Tocotrienol Content in Hen Eggs: Its Fortification by Supplementing the Feed with Rice Bran Scum Oil

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Tocotrienol (T3) is an unsaturated vitamin E having health benefits (e.g., anti-angiogenesis). We measured T3 in commercial eggs, and developed T3-fortified eggs by adding rice bran scum oil (RBO, containing 1.3% T3) to the feed. Commercial eggs contained about 0.11 mg of T3/egg, while the T3 content was improved to 0.62 mg/egg after RBO supplementation to the feed of hens for 7 d.

Key words: tocoptrienol; egg; fortification

Recent studies have shown that tocotrienol (T3, unsaturated vitamin E) had better anti-oxidative, anti-hypercholesterolemic, and anti-cancer activities than those of tocopherol (Toc, a well-known vitamin E).1) We found that T3 acted as an effective anti-angiogenic compound2–4) that would be useful for preventing angiogenic-related disorders (i.e., diabetic retinopathy, rheumatoid arthritis, and cancers). These findings1–4) suggest that T3 has a wide variety of health benefits. However, the intake of T3 from dietary foods might be quite low (probably below several mg/day), because T3 is limited to certain food sources (e.g., rice bran and palm oil).5,6) We are interested in T3 as a potent therapeutic agent working against angiogenic-related disorders, and developing T3 as a food product is one of our objectives.

Hen eggs have recently been receiving considerable attention, since they can be fortified with functional compounds by supplying such compounds to the hen feed. For example, Bourre et al. have reported that docosahexaenoic acid, folic acid, and Toc could be concentrated up to 60 mg (3-fold docosahexaenoic acid to normal egg), 0.1 µg (4-fold folic acid), and 4 mg (6-fold Toc)/egg.7) These kinds of fortified eggs have been increasingly commercialized and are consumed as ordinary food or as food for health purposes. Considering the results reported by Bourre et al.,7) it may be possible that T3 can be accumulated in hen eggs by adding a T3 source to the feed, because T3 has a similar structure to that of Toc (T3 has an unsaturated isoprenoid chain, whereas Toc has a saturated phytol chain).

In this study, due to a lack of quantitative data concerning the T3 concentration in egg, we measured the T3 concentration in eleven kinds of commercial eggs by using high-performance liquid chromatography (HPLC) with fluorescence detection.6) We then investigated whether T3 had been accumulated and concentrated in hen eggs by supplementing the hen feed with rice bran scum oil (RBO, Sanwa-Yushi Co., Ltd., Tendo, Japan). The RBO was composed of 1.3% wt. T3 (0.53, 0.75 and 0.05% of δ-C11-, δ-C13- and δ-C14-T3, respectively) as well as 1.7% wt Toc (1.5, 0.04, 0.08 and 0.03% of α-, β-, γ- and δ-Toc, respectively).

To analyze the T3 contents in commercial hen eggs, eleven kinds of hen eggs (four brown eggs and seven white eggs) were purchased from a local market (Sendai, Japan). The egg yolk was separated from the egg white, lyophilized, and ground into yolk powder. This yolk powder (0.25 g) was suspended in 0.5 ml of a 1% (w/v) NaCl aqueous solution. To the suspension, 9 ml of 3% ethanolic pyrogallol, 1 ml of 50 µM ethanolic 2,2,5,7,8-pentamethyl-6-hydroxychromane (PMC, internal standard), and 0.5 ml of a 60% KOH aqueous solution were added and thoroughly mixed. The resulting mixture was incubated at 70°C for 30 min. The saponified solution

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Abbreviations: HPLC, high-performance liquid chromatography; PMC, 2,2,5,7,8-pentamethyl-6-hydroxychromane; RBO, rice bran scum oil; Toc, tocopherol; T3, tocotrienol
was cooled by ice, 22.5 ml of a 0.9% NaCl aqueous solution was added, and the mixture was extracted with 15 ml of hexane:ethyl acetate (9/1, v/v). After centrifuging at 1,000 × g for 5 min, the upper layer was collected. The extraction with hexane:ethyl acetate (9/1, v/v) was then repeated. The upper layers were combined and dried. The residue was dissolved in hexane, and a portion of the extract was subjected to HPLC for a vitamin E analysis.

The HPLC system consisted of a Jasco PU-980 pump (Japan Spectroscopic Co., Tokyo, Japan), a Jasco CO-860 column oven, and a Reodyne 7125 injector (Cotati, CA, USA). Inertsil SIL 100A-5 (4.6 × 250 mm; GL Science, Tokyo, Japan) was used as the HPLC column, and a mixture of hexane/1,4-dioxane/2-propanol (1000:40:5, v/v/v) was used as the mobile phase. The flow rate was adjusted to 1.0 ml/min, and the column temperature was maintained at 35 °C. T3 and Toc were detected by an RF-10AXL FLD detector (excitation at 294 nm, emission at 326 nm; Shimadzu, Kyoto, Japan). All peak areas were recorded with a SIC Chromatocorder 21J integrator (System Instruments, Tokyo, Japan). The concentrations of T3 and Toc in the egg samples were calculated against calibration curves for standard T3 (Eisai, Tokyo, Japan) and Toc (Wako, Osaka, Japan), and then corrected by using the peak area ratios of the vitamin E isoforms to PMC (internal standard). The determination was made three times for each sample.

To investigate the T3 accumulation in eggs, seventy-two Julia hens were divided into 3 groups (24 hens/group, 4 hens/cage). The experimental diets (Toyohashi Feed Mills Co., Ltd., Shinshiro, Japan) contained essential nutrients (e.g., 17% crude protein) and 4% fat. The fats used were varied as follows: 0% RBO group containing 4% animal fat; 2% RBO group containing 2% animal fat and 2% RBO; and 4% RBO group containing 4% RBO. The laying hens were given free access to the feed and tap water, and eggs were collected on days 0, 7, 14, and 28. The egg samples (three eggs randomly selected from about twenty-four eggs from each collection) were determined for their contents of T3 and Toc in the same way as that for the commercial eggs.

Table 1 shows the T3 and Toc concentrations in the eleven kinds of commercial eggs. Egg white did not contain T3 or Toc (data not shown), but we detected vitamin E in the egg yolk. The amount of T3 was in the range of 0.04 to 0.39 mg/egg (0.11 mg/egg as an average), and α-T3 was found as the predominant form of all T3 isoforms. On the other hand, Toc in the eggs varied from 0.77 to 17 mg/egg. It is therefore likely that humans receive T3 and Toc from eggs.

In the laying hen experiment, the T3 and Toc contents in the experimental diets were 18 mg of T3/kg and 76 mg of Toc/kg in the 0% RBO group, 170 mg of T3/kg and 210 mg of Toc/kg in the 2% RBO group, and 260 mg of T3/kg and 300 mg of Toc/kg in the 4% RBO group. The average feed intake was 127, 131, and 124 g/d/bird for the 0%, 2%, and 4% RBO groups, respectively. The eggs collected from each group on days 0, 7, 14, and 28 showed no significant differences in total weight (around 61 g), egg white weight (36 g), and yolk weight (17 g). Chromatograms of T3 and Toc from the eggs collected on days 0 and 28 from the 2% RBO group are shown in Fig. 1A, and changes in the T3 and Toc contents during the experiment period are presented in Fig. 1B. Considering the day-0 eggs (Fig. 1A), the predominant types of vitamin E were α-Toc and γ-Toc, with little α-T3 and γ-T3 being found.

The amounts of total T3 and total Toc in the day-0 eggs were 0.08–0.09 mg of T3/egg and 1.4–1.7 mg of Toc/egg. In the groups fed with RBO, the contents of both T3 and Toc were elevated to the maximum levels in the day-7 eggs (2% RBO group, 0.62 mg of T3/egg and 7.2 mg of Toc/egg; 4% RBO group, 0.56 mg of T3/egg and 7.6 mg of Toc/egg). The T3 and Toc contents then gradually decreased in the day-14 eggs (2% RBO group, 0.49 mg of T3/egg and 7.4 mg of Toc/egg; 4% RBO group, 0.41 mg of T3/egg and 7.1 mg of Toc/egg) and day-28 eggs (2% RBO group, 0.30 mg of T3/egg and 5.2 mg of Toc/egg; 4% RBO group, 0.21 mg of T3/egg and 4.5 mg of Toc/egg). A clear accumulation was apparent in increasing amounts of α-T3 (day-7: 0.06, 0.51 and 0.45 mg/egg in the 0%, 2% and 4% RBO groups, respectively), γ-T3 (day-7: 0.02, 0.11 and 0.11 mg/egg in the 0%, 2% and 4% RBO groups,
respectively), and α-Toc (day-7: 0.93, 6.54 and 6.99 mg/egg in the 0%, 2% and 4% RBO groups, respectively), these being the predominant forms of vitamin E present in RBO. The results therefore indicate that the increase of T3 and Toc in the eggs would have resulted from the larger amounts of T3 and Toc present in the feed, and that T3 can be accumulated to some extent in eggs by supplementing the feed with RBO. There have been some reports about the production of eggs enriched with Toc,8–11) but not with T3. Toc accumulation in the 2% RBO group (7.2 mg of Toc/egg) was lower or higher than previous studies when supplementing the feed with a similar amount (200 mg/kg) of Toc or tocopheryl acetate.8,9) As shown in Fig. 1B, a reduction of T3 and Toc in the eggs after they had reached the maximum levels was observed. The reduction of T3 and Toc was by 52–62% and 27–42%, respectively. Some studies8–10) have reported such a reduction of Toc (10–50%), but others11) showed no reduction. In our experiment, the reduction of T3 and Toc in eggs may be explained by them being used as antioxidants for protecting the hens from unavoidable heat stress,12) because this study was conducted and finished in the mid-summer of 2007. On the other hand, in previous studies,11,13) the Toc accumulation in eggs seems to have occurred in a dose-dependent manner. However, in our experiment, no dose-dependant increase of either T3 or Toc was apparent. This might be partly due to the total amount of lipophilic compounds (22 mg/kg of xanthophylls and 560 mg/kg of vitamin E) in the feed of the 4% RBO group being excessive. Therefore, no differences in the Toc and T3 contents between the 2% RBO and 4% RBO groups were seen.

We used Julia hens in this study because they are the main strain of laying hens used for commercial egg production. By adding RBO to the feed, T3 and Toc were accumulated in the eggs of Julia hens. However, it seems possible to accumulate more T3 in eggs by considering some factors: for example, brown laying hens might be introduced instead of Julia hens, because brown laying hens have been reported to be more efficient in vitamin E retention in their eggs (2 or 3 times higher than the white ones).14) In our experiment, the feed contained vitamin A for color enhancement, but it has been reported that vitamin A competitively interacted with vitamin E in its intestinal absorption.14) Therefore, supplementation with vitamin A (the proper quantity) might be more effective. In addition, protecting the laying hens from heat stress might also be considered, because, in high-temperature conditions, hens take less feed and the antioxidants (vitamin E) in eggs are used and reduced for protection against heat stress.12)

In summary, we found that commercial eggs contained only a small content of T3 (around 0.11 mg/egg). By adding a T3 source (RBO) to the feed of laying hens, T3 was absorbed and accumulated in the hen eggs. The maximum level of T3 was 0.56–0.62 mg/egg after 7 d of the experiment. The accumulation of T3 in the eggs could be further improved by considering different types of laying hen and a proper amount of other lipophilic
compounds that might be competitive in the absorption of vitamin E. Eggs enriched with T3 could therefore be produced and are recommended as a source of T3 for its health benefits.

References


