Dietary supplementation of *Gracilariopsis persica* is associated with some quality related sera and egg yolk parameters in laying quails

Behnam Abbaspour, Sharifi S Davood* and Abdollah Mohammadi-Sangcheshmeh

Abstract

BACKGROUND: *Gracilariopsis persica* (Gp) is one of the most abundant red algae distributed in the Persian Gulf, containing various bioactive components with hypolipidemic, hypoglycemic and antioxidant properties. Therefore using laying quails as a model we aimed to investigate the effect of dietary Gp on body weight, feed conversion, estradiol, progesterone, calcium and lipid levels in serum, as well as the high-density:low-density lipoprotein (HDL:LDL) ratio. Yolk cholesterol and yolk lipid oxidation were also evaluated. To accomplish this, diets containing 0, 10, 30 and 50 g kg\(^{-1}\) Gp were fed to 5-week-old laying quails for 12 weeks.

RESULTS: Our data revealed that Gp had no effect on body weight, feed conversion, triglycerides and estradiol levels of serum. Dietary Gp decreased the serum and yolk cholesterol in a dose-dependent manner. In addition, the sera progesterone and calcium levels and HDL:LDL ratios were increased by feeding diets containing 50 g kg\(^{-1}\) Gp. Our results relating to yolk lipid oxidation showed that malondialdehyde content was decreased in Gp-fed laying quails.

CONCLUSIONS: The results of the present study demonstrate that not only serum and egg yolk cholesterol levels, but also susceptibility of yolk lipids to oxidation, can be decreased by feeding Gp to laying quails.

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Keywords: quail; cholesterol; malondialdehyde; progesterone; *Gracilariopsis persica*

INTRODUCTION

Egg is an excellent source of high-quality protein as well as many vitamins and minerals.\(^1\) It is well known that egg yolk is rich in fat and fat-soluble vitamins such as vitamin A, D and E, which are essential for human health. Despite the nutritional value of eggs, there are some potential health issues arising from egg quality. Egg is a major source of cholesterol, which can contribute to high blood cholesterol levels.\(^2\) It has been reported that foods with high cholesterol cause artery stenosis, which is one of the major causes of coronary heart disease.\(^3\) The cholesterol and fat content in egg yolk highly depend on the birds’ diet.

Several studies have been conducted in laying hens to reduce the cholesterol content of egg through diet manipulation strategies.\(^4\)–\(^7\) Seaweeds have been used directly as a source of health food for human consumption since very early times.\(^8\) It is evident that the seaweeds are rich sources of bioactive components as well as micro and macro nutrients.\(^9\) Thus the nutritional value of seaweeds as a food for humans\(^10\) or feedstuff for animals\(^11\) and poultry\(^12\) has been widely investigated.

*Gracilaria* is a genus of red algae (Rhodophyceae) and a member of the Gracilariaeae family. This seaweed is well known for its economic importance as a source of agar, as well as its use as a food for humans. *Gracilaria persica* (Gp) is one of the most abundant red algae in the Persian Gulf, which introduced based on morphology and DNA sequence data.\(^13\) Recently, the nutritional value of Gp was evaluated as a feedstuff for laying hens. In that study Gp was demonstrated to be valuable for inclusion in the diet of laying chickens up to a level of 100 g kg\(^{-1}\).\(^14\)

In this regard it has been reported that Gp contains various bioactive sterols such as fucosterol, stigmasterol and \(\beta\)-sitosterol, which can effectively decrease plasma cholesterol, glucose and inflammation.\(^15\) Little data are available on the nutritional value of Gp or its effects on the metabolism of poultry. Therefore, a prospective study has been performed to determine possible effects of using dietary Gp on metabolism of laying quails, especially on their serum lipids, calcium, estrogen and progesterone levels, as well as on egg lipid oxidation.

MATERIAL AND METHODS

Experimental diets and bird husbandry

Before diet formulation, whole Gp seaweeds were ground in a hammer mill using a 1 mm mesh. A GP sample (1 kg) was prepared and after grinding finely the sample was subjected to chemical analysis (Table 1). Moisture, ash, crude fiber, ether extract, crude

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protein, Ca, P and Na content of were determined using standard AOAC methods.16

A total of 96 laying Japanese quail (Coturnix coturnix japonica) 5 weeks of age with an average weight of 200 ± 20 g were randomly assigned to 16 cages (n = 4 cages per treatment; six birds per cage). The feeding experiment was conducted for 12 weeks. However, all quails were fed with the control diet for 2 weeks before the feeding experiment began. The dietary inclusion rates of Gp seaweed was as follows: 0 (control group), 10, 30 and 50 g kg⁻¹.

The diets were isocaloric and isonitrogenous and were formulated to meet or exceed the nutritional recommendations of laying quails (Table 2).17 Birds were raised under environmentally controlled conditions following a standard temperature regimen (20 ± 5 °C) and a 16L:8D lighting program. Feed and water were provided for ad libitum intake.

During the experimental period, egg numbers were recorded daily and feed consumption was determined weekly. The body weights of birds were measured at the beginning and end of the study to determine body weight changes during the experiment.

Analysis of yellow follicles
After weighing the ovaries, follicles were dissected using the method described by Gilbert et al.18 The follicles were classified into three groups according to their size: large yellow follicles (>8 mm), small yellow follicles (2–8 mm) and white follicles (2–5 mm). In this study, the diameter of the largest (F1) and the second largest (F2) ovarian follicles of the hens were measured using a digital Vernier caliper and then calculated based on the total ovarian follicles (%).

The eggs laid during the last 3 days of every 4 weeks were weighed individually. Then, eggs from the same replicate (20 eggs per treatment) were broken out carefully and the yolks separated from albumen, weighed, pooled and blended. Three-gram samples of egg yolks were dissolved in a 2% NaCl solution to measure egg yolk cholesterol. Remaining egg yolk was frozen at −20 °C pending antioxidant activity assessment. At the end of the experiment, two birds per cage (n = 8 per treatment) were randomly selected and their blood samples were taken from the wing vein using a sterile syringe. The blood samples were centrifuged at 1000 × g for 10 min and serum samples were stored at −20 °C for later analysis.

Analysis of egg yolk cholesterol
Cholesterol concentration in yolk samples was determined enzymatically using commercial kits (Pars Azmoun, Karaj, Iran, CHAD-PAP 2012) according to the manufacturer’s recommendations. Briefly, 3 g yolk was dissolved in 27 mL of 2% (w/v) NaCl. The solution was then shaken slowly for 2 h. Subsequently, 1 mL of these solutions was diluted tenfold with 2% NaCl solution and then used as test sample.

Iron-induced lipid oxidation
Iron-induced lipid oxidation (induced TBA) was carried out as a modification of the method of Kornsbrust and Mavis,19 described by Botsoglou et al.20 Briefly, four 1-g subsamples from each egg yolk were weighed into 50 mL centrifuge tubes. Then, 1.5 mL of a solution containing 1.138 mmol L⁻¹ ferrous sulfate and 0.368 mmol L⁻¹ ascorbic acid were added to three of the sub-samples and incubated at 37 °C for 50, 100 or 150 min. Following incubation, all three iron-induced subsamples, along with the fourth non-induced subsample, were immediately submitted to malondialdehyde assay for assessing the extent of lipid oxidation.

Malondialdehyde assay
Malondialdehyde (secondary oxidation product) values were determined by a spectrophotometric method described by Botsoglou et al.21 For this, 4 mL aqueous trichloroacetic acid (50 g L⁻¹) and 2.5 mL butyrate hydroxytoluene in hexane (8 g L⁻¹) were added to samples and then vortexed for 60 s. Then, the mixture was centrifuged for 3 min at 3000 × g; the top hexane layer was discarded and a 2.5 mL aliquot from the bottom layer was mixed with 3 mL aqueous 2-thiobarbituric acid (8 g L⁻¹). TBA-containing tubes were incubated for 30 min at 70 °C in a water bath. Following incubation, the mixture was cooled on ice and submitted

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### Table 1. Metabolizable energy (MJ kg⁻¹ dry matter) and chemical composition (g kg⁻¹ dry matter) of Gracilaria persica11

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>964.0</td>
</tr>
<tr>
<td>Metabolizable energy</td>
<td>9.2</td>
</tr>
<tr>
<td>Crude protein</td>
<td>230.5</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>72.0</td>
</tr>
<tr>
<td>Ether extract</td>
<td>1.0</td>
</tr>
<tr>
<td>Calcium</td>
<td>9.0</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>3.4</td>
</tr>
<tr>
<td>Sodium</td>
<td>12.1</td>
</tr>
<tr>
<td>Ash</td>
<td>255.0</td>
</tr>
</tbody>
</table>

### Table 2. Ingredient and nutrient composition of experimental diets (as fed)

<table>
<thead>
<tr>
<th>Composition (g kg⁻¹)</th>
<th>Dietary Gp (g kg⁻¹)</th>
<th>AMEn (MJ kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12.39</td>
</tr>
<tr>
<td>Yellow corn</td>
<td></td>
<td>12.39</td>
</tr>
<tr>
<td>Soybean meal- 48%</td>
<td></td>
<td>12.39</td>
</tr>
<tr>
<td>Soybean oil</td>
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<td>12.39</td>
</tr>
<tr>
<td>Gluten meal</td>
<td></td>
<td>12.39</td>
</tr>
<tr>
<td>Seaweed</td>
<td></td>
<td>12.39</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td></td>
<td>12.39</td>
</tr>
<tr>
<td>Oyster shell</td>
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<td>12.39</td>
</tr>
<tr>
<td>Sodium chloride</td>
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<td>12.39</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td></td>
<td>12.39</td>
</tr>
<tr>
<td>Vitamin–mineral premix⁸</td>
<td></td>
<td>12.39</td>
</tr>
</tbody>
</table>

Calculated analysis (g kg⁻¹ as fed)

<table>
<thead>
<tr>
<th>AMEn (MJ kg⁻¹)</th>
<th>CP</th>
<th>CF</th>
<th>Ca</th>
<th>Available P</th>
<th>Met + Cys</th>
<th>Lys</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.39</td>
<td>180</td>
<td>180</td>
<td>31</td>
<td>4.50</td>
<td>8.20</td>
<td>8.50</td>
</tr>
<tr>
<td>12.39</td>
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<td>180</td>
<td>31</td>
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<td>180</td>
<td>180</td>
<td>31</td>
<td>4.50</td>
<td>8.20</td>
<td>8.50</td>
</tr>
</tbody>
</table>

⁸ Providing, per kilogram of diet: vitamin A, 12 000IU; vitamin D3, 20 000 IU; vitamin E, 35 mg; vitamin K3,4 mg; vitamin B1, 3 mg; vitamin B2, 7 mg; niacin, 20 mg; vitamin B6, 5 mg; vitamin B12, 0.015 mg; folic acid, 1 mg; biotin, 0.045 mg; ascorbic acid, 50 mg; canthaxanthine, 1.5 mg; apocarotenoic acid ester, 0.5 mg; choline chloride, 125 mg; manganese, 80 mg; iron, 60 mg; zinc, 60 mg; copper, 5 mg; cobalt, 0.2 mg; iodine, 1 mg; selenium, 0.15 mg.
to conventional spectrophotometry (UV–visible S2100, Scinco, Seoul, South Korea) at a wavelength of 521.5 nm.

Analysis of blood biochemical traits
Lipids (cholesterol, triglycerides and HDL:LDL ratios) and calcium of serum were measured by enzyme colorimetric methods, using a commercial kit (Pars Azmoun, Karaj, Iran). All analyses were performed on a multiple reader (Roche Cobas Mira, Elisa Reader and Dana-3200). Serum estradiol and progesterone levels were determined using radioimmunoassay, and commercial kits (DKO003 and DKO006, respectively, Delaware Biotech, Wilmington, DE, USA). Levels were determined using Tosoh AIA-360 Immunoassay Analyzer (WS-AIA360).

Statistical analysis
The data were analyzed using one-way analysis of variance. Differences among treatment means were tested for significance using Duncan’s multiple-range test.

RESULTS
Effect of Gp on live body weight and egg production
Dietary treatments had no effect on the weight of egg and yolk. Also, there were no live body weight changes, daily feed intake or feed conversion due to the dietary treatments (Table 3). No differences (P > 0.05) in egg production and egg mass were detected between birds on Gp diets and control. However, egg production and egg mass produced by birds receiving the diet containing 50 g kg⁻¹ Gp were lower (P < 0.05) when compared to birds fed 10 or 30 g kg⁻¹ Gp diets.

Effect of Gp on cholesterol and blood biochemical parameters
There were no differences in triglycerides and estradiol of serum between treatments (Table 4). However, the concentration of triglycerides and estradiol of serum tended to be lower and higher, respectively, by increasing the level of seaweed in diets. The HDL:LDL ratio was higher (by 31%; P < 0.05) in birds receiving the diet containing 50 g kg⁻¹ Gp as compared to the control diet (Table 4). The levels of cholesterol, progesterone and calcium concentrations in serum were significantly influenced by dietary treatments. Serum cholesterol level was lower in birds fed on diets containing Gp (P < 0.05). In contrast, in birds receiving the 50 g kg⁻¹ Gp diet, concentrations of progesterone and calcium in serum were higher when compared to birds fed the control diet (P < 0.05).

Effect of Gp on ovarian follicles, egg cholesterol content and oxidative stability of the yolk
Table 4 shows our data regarding the proportion of yellow follicles (as a percentage of total ovarian follicles) in the birds. As can be seen, birds that received a diet containing 50 and 30 g kg⁻¹ Gp represent a higher level of follicles than that of control group (P < 0.05). The effect of the dietary Gp on the cholesterol content and oxidative stability of the yolk is illustrated in Figs 1 and 2, respectively. No significant effect of dietary treatments was
observed on the yolk cholesterol at week 10. However, the cholesterol level in the egg yolks, as determined in eggs laid at weeks 14 and 18, were lower in the Gp-fed quail as compared to the respective value of the control group ($P < 0.05$). In egg yolk, the concentrations of malondialdehyde for birds that fed Gp diets were lower than those for birds fed the control (Fig. 2). However, significant differences were observed after 50, 100 and 150 min incubation for iron-induced lipid oxidation ($P < 0.05$).

**DISCUSSION**

According to our findings there were no differences in body weight, egg mass, egg or yolk weight following feeding Gp. This is in great agreement with previous findings where red algae have been found to have no effect on the aforementioned parameters. The birds fed on diets containing Gp tended to show a lower body weight; however, the differences were not significant. The lower body weight, egg mass, egg and yolk weight observed in this study, especially in the 50 g kg$^{-1}$ group, can be explained to some extent by the fiber- and ash-rich properties of the Gp.

In the present study, we speculated that usage of Gp in the diet could be a potential to modify egg and serum cholesterol as well as some blood biochemical parameters. According to our finds, feeding Gp caused a significant reduction in the cholesterol concentration in serum, with a concomitant decrease in serum triglycerides (numerically) and yolk cholesterol content. The increased levels of serum HDL:LDL in birds receiving the diet containing 50 g kg$^{-1}$ Gp may be due to hypertriglyceridemia and hypocholesterolemia induced by reactive metabolite formed during biotransformation. Similarly, Dvir et al. reported that the addition of biomass red micro alga (*Porphyridium* sp.) to rats’ diets increased levels of serum HDL:LDL and decreased levels of plasma cholesterol, whereas no changes were observed in plasma triglyceride levels. These results were also consistent with previous findings. It appears from these results that feeding laying quails with Gp led to a significant reduction in yolk cholesterol content in response to decreased sera cholesterol concentration.

In this study, however, the yolk cholesterol was not different among dietary groups during the first 4 weeks of Gp feeding. This finding is in agreement with a previous report by Ginzberg et al. Previous studies have shown that red marine algae, including *Gracilaria* spp., are a rich source of dietary fiber (338–700 g kg$^{-1}$ dry weight), unsaturated fatty acids and sterols. It is known that the dietary fiber, plant sterols and polyunsaturated fatty acids reduce blood cholesterol in human and animals.

It is reported that dietary fiber increases intestinal viscosity and modifies the gastrointestinal transit time, increases fecal bile acid and neutral steroid excretion, inhibits lipase activity and consequently reduces the absorption of lipids in the small intestine. Also, in some studies, it has been reported that dietary fiber may possess hypocholesterolemic activity by inhibiting hydroxymethylglutaryl-CoA reductase (the key enzyme in cholesterol synthesis) and hepatic sterol synthesis. In another study, it has been shown that algal polysaccharide have no direct effect on the inhibition of hepatic cholesterol synthesis. Therefore, all the above findings may well explain the results of this study.

Increasing serum estradiol and progesterone concentration in Gp-fed birds compared to the control group indicates that there is
Gracilariaopsis persica has been found to reduce serum and egg yolk cholesterol levels... www.soci.org

**Figure 2.** Effect of dietary Gp on yolk malondialdehyde concentration (mean ± SD). Columns with different letters (a, c) are significantly different ($P < 0.05$).

probably some phytoestrogenic components in Gp. Accelerated serum estradiol concentration has been demonstrated in layer hens given exogenous estrogens. In humans, nutrition studies of seaweed have reported increased plasma progesterone and decreased estrogen. Also, it has been proposed that seaweed has potent antiestrogenic effects that may be associated with high fiber content as well as low cholesterol absorption in the intestine.

The proportions of yellow follicles were higher for birds fed Gp diets than control birds, which might be a result of the better development of the ovaries in these birds. The fact that the steroid hormones such as estrogen and progesterone originate from cholesterol may to some extent explain the observed increased steroid hormone and reduced plasma cholesterol in this study.

The observed increase in the calcium content of serum in 50 g kg$^{-1}$ Gp treatment could be related to the higher concentration of estradiol and progesterone of serum in comparison with the other treatments. Increase in plasma calcium as a consequence of estrogen administration agrees with several previous reports. Bar and Hurwitz reported that estrogen hormones increased intestinal absorption of calcium in laying birds. Also, Elkomy et al. reported that estrogen treatment increased calcium absorption and blood calcium concentration in young and old Japanese quail hens. On the other hand, the increases in quail’s serum calcium concentration may be due to the high level of iodine in Gp diets. Seaweeds have the unique ability to concentrate minerals from the ocean; thus they are rich in many minerals and trace elements such as calcium, iron and iodine. Accordingly, Harms et al. reported that feeding iodine resulted in increased blood calcium levels in laying hens.

The Gp used as a dietary constituent in the current research decreased malondialdehyde concentrations of egg yolk after 50, 100 and 150 min incubation for iron-induced lipid oxidation. This indicates that Gp contains chemical compounds with antioxidant activity which can be transferred to the egg. It has been reported that Gp is a rich source of antioxidants. Takamatsu et al. reported that chlorophylls, carotenoids, tocopherol derivatives such as vitamin E, and related isoprenoids that are structurally related to plant-derived antioxidants, are found in seaweeds. Thus it appears that antioxidant constituents of Gp had passed into the developing yolk through feeding, and protected yolk lipids against iron-induced oxidation. This observation is consistent with previous work demonstrating the antioxidant activity of seaweed and marine algae.

In conclusion, our data imply that the cholesterol levels in serum and egg yolk can be decreased by feeding Gp to laying quails. Moreover, susceptibility of yolk lipids to oxidation was found to be reduced by supplying Gp in the quail diet. It is important, however, to note that such an improvement had no detrimental effect on performance of laying quails until Gp was included up to 30 g kg$^{-1}$ in diets.

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