Transfer of α-Tocopherol Stereoisomers from Feeds to Eggs

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The transfer of the stereoisomers of α-tocopherol from three feeds to eggs was studied by using a capillary gas chromatographic method capable of separating the eight isomers into four pairs of enantiomers. The proportions of the isomers in eggs for sale in retail outlets were also examined. The four enantiomeric pairs were found in all egg samples. In all feed vs egg comparisons, the eggs contained significantly more of the enantiomeric pair *RRR* + *SSS* than did the feeds. The proportions of the other pairs were lower in the eggs than in the feeds. The results indicate that the transfer efficiencies of the α-tocopherol stereoisomers are proportional to the order of their biological activities. In the eggs sold in retail outlets, the proportions of the pairs *RRS* + *SSR*, *RRR* + *SSS*, *RSR* + *SRS*, and *RSS* + *SSR* were 13.9%, 56.1%, 13.3%, and 16.6%, respectively.

INTRODUCTION

all-rac-α-Tocopheryl acetate is the most common vitamin E preparation used to supplement feeds. This synthetic product is a mixture of eight stereoisomers or four enantiomeric pairs of α-tocopheryl acetate. The enantiomeric pairs, racemates, have been shown to be present in equimolar amounts (Cohen et al., 1981; Weiser and Vecchi, 1981, 1982; Scott et al., 1982). This finding indicates that the synthetic processes lead to *all-rac*-α-tocopheryl acetate with similar proportions of all eight stereoisomers (Weiser and Vecchi, 1982).

The eight stereoisomers of α-tocopheryl acetate have been shown to have distinctly different biological activities (Weiser and Vecchi, 1982). Acetate of the only naturally occurring isomer, (*R*,*R*,*R*)-α-tocopherol, has the highest activity. The standardized rat resorption–gestation test was used to assay the activities of the other isomers; they were found to have from 21% to 90% of the activity of (*R*,*R*,*R*)-α-tocopheryl acetate.

The different isomers of α-tocopherol are probably transferred from supplemented feeds to animal products (meat, milk, eggs, fish). To date, however, the analytical methods used for determining α-tocopherol in animal products have not separated the different isomers; instead, all α-tocopherol has been imputed as (*R*,*R*,*R*)-α-tocopherol (Piironen et al., 1985; Syväoja et al., 1985a,b). It is therefore possible that their vitamin E activities have been overestimated to some extent.

This study examined the transfer of the different stereoisomers of α-tocopherol from feeds to eggs by comparing their stereoisomeric compositions. A capillary gas–liquid chromatographic (GLC) method capable of separating the eight isomers into four pairs of enantiomers was used. The proportions of the isomers in eggs on sale through retail outlets were also studied. Because of their fairly high α-tocopherol content, in this preliminary study eggs were chosen to represent animal products.

EXPERIMENTAL PROCEDURES

Samples of Feed vs Egg Comparisons. Samples of three feeds and the corresponding eggs were obtained from feeding experiments carried out at the Department of Animal Husbandry, University of Helsinki. The experiments investigated the effects of the cereal composition of feed on various parameters in egg production (Aimonen, 1989). The feeds were prepared by the Animal Feed Division, Cultor Ltd. (Finland).

The cereal compositions of the three feeds selected for this study (A–C) were as follows: 100% barley in feed A; 60% oats and 40% barley in feed B; and 100% oats in feed C. They were all supplemented with the same *all-rac*-α-tocopheryl acetate preparation (Rovimix E-50 adsorbate, F. Hoffmann-La Roche Ltd., Basle, Switzerland). The moisture content of the feeds was about 10%. The amount of added α-tocopherol was 16.0 mg/kg of feed.

Forty-five hens in two-tier pyramid cages (three hens per cage) were fed on each feed. Egg samples were taken after about 4 months of feeding. One egg was taken from each cage (15 eggs/feed).

The eggs were separated, and the determinations were made only from the yolks. One pooled sample representing each feed was made by homogenizing the yolks in a blender (Moulinex). The homogenized samples were vacuum-packed into polyethylene–nylon laminate bags and stored, frozen at −70 °C, until analyzed (after no more than 3 weeks of storage).

Samples of the feeds were taken from four 50-kg sacks (a portion of 1.5–2.0 kg from each sack). One pooled sample was made for each feed by mixing the subsamples. The pooled samples were vacuum-packed and stored at −70 °C until analyzed (about 8 weeks later). The samples were homogenized in a blender (Moulinex) on the day of analysis.

Samples of Eggs on Sale through Retail Outlets. The egg samples (one carton with 10 or 12 eggs) were bought from eight retail stores belonging to Finland's four major food chains. One pooled sample from the yolks of 56 eggs (seven from each carton) was made by homogenizing the yolks. The homogenized samples were analyzed immediately.

Analysis of Stereoisomers of α-Tocopherol. A GLC method was developed for analyzing the stereoisomers of α-tocopherol as their methyl ethers.

The samples were prepared by using the room temperature saponification method (Piironen et al., 1984, 1985). The samples were prepared in triplicate. Sample size was 5 g. The amount of 50% (w/w) KOH in the saponification solution was 40 mL. The other saponification conditions were as described earlier (Piironen et al., 1985) except that BHT (0.02% w/v) was used as the antioxidant in the hexane extraction step.

For GLC analysis, the extracted α-tocopherol isomers were made into the corresponding methyl ethers according to the method published by Cohen et al. (1981). The amount of total α-tocopherol taken for methylation was about 0.05–0.15 mg.

A Micromat HRGC 412 gas chromatograph equipped with a flame ionization detector (Orion Analytica), a Hewlett-Packard Model 3390 A integrator, and a recorder (SE 120, BBC Goerz Metrawatt) were used under the following conditions: column, a fused silica capillary column, CP-Sil 88, 50 m × 0.22 mm i.d. (Chrompack); temperature program from 150 (with a 2-min hold) to 210 °C at 2 °C/min (with a 10-min hold at 210 °C), and from 210 to 230 °C at 1 °C/min (with a 20-min hold at 230 °C); carrier gas, helium at 1.8 mL/min; detector gases, hydrogen at 0.5 bar,

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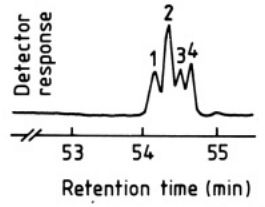

Figure 1. Chromatogram of the gas–liquid chromatographic separation of the enantiomeric pairs of α-tocopherol in an egg yolk sample. Peak 1, *RRS* + *SSR*; peak 2, *RRR* + *SSS*; peak 3, *RSR* + *SRS*; peak 4, *RSS* + *SRR*.

air at 1.0 bar; detector temperature, 260 °C; injector port temperature, 240 °C; split ratio, 1/30; sample size 0.7–2.0 μL.

The peaks of the enantiomeric pairs of α-tocopherol in the feed and egg samples were identified on the basis of the retention times of the peaks separated from the *all-rac*-α-tocopherol standard (Merck, Darmstadt, FRG). The proportions of the separated peaks from the standard were 24.8%, 24.4%, 25.2%, and 25.6%, respectively. The identities of the four standard peaks have been assigned by Cohen et al. (1981). The polarity of the column used by Cohen et al. (1981) was roughly the same as that of the column used in this study.

The proportions of the individual enantiomeric pairs of total α-tocopherol were calculated on the basis of the peak areas (percentage of the sum of all four peaks). For each methylated sample, the chromatographic run was carried out in triplicate. Analysis of variance and Student's *t*-test were used for the statistical evaluation of the results.

The reproducibility of the chromatographic runs was tested by injecting one feed and one egg sample eight consecutive times. The coefficient of variation was 1.6% for the feed sample and 4.0% for the egg sample (means of the coefficients of variation for the four individual peaks). The coefficient of variation for the whole determination was 1.9% when calculated for all feed determinations and 3.0% when calculated for all egg determinations.

Determination of Total α-Tocopherol Contents. The total α-tocopherol contents of the hexane extracts obtained after the room temperature saponification were determined by the normal-phase high-performance liquid chromatographic (HPLC) method, previously described (Piironen et al., 1984, 1985). A Varian Vista 5500 liquid chromatograph was used. The mobile phase was *n*-hexane/diisopropyl alcohol (99:1 v/v) at a flow rate of 0.5 (feed samples) or 1.0 mL/min (egg samples). Other apparatus and chromatographic conditions were as previously described.

Recoveries of α-tocopherol (n = 4) added to egg and feed samples were 79.1% and 88.7%, respectively.

RESULTS AND DISCUSSION

The four enantiomeric pairs of α-tocopherol were found in all feed and egg samples. This demonstrated that the different stereoisomers were transferred from the feeds to the eggs. An example of the separation of the pairs in the egg samples is shown in Figure 1.

The total α-tocopherol contents of the feeds determined by the HPLC method were 18.3 ± 0.3 mg/kg of fresh weight (mean ± standard deviation) for feed A, 19.0 ± 0.7 mg/kg for feed B, and 26.5 ± 0.4 mg/kg for feed C. The corresponding results for the egg yolks were 53.2 ± 2.6 mg/kg, 51.1 ± 2.9 mg/kg, and 75.9 ± 1.0 mg/kg.

The proportions of the enantiomeric pairs of the total α-tocopherol contents in the feed and egg samples are shown in Figure 2. In all feed vs egg comparisons, the eggs contained significantly more ($p < 0.001$ or 0.01) of the enantiomeric pair *RRR* + *SSS* than did the feeds. When the value is calculated according to the biological activity values given by Weiser and Vecchi (1982), this enantiomeric pair has the highest aggregate activity. The proportions of the other enantiomeric pairs were lower in the eggs than in the feeds. The differences were, however, not always significant (*RRS* + *SSR* when hens were given feeds A and C; *RSS* + *SSR* when given feed C). The lower the aggregate biological activity of the enantiomeric pair, the greater was the difference.

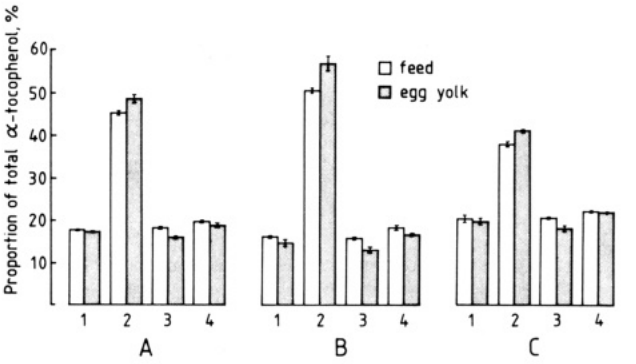

Figure 2. Enantiomeric pairs of α-tocopherol in three feeds (A–C) and the corresponding eggs. Numbers of pairs as in Figure 1.

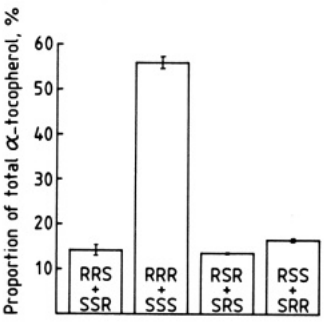

Figure 3. Enantiomeric pairs of α-tocopherol in eggs for sale in retail outlets.

The differences in the proportions of the enantiomeric pairs in the feeds and the corresponding eggs reflect the transfer efficiencies of the different stereoisomers. The results thus indicate that the transfer efficiencies of the stereoisomers from feeds to eggs, and probably also to other animal products, are proportional to their biological activities. Previously, accumulation of (*R*,*R*,*R*)- and (*S*,*R*,*R*)-α-tocopherols in rat tissues has been studied (Weber et al., 1964; Ingold et al., 1987). The more active *RRR* isomer was stored more efficiently than was the *SRR* isomer.

The proportions of the four racemates in the eggs sold by retail outlets are shown in Figure 3. The total α-tocopherol content was 72.0 ± 2.2 mg/kg of yolk. The proportion of the pair containing the naturally occurring *RRR* isomer was 56.1 ± 1.3%; the proportions of the other pairs were as follows: *RRS* + *SSR*, 13.9 ± 1.2%; *RSR* + *SRS*, 13.3 ± 0.1%; *RSS* + *SRR*, 16.6 ± 0.4%. The difference between the middle two figures was not significant.

The findings of this study enable us to estimate the significance of the presence of the non-*RRR* isomers in eggs sold by retail outlets for their vitamin E activity. In the peaks of enantiomeric pairs containing only synthetic isomers (amounts of the isomers equal in *all-rac*-α-tocopheryl acetate preparation and thus also in feeds), the proportions of the two individual isomers were assumed to be according to their biological activities. For the *RRR* + *SSS* pair, it was taken into account that the amounts of the isomers are not equal in feeds but that the *RRR* isomer also derives from natural sources. The actual vitamin E activity of eggs was estimated to be about 80% of the value obtained when all α-tocopherol is determined as (*R*,*R*,*R*)-α-tocopherol. The possible synergism of the

isomers (Weiser and Vecchi, 1982) was not taken into account in this estimation.

A chiral column or some other chiral working method, however, would be needed for determining the exact transfer efficiencies of the eight individual stereoisomers of α-tocopherol from feeds to eggs and other animal products. Such methods are also needed to determine more accurately the importance of the non-*RRR* isomers for the vitamin E activity of animal products.

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Registry No. α-Tocopherol, 59-02-9; *RRS/SSR*-α-tocopherol, 78656-09-4; *RRR/SSS*-α-tocopherol, 2074-53-5; *RS/SR*-α-tocopherol, 78656-10-7; *RSS/SRR*-α-tocopherol, 78656-11-8; *RRS/SSR*-α-tocopherol acetate, 79200-46-7; *RRR/SSS*-α-tocopherol acetate, 52225-20-4; *RSR/SRS*-α-tocopherol, 79200-45-6; *RSS/SRR*-α-tocopherol acetate, 79200-47-8.