.0% red mold rice caused the greatest body weight loss. The reason might be due to the alteration of the diet composition, which may have induced an appetite change. Weight loss of hens in other groups might be due to the fact that the experiment was conducted under high ambient temperature during the hot season; therefore, heat stress might be the cause for body weight loss (*33*). These groups showed no significant difference in egg production. The group fed 2.0% red mold rice showed the lightest egg weight, which was significantly different from that of other groups. Yolk weight had no significant difference across groups. Feed consumption increased by 2−8% as the content of red mold rice increased. Feed conversion increased by 6−14% depending on the red mold rice content and was significantly different among the four dietary treatments.

Egg Cholesterol Concentration. The degree of reduction on egg cholesterol varied with the content of red mold rice in the feed. Egg cholesterol of the control group is 11.77 mg/g of yolk or 194.14 mg/egg. After 6 weeks of feeding, the group fed 2.0% red mold rice showed maximum reduction of cholesterol by 13.89%, and the cholesterol concentration was found to be 10.47 mg/g of yolk or 167.17 mg/egg. In addition, cholesterol reductions of 12.98 and 5.73% were found for the 5.0% and 8.0% red mold rice diets, respectively. On the basis of the data, addition of red mold rice to the hens; diets did help to reduce egg cholesterl concentation (**Table 3**).

Serum Cholesterol, HDL, LDL, and Triglyceride Concentration. Addition of red mold rice in the hens' diets showed effects on serum cholesterol, HDL, LDL, and triglyceride concentration, and the data are shown in **Table 4**. The result indicated that addition of red mold rice can effectively reduce LDL, and the addition of 8.0% of red mold rice to the diet can significantly reduce LDL by 21.73%. Triglyceride analysis showed that when 5.0% red mold rice was incorporated in the hens' diet, the triglycerides can be significantly reduced by 20.41%. The result also indicated that the red mold rice can

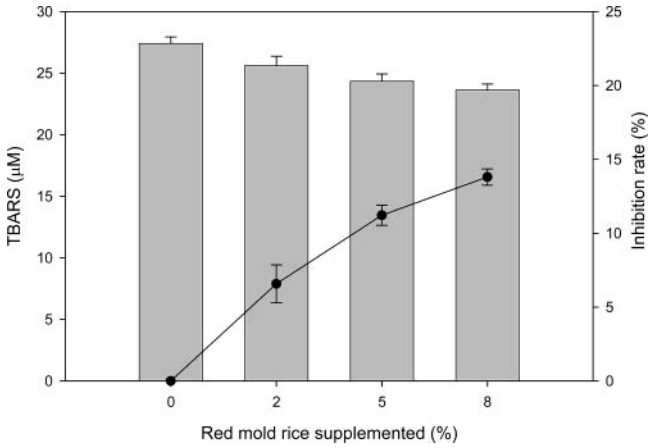

Figure 1. Effect of red mold rice supplementation on serum TBARS levels.

increase ratios of HDL over cholesterol and HDL over LDL. The result agreed with that reported by Qureshi and Peterson (*25*).

Effect on Serum Lipid Peroxidation. Malondialdehyde (MDA) is a byproduct of lipid peroxidation. When reacted with TBA, a red compound named MDA-TBA (TBARS) would be produced. TBARS has maximum absorption under a wavelength of 532 nm and is positively correlated to MDA (*34*). As shown in **Figure 1**, TBARS concentration was tested after 6 weeks of feeding red mold rice added to the diet. Red mold rice showed a 6−14% inhibition rate to serum lipid peroxidation and proved that red mold rice can reduce lipid peroxidation in serum. Inhibition rate (%) = [1 − (serum TBARS value of red mold rice treatment/serum TBARS value of control treatment)] × 100.

DISCUSSION

Secondary metabolites produced from filamentous fungus *Monascus* species include (1) pigment groups (red, rubropuctamine and monascorubranine; yellow, ankaflavin and monascin; orange, monascorubrin and rubropunctanin) (*35*); (2) antihypercholesterolemic agents such as monacolin K; and (3) antioxidant ingredients (*36*). Yolk color is a crucial factor during evaluation and was determined by the variation of pigment in oxycarotenoids. However, the factors that influence egg yolk color include genetics, diets, and environmental systems (*37*). Saunders et al. (*38*) concluded that antioxidants had a synergistic effect on improving egg yolk color. *Monascus* has been known as an edible pigment. In our laboratory, the antioxidant property of *Monascus* species had been verified in our previous studies (*39*). Therefore, the addition of red mold rice to the hens' diet proved to exhibit a positive effect on yolk color as the experimental groups show 3−16% increases of yolk color when compared to the control group. As far as eggshell quality is concerned, although red mold rice can increase shell weight, it seems to exhibit very little effect on shell strength. Whether red mold rice has an effect on the use of calcium in hens' bodies, which, in turn, affect shell strength, needs to be investigated in further research. Groups that received red mold rice showed higher daily feed consumption and feed conversion than the control group. However, egg productions for all of the groups that received red mold rice were almost the same, and the difference lies in the egg size only. The results agreed with those of Elkin and Rogler (*22*).

Sim and Bragg (*40*) believed that the cholesteorl concentration in serum should be reduced prior to the reduction of yolk cholesterol concentration. Therefore, reducing serum cholesterol by applying drugs seems to be the best approach to reduce yolk cholesterol. However, whether there is any drug residue remaining in eggs also need to be considered. In this research, red mold rice with monacolin K was used as the additive to lower cholesterol. Monaolin K was considered to be a statins substance and was used as the cholesterol-reducing drug in medical treatment (*41*). In cholesterol biosynthesis, HMG-CoA reductase (EC 1.1.1.34) carries the reaction of HMG-CoA in synthesizing mevalonate. In the early stage of biosynthsis, it is a rate-limiting enzyme. Therefore, monacolin K could be used to inhibit enzyme reaction and lower cholesterol concentration. However, the amount of monacolin K added to the feed diet was determined by the rate of biosynthsis and the hens' body weight. A normal hen weighs 1.5−1.7 kg, and the rate of biosynthesis is 300 mg of cholesterol/day. However, a 70 kg human adult can synthsize 800 mg (*42*), thus setting the normal daily human dose at 40 mg. The normal daily hen dose of monacolin K should then be 15 mg of monacolin K [(300 × 40)/800]. Thus, the human equivalent dose is ∼15 mg of monacolin K/100 g, or 0.015% monacolin K. The dose used in this research was based on the above reasoning, and 2.0, 5.0, or 8.0% of red mold rice was incorporated into hens diet to reach 0.014, 0.035, and 0.056% of monacolin K, respectively, to study the effect of the addition of red mold rice in the hens' diet on serum cholesterol, HDL, LDL and triglyceride concentrations.

The results showed that the addition of 2.0, 5.0, and 8.0% red mold rice to hens' diet could reduce serum cholesterol by 8.35, 19.35, and 10.93%, respectively, reduce triglyceride concentration by 14.32, 20.41, and 7.56%, respectively, and reduce LDL concentration by 12.39, 18.34, and 21.37%, respectively. Monacolin K could inhibit the activity of HMG-CoA reductase and increase the activity of LDL receptor. Therefore, it could strengthen direct absorption of VLDL in the liver and reduce the amount of VLDL transformed to LDL to achieve the goal of cholesterol reduction (*43*). HDL showed no significant change when red mold rice was added to the hens' diet and showed slight decreases at high concentrations of monacolin K. When HDL/LDL and HDL/cholesterol ratios were compared, it was found that groups receiving red mold rice diets showed higher levels (HDL/LDL, 16.1−10.74%; HDL/cholesterol, 0−14.6%). In addition, egg cholesterol contents varied inversely with the HDL/LDL ratio. The serum LDL and HDL are dose dependent as shown in **Table 4**. The serum LDL and HDL would decrease as the concentration of red mold rice in the diet increased. However, the egg cholesterol content was not dose dependent. Rather, it was related to the ratio of serum HDL and LDL. As can been seen from **Tables 3** and **4**, the egg cholesterol would decrease with increasing HDL/LDL ratios. Therefore, the best way to achieve maximum egg cholesterol reduction would be to control the HDL/LDL ratios rather than to control the diet dosage only. Compared to hypocholesterolemic agents in humans, red mold rice showed a similar function in reducing cholesterol, trglyceride, HDL, and LDL in laying hens. However, the degree of reduction was correlated to the hens' age, lovastatin concentraiton, and feed time (*21*, *24*, *44*).

Some research has shown that atherosclerosis is correlated not only to cholesterol and LDL in blood but also to the oxidation of LDL (*45*). Chen et al. pointed out that antioxidant ability and cardiovascular diseases were closely related (*46*). Aviram et al. (*47*) argued that lovastatin had a special chemical composition; it was easier to react with metal ions to produce coordination compounds. In other words, lovastatin was an antioxidant and could inhibit lipid peroxidation. They also pointed out that during the oxidation of LDL, decreasing the

TBARS concentration of lovastatin was related to the duration and dose of lovastatin. Jeon et al. (*48*) pointed out that lovastatin can not only significantly reduce hepatic CAT activity but also reduce peroxidation of lipid in plasms and liver. This research showed that the antioxidant ability of serum can be significantly increased by red mold rice and proved that red mold rice could increase the antioxidant ability of laying hens.

As far as reducing egg cholesterol concentration was concerned, Elkin and Rogler (22) suggested that the addition of 0.2407% lovastatin in feed for 9 days could decrease the amount of cholesterol by 13%. Elkin and Yan (*23*) pointed out that the addition of 0.03 or 0.06% lovastatin could lower egg cholesterol concentration by 4 or 7% (18-week-old hens fed for 5 weeks). Mori et al. (*24*) pointed out that the addition of 0.0005 or 0.0015% lovastatin could lower egg cholesterol concentration by 7.5 or 12.7% (30-week-old hens fed for 12 weeks). On the other hand, Luhman et al. *(44*) aruged that the addition of 0.0035% lovastatin showed no significant decrease in egg cholesterol concentration (69-week-old hens fed for 5 weeks), and the lovastatin used would remain in the hens' liver. On the basis of the above research, it was obvious that monacolin K had no consistent effect on reducing egg cholesterol concentration, and the reason might be due to hen age, feed amount, and feed time. This research uses Hy-line hens of 58 weeks of age and fed red mold rice for 6 weeks. The result showed that the egg cholesterol was reduced by 13.89% for the group of hens that receved 0.0145% monacolin K, and there was no monacolin K residue found in eggs. The result is better than that reported in previous studies (data not included).

On the basis of the above experiment, it is reasonable to conclude that incorporating red mold rice in hens' diets can reduce egg cholesterol concentration, serum cholesterol, triglyceride level, and LDL concentration.

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LITERATURE CITED

(1) Carlson, L. A. Serum lipids and atherosclerotic disease. In *Metabolic Risk Factors in Ischemia Cardiovascular Disease*; Carlson, L. A., Pernow, B., Eds.; Raven Press: New York, 1982; pp 1−6.

(2) Pekkanen, A. N.; Punsar, S.; Karvonen, M. J. Short- and long-term association of serum cholesterol with mortality. *Am. J. Epidemiol.* **1992**, *135*, 1252−1258.

(3) American Heart Association. Dietary guidelines for healthy American adults. *Circulation* **1986**, *74*, 1465A−1468A.

(4) American Heart Association. Egg: AHA scientific/medical position. *Heart and Stroke A−Z Guide*; American Heart Association: Dallas, TX, 1996.

(5) Andrews, J. W.; Wagstaff, R. K.; Edwards, H. M. Cholesterol metabolism in the laying fowl. *Am. J. Physiol.* **1968**, *214*, 1078−1083.

(6) Burley, R. W.; Sleigh, R. W.; Shenstone, F. S. Lipoproteins from the blood and egg yolk of the hen: The transfer of very low-density lipoprotein to egg yolk and possible changes to apoprotein B. *Eur. J. Biochem*. **1984**, *142*, 171−176.

(7) George, R.; Barber, D. L.; Schneider, W. J. Characterization of the chicken oocyte receptor for low and very low-density lipoproteins. *J. Biol. Chem*. **1987**, *262*, 16838−16847.

(8) Nimpf, J.; Schneider, W. J. Receptor-mediated lipoprotein transport in laying hens. *J. Nutr*. **1991**, *121*, 1471−1474.

(9) Marks, H. L.; Washburn, K. W. Divergent selection for yolk lipid and reproductive efficiency of the hen. *Br. Poult. Sci*. **1977**, *18*, 179−188.

(10) Ansah, G. A.; Chan, C. W.; Touchburn, S. P.; Buckland, R. B. Selection for low yolk cholesterol in leghorn-type chickens. *Poult. Sci*. **1985**, *64*, 1−5.

(11) Menge, H.; Littlefield, L. H.; Frobish, L. T.; Weinland, B. T. Effect of cellulose and cholesterol on blood yolk lipids and reproductive efficiency on the hen. *J. Nutr*. **1974**, *104*, 1551−1566.

(12) Hammad, S. M.; Siegel, H. S.; Marks, H. L. Dietary cholesterol effects on plasma and yolk cholesterol fractions in selected lines of Japanese quail. *Poult. Sci*. **1996**, *75*, 933−942.

(13) Jiang, Z.; Cherian, G.; Robinson, F. E.; Sim, J. S. Effect of feeding cholesterol to laying hens and chicks on cholesterol metabolism in pre- and posthatch chicks. *Poult. Sci*. **1990**, *69*, 1694−1701.

(14) Grundy, S. M. What is the desirable ratio of saturated, polyunsaturated, and monounsaturated fatty acids in the diet? *Am. J. Clin. Nutr*. **1997**, *66*, 988S−990S.

(15) Pesti, G. M.; Bakalli, R. I. Studies on the effect of feeding cupric sulfate pentahydrate to laying hens on egg cholesterol content. *Poult. Sci*. **1998**, *77*, 1540−1545.

(16) Hammersh, M.; Kjaer, J. B. Phase feeding for laying hens: Effect of protein and essential amino acids on egg quality and production. *Acta Agric. Scand*. **1999**, *49*, 31−41.

(17) Ayerza, R.; Coates, W. Dietary levels of chia: Influence on yolk cholesterol, lipid content and fatty acid composition for two strains of hens. *Poult. Sci*. **2000**, *79*, 724−739.

(18) Ginzberg, A.; Cohen, M.; Sod-Moriah, U. A.; Shany, S.; Rosenshtrauch, A.; Arad, S. Chickens fed with biomass of the red microalga *Porphyridium* sp. have reduced blood cholesterol level and modified fatty acid composition in egg yolk. *J. Appl. Phycol*. **2000**, *12*, 324−330.

(19) Naber, E. C.; Elliot, J. F.; Smith, T. L. Effect of probucol on reproductive performance, egg yolk cholesterol content, and lipid metabolism in the laying hen. *Poult. Sci*. **1982**, *61*, 1118−1124.

(20) Beyer, R. S.; Jensen, L. S. The hypocholesterolemic agent dichloroacetate increases egg cholesterol content of laying hens. *Poult. Sci*. **1993**, *72*, 1063−1069.

(21) Elkin, R. G.; Freed, M. B.; Kieft, K. A.; Newton, R. S. Alteration of egg yolk cholesterol content and plasma lipoprotein profiles following administration of a totally synthetic HMG-CoA reductase inhibitor to laying hens. *J. Agric. Food Chem*. **1993**, *41*, 1094−1101.

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

(23) Elkin, R. G.; Yan, Z. Relationship between inhibition of mevalonate biosynthesis and reduced fertility in laying hens. *J. Reprod. Fertil*. **1999**, *116*, 269−275.

(24) Mori, A. V.; Mendonca, C. X. J.; Santos, C. O. Effect of dietary lipid-lowering drugs upon plasma lipids and egg yolk cholesterol levels of laying hens. *J. Agric. Food Chem*. **1999**, *47*, 4731−4735.

(25) Qureshi, A. A.; Peterson, D. M. The combined effects of novel tocotrienols and lovastatin on lipid metabolism in chickens. *Atherosclerosis* **2001**, *156*, 39−47.

(26) Endo, A. Monacolin K, a new hypocholesterolemic agent produced by *Monascus* species. *J. Antibiot*. **1979**, *32*, 852−854.

(27) Tobert, J. A.; Bell, G. D.; Birtwell, J.; James, I.; Kukovetz, W. R.; Pryor, J. S.; Buntinx, A.; Holmes, I. B.; Chao, Y. S.; Bolognese, J. A. Cholesterol-lowering effect of mevinolin, an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, in healthy volunteers. *J. Clin. Invest*. **1982**, *68*, 913−919.

(28) Su, Y. C.; Wang, J. J.; Lin, T. T.; Pan, T. M. Production of the secondary metabolites γ-aminobutyric acid and monacolin K by *Monascus*. *J. Ind. Microbiol. Biotechnol*. **2003**, *30*, 41−46.

(29) Hamilton, R. M. G. Methods and factors that affect the measurement of egg shell quality. *Poult. Sci*. **1982**, *61*, 2022−2039.

(30) Carter, T. C. The hen's egg: Factors affecting the shearing strength of shell material. *Br. Poult. Sci*. **1970**, *11*, 433−449.

(31) Rudel, L. L.; Morris, M. D. Determination of cholesterol using *o*-phthaladehyde. *J. Lipid Res*. **1973**, *14*, 364−366.
(32) Tarladgis, B. G.; Pearson, A. M.; Duran, L. R. Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in the foods. *J. Sci. Food Agric*. **1964**, *15*, 602−607.
(33) Peguri, A.; Coon, C. Effect of temperature and dietary energy on performance. *Poult. Sci*. **1991**, *70*, 126−138.
(34) Draper, H. H.; Hadley, M. Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol*. **1990**, *186*, 421−431.
(35) Wong, H. C.; Koehler, P. E. Production and isolation of an antibiotic from *Monascus purpureus* and its relationship to pigment production. *J. Food Sci*. **1981**, *46*, 589−592.
(36) Aniya, Y.; Ohtani, I. I.; Higa, T.; Miyagi, C.; Gibo, H.; Shimabukuro, M.; Nakanish, H.; Taira, J. Dimerumic acid as an antioxidant of the mold, *Monascus anka*. *Free Radical Biol. Med*. **1999**, *28*, 999−1004.
(37) Fletecher, D. L. Methodology for achieving pigment specifications. *Poult. Sci*. **1992**, *71*, 733−743.
(38) Saunders, C. F.; Hayes, J. P.; Erasmus, J.; Jooste, J. P.; Duplessis, P. H. C.; Venter, E. L. Egg yolk pigmentation as influenced by BHT, vitamin E and sunflower oil. *S. Afr. J. Anim. Sci*. **1967**, *10*, 373−380.
(39) Wang, J. J.; Lee, C. L.; Pan, T. M. Evaluation on the antioxidant activity of fermentatedred mold rice and dioscorea. *J. Food Sci.* **2003**, submitted for publication.
(40) Sim, J. S.; Bragg, D. B. Effect of dietary factors on serum and egg yolk cholesterol levels of laying hens. *Poult. Sci*. **1977**, *56*, 1616−1621.
(41) Manzoni, M.; Bergomi, S.; Rollini, M.; Cavazzoni, V. Production of statins by filamentous fungi. *Biotechnol. Lett*. **1999**, *21*, 253−257.
(42) McNamara, D. J.; Kolb, R.; Parker, T. S.; Batwin, H.; Samuel, P.; Brown, C. D.; Ahrens, E. H. Heterogeneity of cholesterol homeostasis in man: Response to changes in dietary fat quality and cholesterol quality. *J. Clin. Invest*. **1987**, *79*, 1729−1739.
(43) Pitman, W. A.; Osgood, D. P.; Smith, D.; Schaefer, E. J.; Ordovas, J. M. The effects of diet and lovastatin on regression of fatty streak lesions and on hepatic and intestinal mRNA levels for the LDL-C receptor and HMG-CoA reductase in F1B hamsters. *Atherosclerosis (Shannon, Irel.)* **1998**, *138*, 43−52.
(44) Luhman, C. M.; Miller, B. G.; Beitz, D. C. The effect of feeding lovastatin and colestipol on production and cholesterol content of eggs. *Poult. Sci*. **1989**, *69*, 852−855.
(45) Steinberg, D. Lipoprotein and atherosclerosis: a look back and look ahead. *Arteriosclerosis (Dallas)* **1983**, *3*, 283−301.
(46) Chen, L.; Haught, W. H.; Yang, B.; Saldeen, T. G.; Parathasarathy, S.; Metha, J. L. Preservation of endogenous antioxidant activity and inhibition of lipid peroxidation as common mechanisms of antiatherosclerotic effects of vitamin E, lovastatin and amlodipine. *J. Am. Coll. Cardiol*. **1997**, *30*, 569−575.
(47) Aviram, M.; Dankner, G.; Cogan, U.; Hochgraf, E.; Brook, J. G. Lovastatin inhibitors low-density lipoprotein oxidation and alters its fluidity and uptake by macrophages: In vitro and in vivo studies. *Metabolism* **1992**, *41*, 229−235.
(48) Jeon, S. M.; Bok, S. H.; Jang, M. K.; Lee, M. K.; Nam, K. T.; Park, Y. B.; Rhee, S. J.; Choi, M. S. Antioxidative activity of naringin and lovastatin in high cholesterol-fed rabbits. *Life Sci*. **2001**, *69*, 2855−2866.

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