CHAPTER 6

Evaluation of red mold rice for cholesterol reduction in the serum and yolks of Japanese quail eggs and its effect on growth performance

6.1 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 (Applegate, 2000; Fraeye *et al*., 2012). However, eggs have been associated with an adverse affect on human health, mainly due to the cholesterol content found in the yolk (Miranda *et al*., 2015). 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) (Weggemans *et al*., 2001; Bragagnolo and Rodriguez-Amaya, 2003). Many people have been advised to limit their consumption of eggs to one yolk per day (Assmann *et al*., 1999). However, there has been a limited amount of scientific data to support limiting the consumption of eggs (Kritchevsky, 2004), while some studies have shown that the consumption of eggs among diabetic patients has increased the risks of acquiring CVDs (Spence *et al*., 2010; Fuller *et al*., 2015). 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 (Kayang *et al*., 2004; Randall and Bolla, 2008). Quail eggs are gaining popularity because of their unique color and mottles, and are favored by consumers (Tolik *et al*., 2014). Quail eggs are smaller in size than chicken and duck eggs but there is very little difference in taste. Moreover, quail eggs are richer in calcium and are higher in content in terms of phosphorus and iron than chicken eggs (Abduljaleel *et al*., 2011; Genchev, 2012). On the other hand, the yolks of quail eggs have higher cholesterol levels (16.05 mg/g) than those of chicken

(7.65 mg/g) and duck eggs (10.36 mg/g); therefore, the consumption of quail eggs may lead to a higher risk of acquiring CVDs (Aziz *et al*., 2012).

Previously, digitonins have been used to decrease the cholesterol content in the yolks of consumptive poultry eggs, but they were known to reduce egg production (Tumova *et al*., 2004). Moreover, this chemical compound has been reported to be toxic (Ulloa and Nervi, 1985; Sudji *et al*., 2015). In addition, 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 (Kim *et al*., 2004a; Elkin, 2007; Elangovan *et al*., 2011). 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 (Nichols and Balloun, 1962; Cecil *et al*., 1981). 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 (Cakir *et al*., 2008; Imik *et al*., 2009; Al-Daraji *et al*., 2010; Ayasan, 2013).

Red mold rice (RMR) or red yeast rice is a traditional food and medicine that has been used for long time in Asia (Ma *et al*., 2000; Lee *et al*., 2013). RMR is obtained from the fermented rice of the *Monascus* species e.g. *M. purpureus*, *M. ruber* and *M. pilosus* (Patakova, 2013). Many countries including China, Japan, the Philippines and Taiwan have used RMR as a food coloring agent, a preservative and an additive (Ma *et al*., 2000). RMR has been used in the treatment of dyslipidemia to reduce cholesterol levels in human blood samples (Erdogrul and Azirak, 2005; Becker *et al*., 2009). 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 (Lin *et al*., 2008). However, RMR may be contaminated with a mycotoxin known as citrinin. Citrinin has been reported to be hepatotoxic and nephrotoxic in mammals (Betina, 1989). Therefore, the level of safety associated with the use of RMR is dependent upon the citrinin content in the product. A safe level of citrinin content in commercial RMR has been limited by the standards of the Commission Regulation (EU) and Taiwan that have set the maximum levels of citrinin at 2,000 ppb (Chung *et al*., 2009; Le Bloc′h *et al*., 2015). 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.

6.2 Material and methods

6.2.1 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). Birds were offered a corn-soy basal diet obtained from Betrago Company (Table 6.1) 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).

Table 6.1 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[a]	0.25
Total	**100**

Nutrients	
Crude protein (%)	24
Crude Fiber (%)	5
Metabolic energy (MJ/Kg)	12.02

[a] 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

6.2.2 Red mold rice preparation

Monascus purpureus CMU002U, a high monacolin K production strain developed from UV radiation (Pengnoi *et al*., 2017), 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 Chairote *et al*. (2007) with some modifications. Twenty grams of glutinous rice was placed in 250-mL Erlenmeyer flasks and the moisture content of the rice was adjusted to 60% (w/w) on a wet basis with distilled water. The flasks containing rice were autoclaved at 121°C for 15 min. One millilitre of inoculum (10^6 spores/mL) was added to the flasks after they had cooled for 24 h, and the inoculated flasks were then incubated at 30°C in darkness. After two weeks, the fermented rice was collected and dried at 60°C overnight using a commercial food dryer for 12 h. The contents of monacolin K and citrinin in dried RMR were analyzed using high performance liquid chromatography following the method described by Wang *et al*. (2004) and Chairote *et al*. (2007). 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, which have restricted citrinin concentration limits to 2,000 ppb (Chung *et al*., 2009; The European Commission, 2014). Dried RMR was milled to a powder using a blender and prepared in capsule form for further experimentation.

6.2.3 Animal and 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/g RMR). 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.

6.2.4 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) following the procedure employed in the study by Agboola *et al*. (2016). The weekly average value of feed intake was calculated.

6.2.5 Egg production

6.2.5.1 Egg production and weight of eggs, eggshells, albumen and yolks

Eggs in each treatment were collected every day and individually weighed. The total amount of egg production per bird for each week was calculated. 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.

6.2.5.2 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 (ΔE*) of the RMR and lovastatin treatments were calculated using the control treatment. The egg yolk color was reported weekly as an average.

6.2.5.3 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.

6.2.6 Feed conversion ratio

The weekly feed conversion ratio of the birds in each treatment was calculated as the data of feed intake divided by the egg weight (Varkoohi *et al*., 2010; Agboola *et al*. 2016).

6.2.7 Blood collection and analysis

Blood samples of approximately two milliliters were collected from the jugular veins of individual birds in each experiment following 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.

6.2.8 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 ($P \leq 0.05$) between treatments.

6.3 Results and discussion

6.3.1 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 6.1. 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 6.1A). 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 6.1B). However, the feed intake values in the 6, 12 and 24 mg RMR treatments were not found to be statistically different. Therefore, the presumptive findings indicate that the RMR supplementation at all dosages used in this study did not affect the bird body weights, but there was a decrease in the feed intake of the birds.

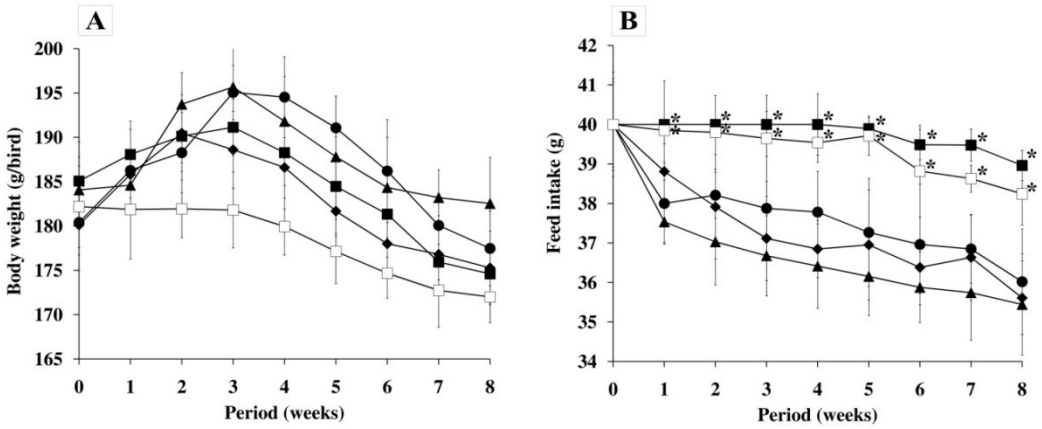

Figure 6.1 Body weight (A) and feed intake (B) of birds in this study. Data are presented as mean averages and the error bar on each graph indicates a ± standard error. Week 0 indicates the beginning value. An asterisk symbol (*) indicates a significant difference ($P \leq 0.05$). (■) = Control, (▲) = 6 mg RMR, (♦) = 12 mg RMR, (●) = 24 mg RMR and (□) = lovastatin drug treatments.

Our study showed that the supplementation of all dosages of RMR used (6, 12 and 24 mg/day/bird) in the Japanese quail decreased levels of feed intake. 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 (Wong *et al*., 2005; Sun *et al*., 2015). However, the supplementation of RMR ranging from 183 to 777 and 1,000 to 3,162 mg/day/bird in white Leghorn (Wang and Pan, 2003) and Isa brown laying hens (Nuraini and Latif, 2012), 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*. (2003) 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.

6.3.2 Egg production

6.3.2.1 Egg production and weights of eggs, eggshells, albumen and yolks

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 6.2A). 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. However, there were no significant differences in the weekly egg production values for all RMR treatments. The total weights of the eggs, eggshells, albumen and yolks are shown in Figure 6.2B–E. It was found that the weights of the eggs, eggshells, albumen and yolks in all treatments in this study were not significantly different. It was presumptively concluded that the supplementation of RMR could increase the egg production value.

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. 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 (Wang and Pan, 2003; Sun *et al*., 2015). 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, Wong *et al*. (2005) reported that the supplementation of RMR at 209 to 422 mg/day/bird in Hyline brown hens could decrease the total egg weights and Nuraini and Latif (2012) found that the supplementation of a high level of RMR at 3,162 mg/day/bird in Isa brown hens could increase the total egg weights.

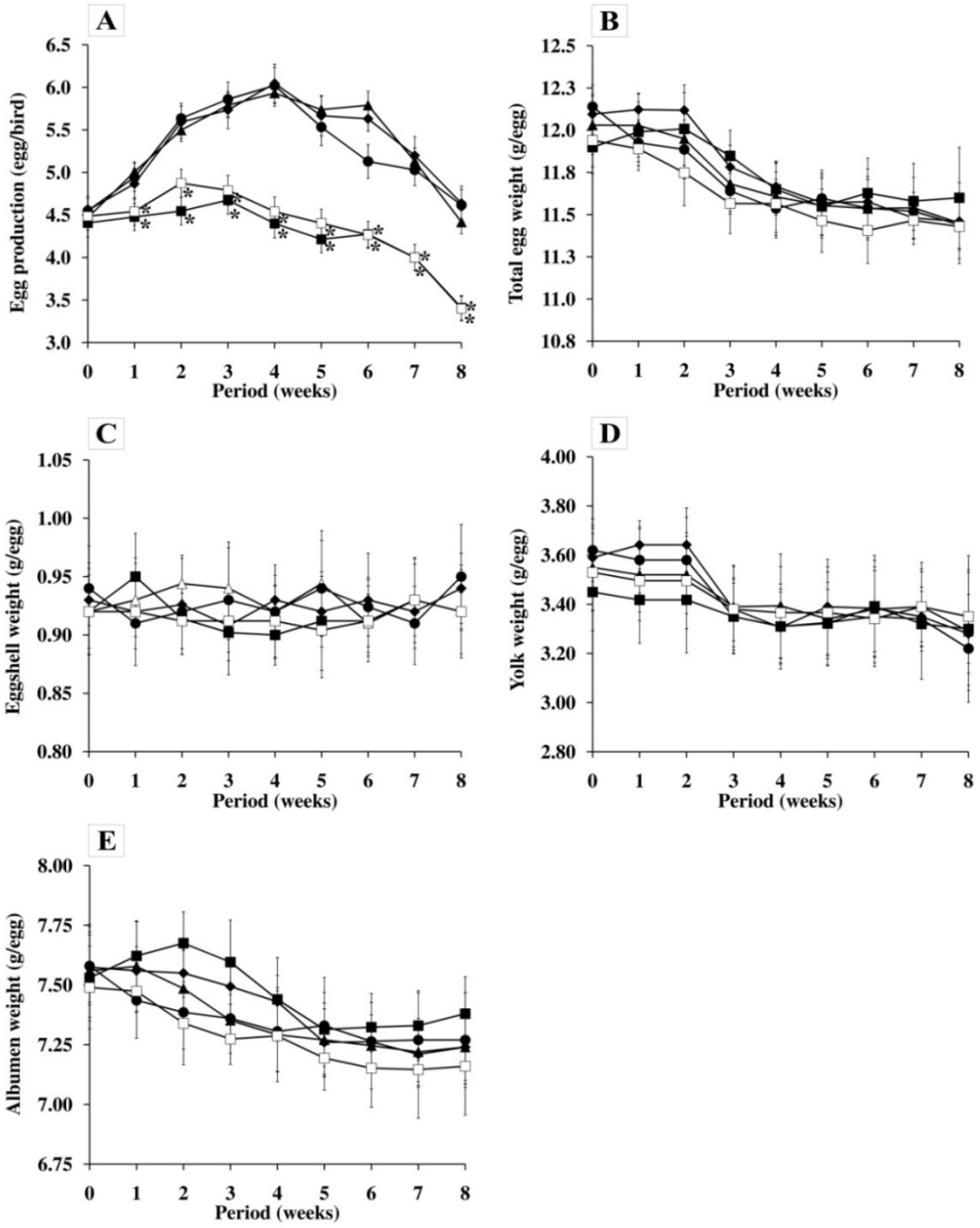

Figure 6.2 Egg production (A) and weights of eggs (B), eggshells (C), egg yolks (D) and albumen (E) in this study. Data are presented as mean averages and the error bar on each graph indicates a ± standard error. Week 0 indicates the beginning value. An asterisk symbol (*) indicates a significant difference ($P \leq 0.05$). (■) = Control, (▲) = 6 mg RMR, (♦) = 12 mg RMR, (●) = 24 mg RMR and (□) = lovastatin drug treatments.

The coloring of the egg yolks depended on the amounts and types of the feed diets used (Galobart *et al*., 2004; Roberson *et al*., 2005). 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. This was similar to the findings of Sun *et al*. (2005), which reported that the yolk color of the eggs of the Lohmann brown hen in the RMR trial was not different from the control. 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 (Wang and Pan, 2003) and at least 1000 mg/day/bird in Isa brown hens (Nuraini and Latif, 2012) could influence the egg yolk color. 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. This might be due to the fact that RMR contains a number of bioactive compounds, e.g. gamma-aminobutyric acid (GABA) and lysine that could increase the level of functional egg production (Chi and Speers, 1976; Kono and Himeno, 2000; Wang *et al*., 2006a; Park and Kim, 2015; Zhu *et al*., 2015).

6.3.2.2 Color and cholesterol content of egg yolks

The results showed that the weekly average L*, a*, b* and ΔE* values of egg yolks in all treatments were not significantly different (Figure 6.3 A–D), while the egg yolks at all dosages of the RMR and lovastatin treatments were slightly lighter than in the control treatment. Additionally, the weekly average a* value of the yolks in the lovastatin treatment was slightly higher than the other treatments.

At the beginning of the experimental period, the cholesterol levels in the egg yolks ranged from 17.54 to 18.03 mg/g. The results indicated that the cholesterol levels of the egg yolks of the control treatment were significantly higher than in all of the RMR and lovastatin treatments after one week until the end of experimental period (Figure 6.3E). The weekly average values of the cholesterol

levels in all RMR and lovastatin treatments decreased after one week of cultivation, while the yolk cholesterol levels at all dosages of the RMR treatment were lower than those of the lovastatin treatment after two weeks of cultivation but no significant differences were observed. 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. According to our results, it was indicated that RMR could decrease the cholesterol content in the egg yolks of Japanese quails.

Generally, improvement of poultry eggs through the use of pharmacological agents is the one of the many methods being employed to reduce egg yolk cholesterol. For many years, azasterols, fibrates, SC-11592 and triparanol have been used to reduce the amount of cholesterol in egg yolks. Unfortunately, the properties of these agents have resulted in a reduction in egg production (Nichols and Balloun, 1962; Nelson *et al*., 1962; Cecil *et al*., 1981). However, there are other substances that could be used to reduce cholesterol levels that do not affect egg production, such as diethylaminoethyl-diphenylvalerate (SKF 525-A) and the lovastatin drug (Naber, 1976; Kim *et al*., 2004a).

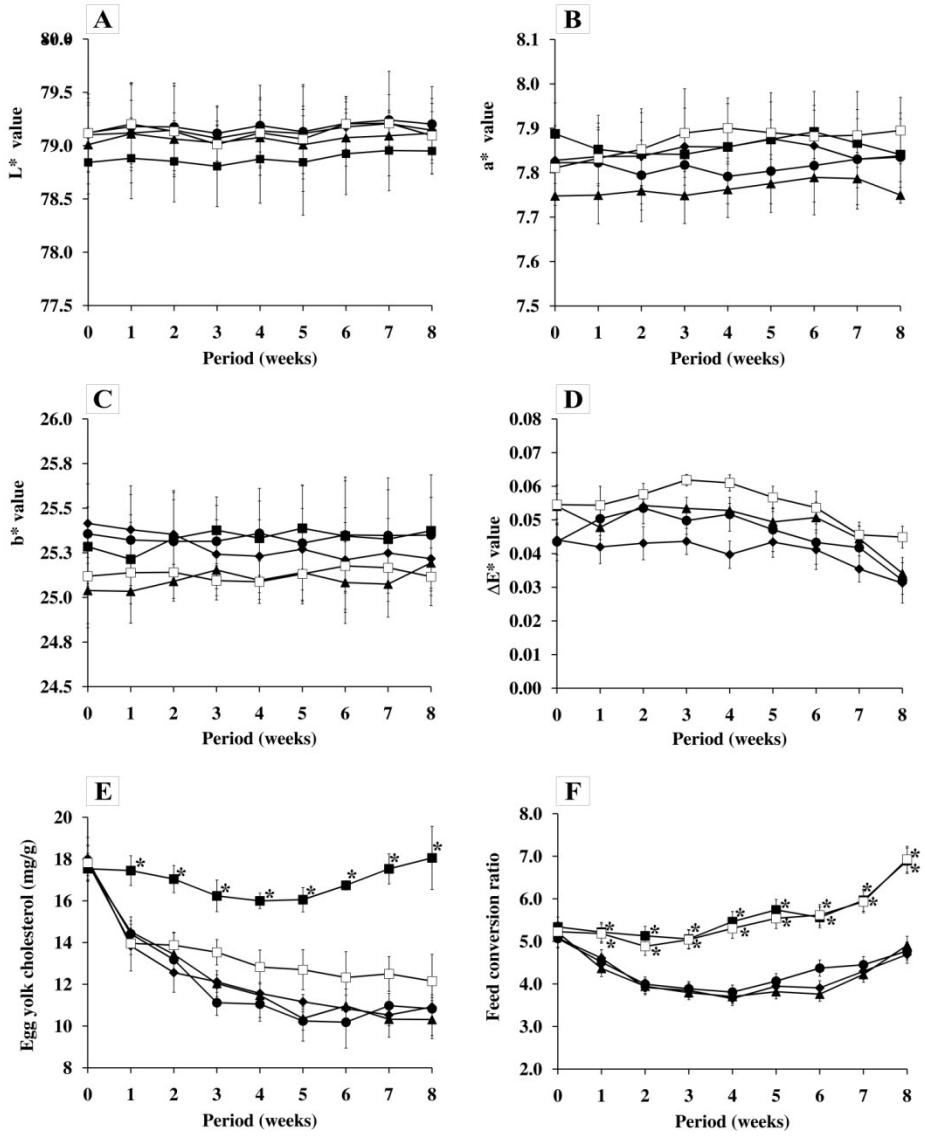

Figure 6.3 The color value (A–D) and cholesterol content (E) of egg yolks, and feed conversion ratio value of bird (F) in this study. Data are presented as mean averages and the error bar on each graph indicates a ± standard error. Week 0 indicates the beginning value. An asterisk symbol (*) indicates a significant difference ($P \leq 0.05$). (■) = Control, (▲) = 6 mg RMR, (♦) = 12 mg RMR, (●) = 24 mg RMR and (□) = lovastatin drug treatments.

Due to the high cost of pharmacological agents, and the fact that some have caused ovarian regression and a reduction in egg production, biological agents obtained from plants and microorganisms are now being used as they are generally assumed to be more affordable and less hazardous. For example,

Christaki *et al*. (2011) and Aydin *et al*. (2008) reported that the supplementation of the anise (*Pimpinella anisum* L.) seed and black cumin (*Nigella sativa* L.) extracts could reduce the level of cholesterol in the egg yolks of the Japanese quail and the white Hyline laying hen, respectively. Yalcin *et al*. (2008) found that the use of yeast culture (*Saccharomyces cerevisiae*) could reduce egg yolk cholesterol levels in the Lohmann brown laying hen. Primarily, RMR products have been used for the reduction of cholesterol levels in the egg yolks of laying hens (Wang and Pan, 2003; Wong *et al*., 2005; Nuraini and Latif, 2012; Sun *et al*., 2015), due to the fact that RMR products contain monacolin K, which is an inhibitor of the hydroxymethyl-glutaryl coenzyme A reductase (HMG-COA) regulates and inhibits the enzymes involved in cholesterol biosynthesis (Manzoni *et al*., 1999; Patrick and Uzick, 2001). However, there have not been any reports on the application of RMR products to reduce the cholesterol levels in the yolks and serum of Japanese quail eggs. This present study showed that the supplementation at a range of dosages (6, 12 and 24 mg/day/bird that contained monacolin K at 0.060, 0.121 and 0.241 mg/day/bird, respectively) of RMR could reduce the level of cholesterol content in the egg yolks of the Japanese quail. 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 (Wang and Pan, 2003; Sun *et al*., 2015). 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 (Mori *et al*., 1999; Elkin *et al*., 1999; Kim *et al*., 2004a). Sun *et al*. (2015) reported that the supplementation of RMR that contained monacolin K of at least 0.003 mg/day/bird could reduce the cholesterol content in the egg yolks of the Lohmann brown laying hen when compared with the control treatment, while the supplementation of monacolin K by at least 4.0 mg/day/bird could reduce the level of egg yolk cholesterol in Isa brown laying hens (Nuraini and Latif, 2012). Furthermore, Wang and Pan (2003) found that the level of monacolin K of at least 13 mg/day/bird could reduce the egg yolk cholesterol level of white Leghorn hens.

6.3.3 Feed conversion ratio

The feed conversion ratio represents the proportion of food that is converted into eggs. The feed conversion ratio value of all treatments in this study was calculated and is presented in Figure 6.3F. 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. It was presumptively concluded that RMR could decrease the feed conversion ratio in the Japanese quail.

This study found that the supplementation of RMR increased egg production and decreased the feed conversion ratio of quail according to the study of Nuraini and Latif (2012), while some previous studies have reported that RMR did not affect the egg production and the feed conversion ratio among laying hens (Kim *et al*., 2003; Wong *et al*., 2005; Sun *et al*., 2015). 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 (Varkoohi *et al*., 2010; Nuraini and Latif, 2012). Therefore, this study indicated that the egg production efficiency of the Japanese quail at all dosages of the RMR treatments was higher than that of the control and lovastatin treatments. 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 (Sazzad, 1992; Singh *et al*., 2009).

6.3.4 Blood biochemical parameters

A lipid profile of the serum after eight weeks of cultivation is shown in Figure 6.4. 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. The statistical analysis showed that the total cholesterol and triglyceride levels in the lovastatin treatment were lower than those of all dosages of the RMR treatments, but they were significantly lower than those of the control treatment. The results show that the LDL levels at all dosages

of the RMR and lovastatin treatments were not significantly different, but they were significantly lower than those of the control treatment. The HDL levels in all treatments were not found to be significantly different. A comparison of all RMR treatments indicated that the levels of total cholesterol in the 6 mg RMR treatment were significantly higher than those of the 12 and 24 mg RMR treatments.

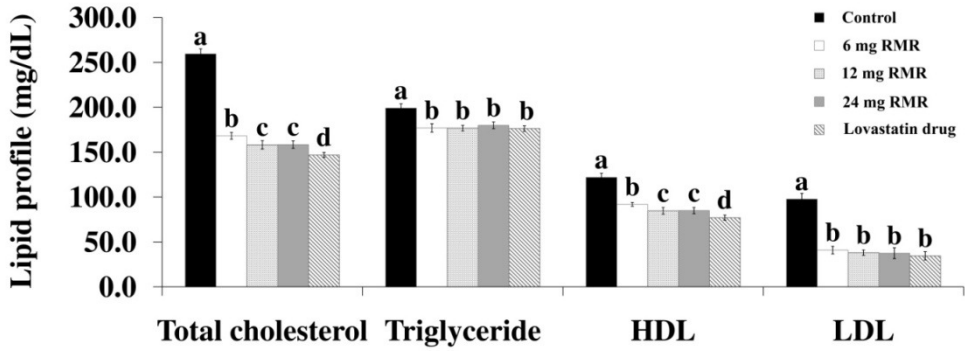

Figure 6.4 Lipid profiles of birds serum after eight weeks of cultivation in this study. Data are presented as means and the error bar at each point indicates the ± standard deviation. The different letter of each point in the same parameters indicates a significant difference ($P \leq 0.05$). HDL = high density lipoprotein and LDL = low density lipoprotein.

In this study, it was presumptively concluded that RMR could decrease total cholesterol, triglyceride and LDL levels in the serum of Japanese quail. RMR products have been successfully used to reduce serum cholesterol, triglyceride and LDL levels not only in humans but also in certain animals, e.g. rabbits, Wistar rats, laying hens and broiler chickens (Jeon *et al*., 2001; Wei *et al*., 2003; Wang *et al*., 2006; Bunnoy *et al*., 2015). In this study, all dosages of RMR used could reduce the total cholesterol, triglyceride and LDL levels in the serum of Japanese quails. Similarly, RMR products have been found to reduce serum cholesterol, triglyceride and LDL levels in the serum of laying hens. However, the degree to which this decrease occurred was dependent upon the RMR dose and poultry breed. For example, Sun *et al*. (2015) reported that the supplementation of RMR containing monacolin K 0.006 mg/day/bird reduced the total cholesterol,

triglyceride and LDL levels in Lohmann brown laying hens, while supplementation with RMR containing monacolin K at 33 mg could reduce the total cholesterol levels in white Leghorn hens (Wang and Pan, 2003). However, Kim *et al*. (2003) reported that the supplementation of *Monascus* culture that contained monacolin K 0.02 at 0.05 mg/day/bird could not decrease total cholesterol levels in Isa brown hens. 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 broiler chickens and laying hens did not affect the HDL levels in the serum (Wang and Pan, 2003; Wang *et al*., 2006; Sun *et al*., 2015).

The biochemical parameters in the blood serum data are shown in Figure 6.5. The results show that the levels of BUN, creatinine, ALT, albumin, and globulin in the birds used in all treatments were not significantly different. The levels of AST and CPK of the blood serum of all dosages of RMR and lovastatin treatments were statistically higher than in the control treatment. A comparison of RMR treatments indicated that the levels of AST in the 24 mg RMR treatment were significantly higher than in the 6 and 12 mg RMR treatments. Generally, a functional application of the kidney has been determined by BUN and the creatinine parameters (Schrier, 2008) and a clinical symptom for monitoring the health of the immune system has been determined by the albumin and globulin levels in the serum (Hamidipour *et al*., 2016). 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; therefore, it maybe 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.

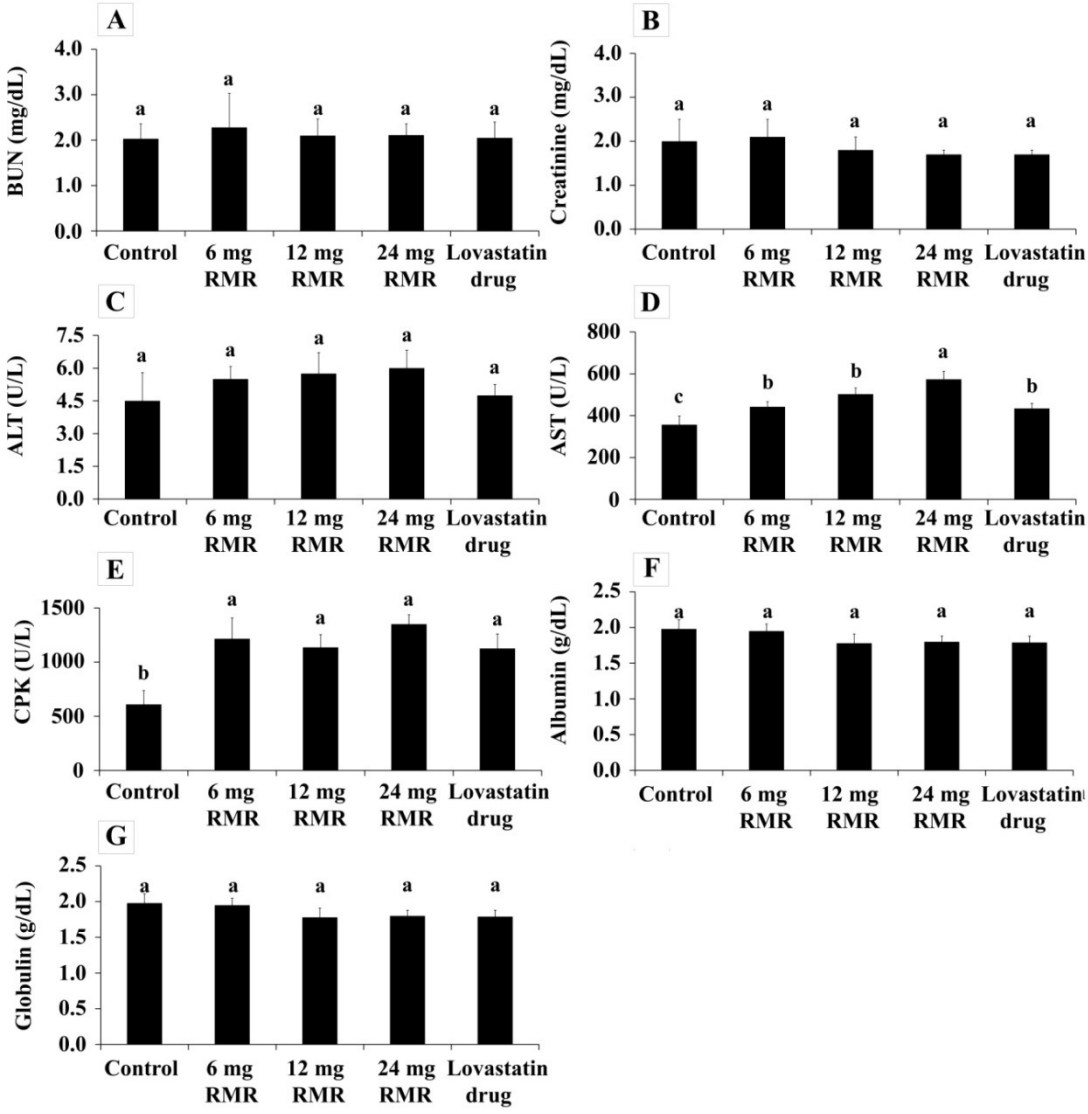

Figure 6.5 Serum parameters of birds at eight weeks of cultivation in this study. Data are presented as means and the error bar at each point indicates the ± standard deviation. The different letter of each point in the same parameters indicates a significant difference ($P \leq 0.05$). BUN = blood urea nitrogen, ALT = alanine aminotransferase, AST = aspartate aminotransferase and CPK = creatine phosphokinase.

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 (Fatemi *et al.*, 2006). Our study showed that the ALT levels (4.5 to 6 U/L) in all treatments were not significantly different, as those levels were within

the ALT normal level (4 to 7 U/L) for Japanese quails (Ukashatu *et al*., 2014). 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 (Patrick and Uzick, 2001; Leung *et al*., 2012). 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 to 880 U/L) levels found in the serum of the Japanese quail from several previous studies (Denli *et al*., 2005; Scholtz *et al*., 2009; Ukashatu *et al*., 2014; Yassein *et al*., 2015; Abou-Kassem *et al*., 2016). Moreover, previous studies have shown that the increase in the AST and ALT levels by lovastatin treatment was dependent upon the dosage used and the animal species. Additionally, the AST and ALT levels increased when the dosage of lovastatin was also increased (Kornbrust *et al*., 1989; Sun *et al*., 2015). 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) (Smith and Olive, 2003; Guis *et al*., 2005; Lapi *et al*., 2008). Moreover, Lee *et al*. (2012) reported that monacolin K could increase the CPK activity in Golden Syrian hamsters.