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Contributed Paper

Evaluation of Red Mold Rice for Cholesterol Reduction in the Serum and Yolks of Japanese Quail Eggs and Its Effect on Growth Performance

Prodpran Pengnoi [a], Jaturong Kumla [a], Chartchai Khanongnuch [b] and Saisamorn Lumyong [a]

[a] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.

[b] Division of Biotechnology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, Thailand.

* Author for correspondence; e-mail: scboi009@gmail.com

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ABSTRACT

Red mold rice (RMR) is a fermented rice product of the *Monascus* species and has been regarded as a cholesterol-lowering functional food. In this study, the potential for fermented RMR by *Monascus purpureus* CMU002U (a mutant treated by UV) to reduce cholesterol levels in the serum and egg yolks of Japanese quail (*Coturnix coturnix japonica*) was investigated. The results showed that supplementation at various dosages of RMR (6, 12 and 24 mg/day/bird) did not affect the body weight of the birds when compared with both the control and the lovastatin drug treatments. However, the feed intake value and feed conversion ratio were significantly lowered. All dosages of RMR treatments could increase egg production, while they did not affect the weights of the eggs, eggshells, albumen and yolks, nor did they affect the color of the yolks. All supplemented RMR treatments showed significantly lower yolk cholesterol levels than those of the control treatment. Non-significant differences were found in the yolk cholesterol levels at all dosages of RMR treatments when compared with the lovastatin treatment. After eight weeks of cultivation, the lipid profile and serum parameters of the birds were determined. The results indicated that the levels of total cholesterol, triglycerides and low-density lipoproteins in birds at all dosages of RMR and lovastatin treatments were significantly lower than in the control treatment. However, the total cholesterol levels in 12 and 24 mg/day/bird RMR treatments were significantly lower than in the 6 mg RMR treatment. The results showed that the levels of blood urea nitrogen, creatinine, alanine aminotransferase, albumin, and globulin in the birds recorded in all treatments were not significantly different. Therefore, RMR could serve as a biological agent for the promotion of egg production and could facilitate a decrease in the cholesterol levels in the serum and egg yolks.

Keywords: cholesterol reduction agent, poultry, red yeast rice, *Monascus purpureus*

1. INTRODUCTION

Eggs are considered a major inexpensive source of many nutrients including proteins, fatty acids, minerals and vitamins that play an important role in a balanced human diet [1]. However, eggs have been associated with an adverse affect on human health, mainly due to the cholesterol content found in the yolk [2]. For many years, it was believed that the consumption of eggs was responsible for a variety of health problems due to their high cholesterol content which can lead to cardiovascular diseases (CVDs) Many people have been advised to limit their consumption of eggs to one yolk per day [3]. However, there has been a limited amount of scientific data to support limiting the consumption of eggs, while some studies have shown that the consumption of eggs among diabetic patients has increased the risks of acquiring CVDs [4]. The Japanese quail has become a popular breed of bird for cultivation because it is acknowledged as a hardy bird that can grow in small cages and can be easily managed with high yields of egg production [5]. Quail eggs are gaining popularity because of their unique color and mottles, and are favored by consumers. On the other hand, the yolks of quail eggs have higher cholesterol levels than those of chicken and duck eggs; therefore, the consumption of quail eggs may lead to a higher risk of acquiring CVDs [6].

There are various commercial compounds, e.g. triparanol, fibrates, azasterols, probucol, clenbuterol and statins (including lovastatin, simvastatin and pravastatin) that have been revealed to decrease the level of cholesterol in egg yolks [7-8]. These products have been associated with a decrease in egg weight and have been attributed to the ovarian regression that leads to the cessation of egg production, as well as an increase in the cost of production [9]. For this reason, researchers have been interested in studying the biologically active compounds that have been acquired from plants and probiotic microorganisms that can be used to decrease the level of cholesterol in egg yolks [10-12].

Red mold rice (RMR) or red yeast rice is a traditional food and medicine that has been used for long time in Asia. Many countries including China, Japan, the Philippines and Taiwan have used RMR as a food coloring agent, a preservative and an additive [13]. Monacolin K (lovastatin), a compound found in RMR, is a secondary metabolite that can inhibit HMG CoA reductase (3-hydroxy-3-methyl-glutaryl-Coenzyme A), which is involved in cholesterol biosynthesis [14]. However, RMR may be contaminated with a mycotoxin known as citrinin. Citrinin has been reported to be hepatotoxic and nephrotoxic in mammals [15]. Therefore, the level of safety associated with the use of RMR is dependent upon the citrinin content in the product. The standards of Japan, which have imposed citrinin concentration limits to 200 ppb while European Union and Taiwan set the maximum levels of citrinin in rice fermented with *M. purpureus* at 2,000 ppb [16]. Therefore, this study aims to evaluate the effects of RMR on the growth performance, lipid profile, serum parameters, egg production and cholesterol levels in the egg yolks of the Japanese quail by comparing it with experiments involving commercial lovastatin.

2. MATERIALS AND METHODS

2.1 Birds

Five-week-old female Japanese quail specimens (*Coturnix coturnix japonica*) at the egg stage (180-185 g/bird) were obtained from TC Farm, San Kamphaeng District, Chiang Mai, Thailand. All quail specimens received the Newcastle disease virus and infectious bronchitis vaccines at one week old and the smallpox vaccine at two weeks of age. Birds were kept in standard metal cages 50 × 52 × 42 cm with five birds per cage in a poultry house at Maejo University (26 ± 2 °C, 60 ± 5% relative humidity and 12 h light period) (Figure 1). Birds were offered a corn-soy basal diet obtained from Betrago Company (Table 1) twice daily. Water with free toxins and germs was provided. The management of the birds and all procedures in the present study were performed according to the principles of the Chiang Mai University Animal Ethics Committee (AEC) (Re 008/13; 15 October 2013).

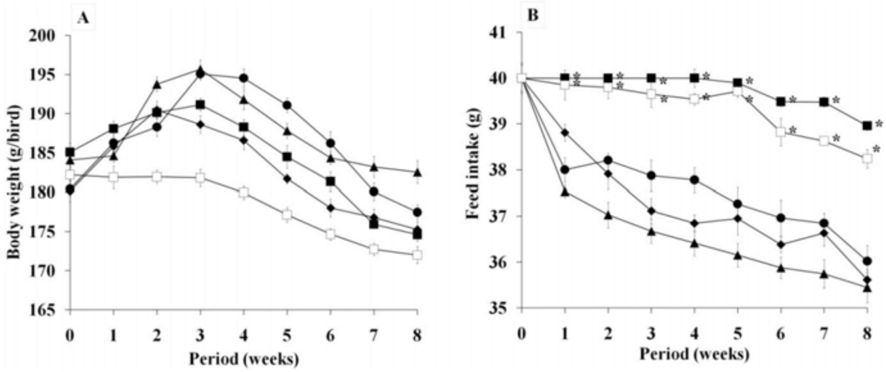

Figure 1. Body weight (A) and feed intake (B) of birds in this study. Data are presented as mean averages and the error bar on each graph indicates a ± standard error. Week 0 indicates the beginning value. An asterisk symbol (*) indicates a significant difference (*P* < 0.05). (■) = control, (▲) = 6 mg RMR, (♦) = 12 mg RMR, (●) = 24 mg RMR and (□) = lovastatin drug treatments.

Table 1. Basal diet of Japanese quail in this study.

Ingredients	Quantity (%)
Soybean meal	36.2
Ground corn	36.0
Wheat bran	10.0
Fish meal	7.0
Acacia leaves powder	3.0
Oyster shell powder	5.0
Dicalcium phosphate	2.0
Sodium chloride	0.35
DL-methionine	0.20
Vitamin mineral premix*	0.25
Total	100
Nutrients	
Crude protein (%)	24
Crude Fiber (%)	5
Metabolic energy (MJ/Kg)	12.02

*One kilogram of Vitamin mineral premix contained: Vitamin A = 15,000,000 IU; Vitamin D3 = 3,000,000 IU; Vitamin E = 2000 mg; Vitamin K3 = 750 mg; Vitamin B1 = 600 mg; Vitamin B2 = 2250 mg; Vitamin B6 = 600 mg Vitamin B12 = 5000 mcg; Nicotinic acid = 6500 mg; Biotin = 5 mg; Calcium-D-Pantothenate = 4500 mg; Choline chloride = 95,000 mg Mn = 20,000 mg; Fe = 6500 mg; Zn = 1000 mg; Cu = 2500 mg; Co = 180 mg; I = 250 mg; Se = 30 mg

2.2 Red Mold Rice Preparation

Monascus purpureus CMU002U, a high monacolin K production strain developed from UV radiation was used in this study. Sanpatong glutinous rice (*Oryza sativa* L.) was used as a substrate for RMR production. The procedure for RMR production was followed according to the method described by Pengnoi et al. [17]. The contents of monacolin K and citrinin in dried RMR were analyzed using high performance liquid chromatography The monacolin K content of RMR in this study was 10.05 ± 0.25 mg/g. The citrinin content in RMR was 640 ± 5 ppb and passed the standards set in both the European Union and Taiwan. Dried RMR was milled to a powder using a blender and prepared in capsule form for further experimentation.

2.3 Experimental Design

Five treatments were established to test the effects of RMR on the growth performance and egg production of the birds. Fifteen birds were used in each treatment. The control was provided with a basal diet feeding. The second, third and fourth treatments consisted of a basal diet feeding that was supplemented with RMR 6, 12 and 24 mg/day/bird, respectively (monacolin K concentration equivalent to 0.060 ± 0.001, 0.121 ± 0.003 and 0.241 ± 0.006 mg). The dose of monacolin K in this study was based on a human dose (20 mg /day/50-60 kg body weight). The average weight of the birds at the beginning of the experiment was about 180 g. Therefore, the initiation dose of monacolin K was 0.06 mg/day/bird. The last treatment involved a basal diet feeding that was supplemented with 0.06 mg (recommended dosage) of a commercial lovastatin drug (Mevacor®; Merck, USA). All birds in each treatment were kept in the same poultry house as mentioned above over the course of eight weeks. The growth performance and egg production of the birds in each treatment were measured every week. Each treatment was repeated twice.

2.4 Growth Performance of Birds

All birds in each treatment were individually weighed and the results were recorded weekly. Moreover, daily feed intake values in each treatment were determined (a total feed offered during a single day minus the amount of feed refused at the end of the day). The weekly average value of feed intake was calculated.

2.5 Egg Production and Feed Conversion Ratio

Eggs in each treatment were collected every day and individually weighed. The total amount of egg production per bird for each week was calculated. The weekly feed conversion ratio of the birds in each treatment was calculated as the data of feed intake divided by the egg weight [18].

2.6 Weight of Eggs, Eggshells, Albumen and Yolks

Ten eggs were randomly selected for each treatment and used for the investigation of eggshells, albumen and yolk weights. Eggs were broken in a Petri dish of 9 cm in diameter. Eggshells, albumen and yolks were separated and weighed. The average values were reported weekly.

2.7 Egg Yolk Color

The surface color of the egg yolk samples was measured using a Minolta Chroma Meter Model CR-410 (Minolta Co Ltd, Japan). The L* (lightness), a* (red/green) and b* (yellow/blue) values were measured in the CIE Lab color space). The different color values (DE*) of the RMR and lovastatin treatments were calculated using the control treatment. The egg yolk color was reported weekly as an average.

2.8 Cholesterol Contents in Egg Yolks

Individual specimens of separated egg yolks were used in this experiment. The cholesterol contents were determined using a commercial Cholesterol/Cholesteryl Ester Quantitation Kit (Merck, Germany) according to the manufacturer's protocol. The average weekly cholesterol content in the egg yolks was presented.

2.9 Blood Collection and Analysis

Blood samples of approximately two milliliters were collected from the jugular veins of individual birds in each experiment according to the method described by Al-Daraji et al. (2010) after eight weeks of cultivation. The lipid profile and blood clinical analysis were analyzed within 6 h. All bloods samples were sent to the Associated Medical Sciences Clinical Service Center, Chiang Mai University, Thailand for total cholesterol, triglyceride, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) assays. Moreover, blood clinical biochemistry including blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine phosphokinase (CPK), albumin and globulin values were determined by Veterinary Diagnostic Laboratory, Small Animal Hospital, Faculty of Veterinary, Chiang Mai University, Thailand.

2.10 Statistical Analysis

All data was analyzed by one-way analysis of variance (ANOVA) by SPSS program version 16.0 for Windows, and Tukey's test was used to identify any significant differences ($P < 0.05$) between treatments.

3. Results and Discussion

3.1 Growth Performance of Birds

In all five treatments, no deaths of birds occurred throughout the experimental period. The growth performance of the birds was recorded in terms of the average weekly value of body weight along with the feed intake of the birds and the results are shown in Figure 1. It was found that the recorded weekly bird body weights among all RMR treatments were not significantly different when compared with birds exposed to the lovastatin and control treatments (Figure 1A). Significantly, the body weights of the birds exposed to the lovastatin treatment were slightly lower than other treatments. The results show that the weekly values of bird feed intake after supplementation with RMR were significantly lower than those of the lovastatin and control treatments for all cultivation periods (Figure 1B). However, the feed intake values in the 6, 12 and 24 mg RMR treatments were not found to be statistically different. However, prior studies have reported that the supplementation of RMR ranging from 66 to 660 mg/day/bird in Hyline brown and Lohmann brown laying hens did not affect the feed intake among the birds [19-20]. The supplementation of RMR ranging from 183 to 777 and 1000 to 3162 mg/day/bird in white Leghorn [21] and Isa brown laying hens [22], respectively, could increase their feed intake. This might be a result of the different bird species being studied, while RMR may have reduced the level of appetites of the birds according to the study of Kim et al. [23] who found that the feed intake of Isa brown laying hens supplemented with 3.1 to 8.6 g of *Monascus* culture experiment was lower than that of the control.

3.2 Egg Production and Feed Conversion Ratio

The results showed that the egg production of birds increased in all treatments from the first week and decreased after four weeks of cultivation (Figure 2). The average value of weekly egg production for all RMR treatments showed a significantly higher value than that of the control and lovastatin treatments after one week until the end of the experimental period. Generally, the feed conversion ratio can be used as an expression of the egg production coefficient, with the lower value indicating a more efficient use of feed to produce eggs [18, 22]. The results showed that the feed conversion ratio at all dosages of RMR treatments after one week were significantly lower than those of the lovastatin and control treatments. But the feed conversion ratio values were not found to be significantly different among all dosages of the RMR treatments (Figure 2B). It was presumptively concluded that the supplementation of RMR increased egg production and decreased the feed conversion ratio of quail according to the study of Nuraini and Latif [22] Additionally, several previous studies have reported that the levels of feed intake, egg production and the feed conversion ratio of poultry were influenced by the age and poultry breeds, the composition of the basal and alternative diets, and the cultivation systems being employed [19-20, 23-24].

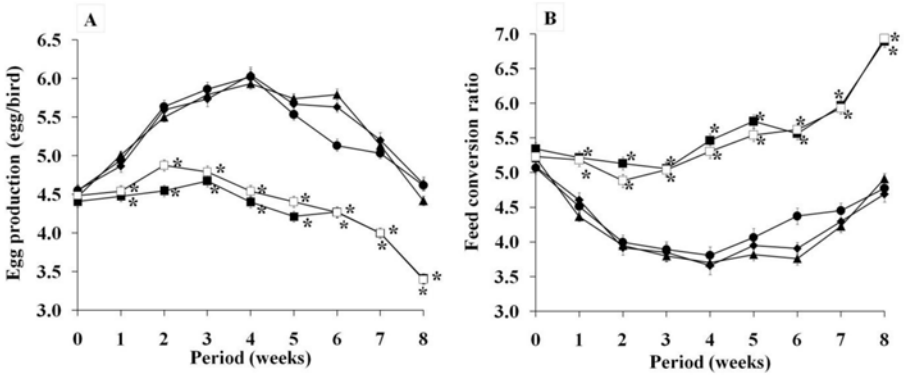

Figure 2. Egg production (A) and feed conversion ratio (B) in this study. Data are presented as mean averages and the error bar on each graph indicates a ± standard error. Week 0 indicates the beginning value. An asterisk symbol (*) indicates a significant difference ($P < 0.05$). (■) = control, (▲) = 6 mg RMR, (♦) = 12 mg RMR, (●) = 24 mg RMR and (□) = lovastatin drug treatments.

3.3 Weight of Eggs, Eggshells, Albumen and Yolks

This study showed that the weights of the eggs, eggshells, albumen and yolks at all dosages of the RMR treatments were not significantly different from those of the control treatment (Figure 3). This result was similar to that of the supplementation of RMR at values of 66 to 777 mg/day/bird in white Leghorn and Lohmann brown hens and did not affect the total egg and yolk weights [20-21]. However, significant changes in the egg weights, eggshell weights and yolk weights between the supplemented RMR trial and the control trials were found to depend on the poultry species and the amount of RMR used. For example, Hyline brown hens could decrease the total egg weights when supplementation of RMR at 209 to 422 mg/day/bird [19] while supplementation of a high level of RMR at 3162 mg/day/bird in Isa brown hens could increase the total egg weights [22].

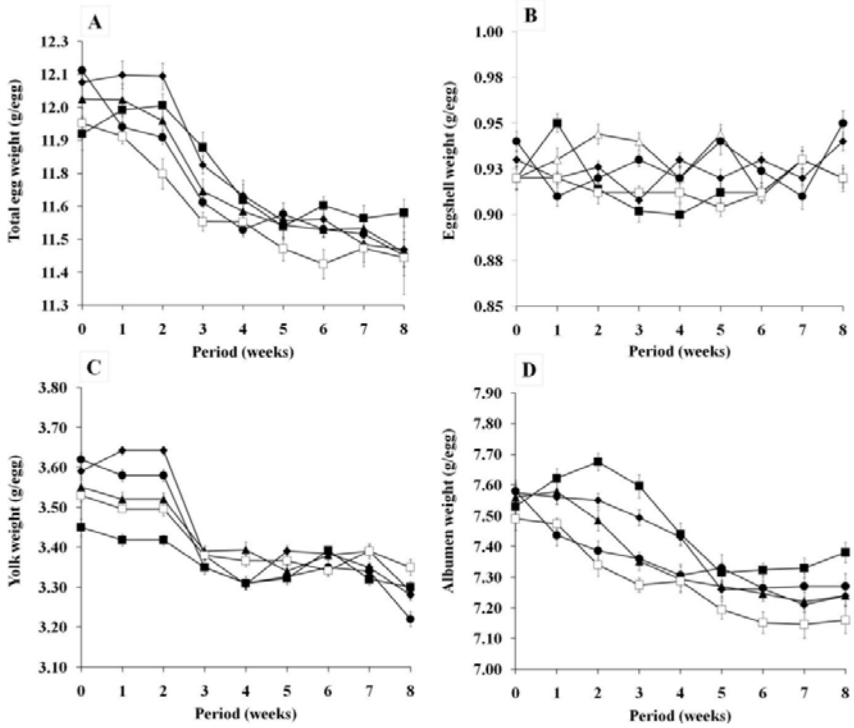

Figure 3. Total eggs weight (A), weights of eggshells (B), egg yolks (C) and albumen (D) in this study. Data are presented as mean averages and the error bar on each graph indicates a ± standard error. Week 0 indicates the beginning value. An asterisk symbol (*) indicates a significant difference ($P < 0.05$). (■) = control, (▲) = 6 mg RMR, (♦) = 12 mg RMR, (●) = 24 mg RMR and (□) = lovastatin drug treatments.

3.4 Egg Yolk Color

In this study, it was found that the egg yolk color of the Japanese quail at all dosages of RMR treatments were not significantly different from that of the control (Figure 4 and 5). This was agreed with the test of Lohmann brown hen [20]. However, several studies have reported that the supplementation of RMR could affect the color of the egg yolks in laying hens depending on the different breeds and high amounts of RMR used. For example, the use of RMR of at least 183 mg/day/bird in white Leghorn hens [21] and at least 1000 mg/day/bird in Isa brown hens [22] could influence the egg yolk color. However, the coloring of the egg yolks depended on the amounts and types of the feed diets used [25].

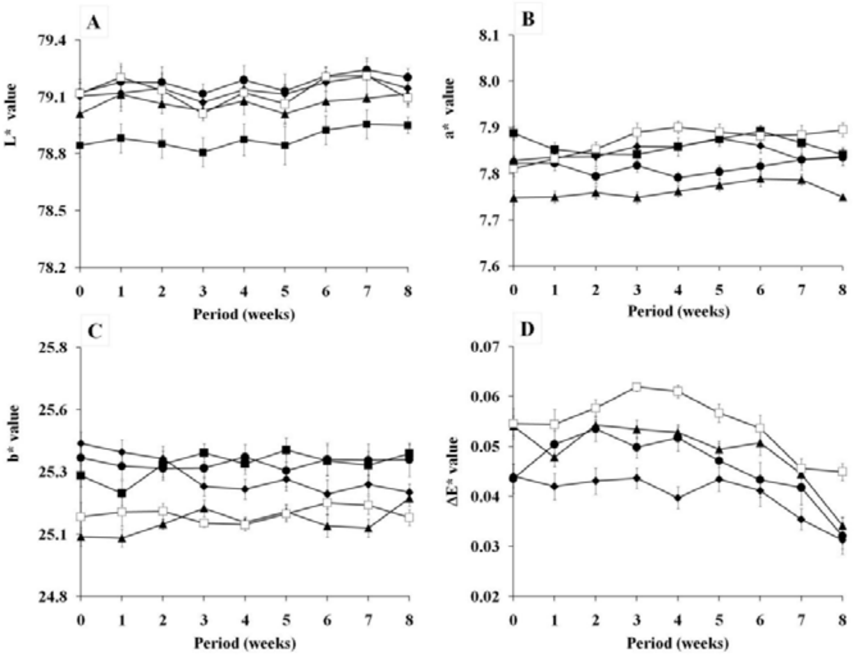

Figure 4. The color value of egg yolks (A-D); L* = lightness, a* = red/green and b* = yellow/blue in this study. Data are presented as mean averages and the error bar on each graph indicates a ± standard error. Week 0 indicates the beginning value. An asterisk symbol (*) indicates a significant difference ($P < 0.05$). (■) = control, (▲) = 6 mg RMR, (♦) = 12 mg RMR, (●) = 24 mg RMR and (□) = lovastatin drug treatments.

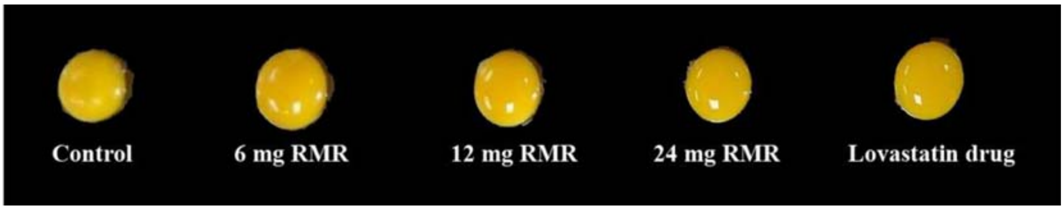

Figure 5. The color value of egg yolks at 8 weeks of cultivation.

3.5 Cholesterol Contents in Egg Yolks

At the beginning of the experimental period, the cholesterol levels in the egg yolks ranged from 17.54 to 18.03 mg/g. (Figure 6). This present study showed that the supplementation at a range of dosages (6, 12 and 24 mg/day/bird) of RMR could reduce the level of cholesterol content in the egg yolks of the Japanese quail. At eight weeks of cultivation, the average cholesterol levels in the control, 6, 12 and 24 mg RMR and lovastatin treatments were 18.05 ± 1.51, 10.31 ± 0.91, 10.92 ± 0.58, 10.83 ± 0.53 and 12.15 ± 1.28 mg/g yolk, respectively. Our study found that the same level of monacolin K content in RMR and lovastatin could reduce the amount of cholesterol in the yolks of quail eggs, but the RMR treatment revealed a significantly higher level of egg production than the lovastatin treatment. The findings obtained from previous studies might have been due to the fact that RMR contains a number of bioactive compounds that could increase the level of functional egg production [26-27]. Primarily, RMR products have been used for the reduction of cholesterol levels in the egg yolks of laying hens [19-22], due to the fact that RMR products contain monacolin K, which inhibits the enzymes involved in cholesterol biosynthesis. However, there have not been any reports on the applications of RMR products in reducing the cholesterol levels in the yolks and serum of Japanese quail eggs. In this study, a mutant strain of *M. purpureus* was used for RMR production instead of the wild type strain because the mutant strain contained a lower level of citrinin than the wild type. This result was similar to those of a number of previous studies that reported that RMR could be used as a biological cholesterol-reducing agent in the eggs of laying hens [20-21]. However, the degree of reduction was correlated with the poultry breed and age, and the amount of monacolin K content in RMR along with the feed time [7, 28]. The amount of RMR that reduced cholesterol could vary from 0.003 mg/day /bird in Lohmann brown laying hen [20] and 4.0 mg/day/bird in Isa brown laying hens [22] and 13 mg/day/bird in white Leghorn hens [21]. Finally, a number of reports have shown that citrinin could have negatively affect pregnant Wistar rats by way of mild maternal toxicity in the form of degenerative liver changes, multiple renal lesions and glomerular congestion. For the purposes of safety, pregnant woman should avoiding consuming Japanese quail eggs that have been treated with RMR [29-30]. The citrinin content in the RMR products used in this study have passed the standards of Taiwan and the EU but could not pass the standards of Japan since the acceptable levels must be less than 200 ppb. However, the same RMR product was toxicity tested in the Wistar rats to estimate the degree of safety of the products. Rats which have received an extract of RMR at a dose of 5,000 mg/kg body weight did not exhibit any clinical signs of toxicity, changes in behavior, food or water consumption, or body weight, or death, immediately after oral administration and during the experimental period [31].

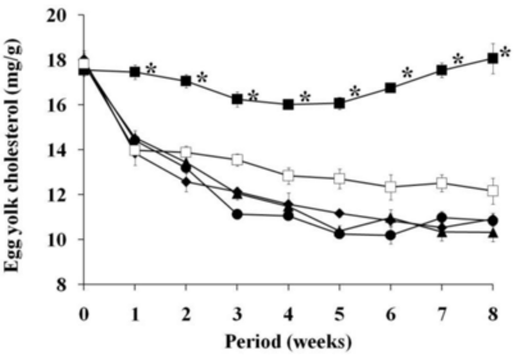

Figure 6. The cholesterol content of egg yolks in this study. Data are presented as mean averages and the error bar on each graph indicates a ± standard error. Week 0 indicates the beginning value. An asterisk symbol (*) indicates a significant difference ($P < 0.05$). (■) = control, (▲) = 6 mg RMR, (♦) = 12 mg RMR, (●) = 24 mg RMR and (□) = lovastatin drug treatments.

3.6 Lipid Profile and Blood Biochemical Parameters

A lipid profile of the serum after eight weeks of cultivation is shown in Figure 7. In this study, all dosages of RMR used could reduce the total cholesterol, triglyceride and LDL levels in the serum of Japanese quails. The total recorded cholesterol levels in the control, 6, 12 or 24 mg RMR and lovastatin treatments were 213.8 ± 4.8, 165.8 ± 4.5, 160.0 ± 3.4, 159.9 ± 5.5 and 145.4 ± 3.2 mg/dL, respectively. This result was similar to those of a number of previous studies that reported that RMR could be used as a biological cholesterol-reducing agent in hens [20-21]. Similarly, RMR products have been found to reduce serum cholesterol, triglyceride and LDL levels in the serum of laying hens [19-20]. Our results showed that the supplementation of RMR at all dosages did not affect the levels of HDL in quail serum when compared with the control and lovastatin treatments. This result is consistent with those of previous studies, which found that the supplementation of RMR in laying hens did not affect the HDL levels in the serum [20-21].

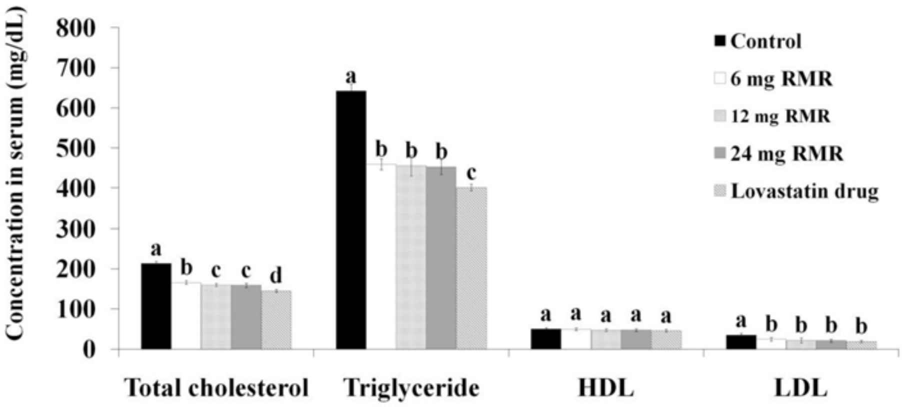

Figure 7. Lipid profiles of birds serum after eight weeks of cultivation in this study. Data are presented as means and the error bar at each point indicates the ± standard error. The different letter of each point in the same parameters indicates a significant difference ($P < 0.05$). HDL = high density lipoprotein and LDL = low density lipoprotein.

Generally, a functional application of the kidney has been determined by BUN and the creatinine parameters and a clinical symptom for monitoring the health of the immune system has been determined by the albumin and globulin levels in the serum. Our results revealed that BUN, creatinine, albumin and globulin levels in the supplementation at all dosages in the RMR and lovastatin treatments were not significantly different when compared to those of the control (Figure 8). Therefore, it may be concluded that the supplementation of all dosages of RMR and lovastatin used did not affect the kidney and liver function or the immune systems of the quails. ALT and AST were used to evaluate liver function, while increases in their levels were related to the malfunction of the liver including the degeneration of hepatocyte [32]. Our study showed that the ALT levels (4.5 to 6.0 U/L) in all treatments were not significantly different, as those levels were within the ALT normal level (4.0 to 7.0 U/L) for Japanese quails. However, the AST levels of the RMR and lovastatin treatments were higher than those of the control treatment. It seems that RMR and lovastatin could increase liver function by increasing the level of AST. This result was in accordance with several studies, which found that lovastatin was involved with hepatic cell injury by increasing AST and ALT levels [33]. However, the values of the AST (356.8 to 573.8 U/L) levels in all treatments in this study were still in the normal range of AST (243.0 to 880.0 U/L) levels found in the serum of the Japanese quail from several previous studies [34-35]. In this study, the levels of CPK in all RMR and lovastatin treatments were higher than in the control treatment. This result was supported by that of a previous study, which reported that RMR and lovastatin yielded a side effect that was associated with rhabdomyolysis (a breakdown of muscle fibers that occurs due to muscle injury) [36].

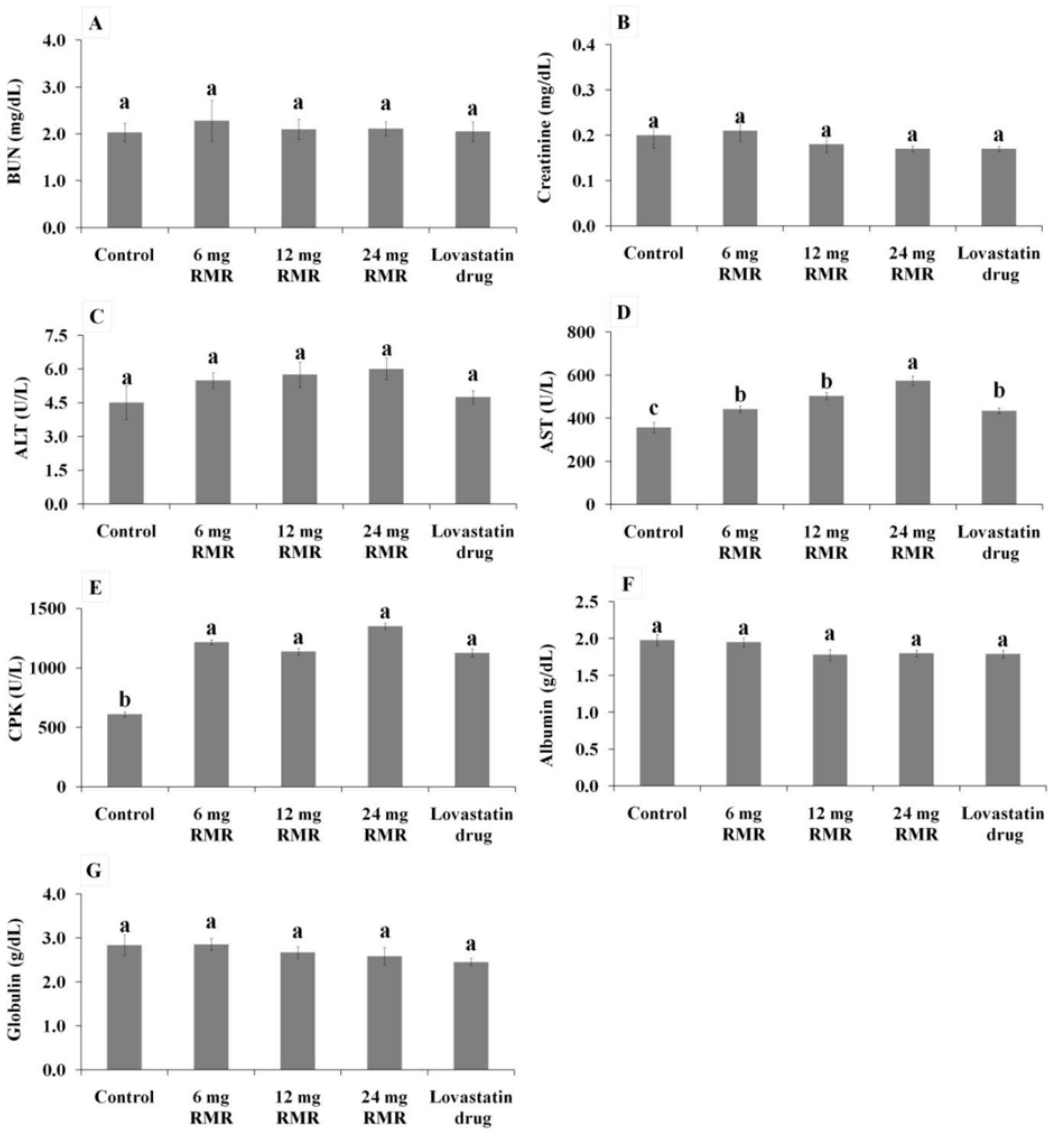

Figure 8. Serum parameters of birds at eight weeks of cultivation in this study. Data are presented as means and the error bar at each point indicates the ± standard error. The different letter of each point in the same parameters indicates a significant difference ($P < 0.05$). BUN = blood urea nitrogen, ALT = alanine aminotransferase, AST = aspartate aminotransferase and CPK = creatine phosphokinase.

4. Conclusions

Based on the results of our experiment, it is reasonable to conclude that the supplementation of RMR as a biological cholesterol-lowering agent in the Japanese quail diet could reduce feed intake, the feed conversion ratio, the egg yolkcholesterol content, and serum cholesterol and triglyceride levels, as well as LDL concentration. Moreover, RMR could increase egg production. The dose of RMR at 6 or 12 mg per day (monacolin K = 0.12 and 0.24 mg, respectively) was very active in terms of cholesterol reduction in egg yolks and serum. Further studies are required to analyze the residue monacolin k and citrinin content in the eggs and serum of Japanese quail, and to meet the expectations of lower citrinin content with regard to RMR production.

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