Cholecalciferol and 25-Hydroxycholecalciferol Content of Chicken Egg Yolk As Affected by the Cholecalciferol Content of Feed

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The predominant source of vitamin D is the synthesis of cholecalciferol in the skin by the action of sunlight; however, due to the relative lack of sunlight, the intake of vitamin D from food is emphasized during winter, especially in the northern countries. Only a few foods (fish, eggs, wild mushrooms, meat, and milk) are natural sources of vitamin D. In addition, the content of vitamin D in foods is generally low, and some groups of people obtain amounts of vitamin D that are too small from their diet. The present study was designed to determine whether it is possible to increase the vitamin D content of egg yolk by giving hens feed containing elevated levels of cholecalciferol. Three cholecalciferol levels were tested: 26.6 (1064), 62.4 (2496), and 216 μg (8640 IU)/kg feed. Egg yolk samples were taken after 0, 4, 5, and 6 weeks and were assayed for the presence of cholecalciferol and 25-hydroxycholecalciferol using an HPLC method. According to the present study, there was strong positive correlation between cholecalciferol content in poultry feed and cholecalciferol (r = 0.995) and 25-hydroxycholecalciferol (r = 0.941) content in egg yolk.

Keywords: *Vitamin D; cholecalciferol; 25-hydroxycholecalciferol; egg yolk; chickens; feed enrichment; HPLC*

INTRODUCTION

It is well-known that man and most other mammals are able to synthesize cholecalciferol in the skin by the photochemical conversion of 7-dehydrocholesterol to previtamin D_3. In addition to being produced in the skin, vitamin D compounds (cholecalciferol, ergocalciferol, and their hydroxylated metabolites) can be absorbed from the diet in the intestinal tract. Due to the relative lack of sunlight during winter, especially in the northern countries, the intake of vitamin D from food is emphasized. Unfortunately, vitamin D is found naturally in only a few foodstuffs: fish, eggs, wild mushrooms, meat, and milk (Mattila, 1995a). The level of vitamin D in fish, eggs, and wild mushrooms is much higher than in meat and milk, which contain only traces of this vitamin.

Eggs, particularly their yolks, are considered to be one of the most important sources of vitamin D in the diet (Parrish, 1979). According to Mattila (1995a) the best dietary source of vitamin D in the average Finnish diet was fish, followed by fortified margarine, meat and liver, and egg yolk. Eggs are an especially interesting vitamin D source because in addition to cholecalciferol they contain significant amounts of the 5 times more vitamin D active (Reeve et al., 1982) hydroxylated metabolite, 25-hydroxycholecalciferol (Koshy and VanDerSlik, 1979; Koskinen and Valtonen, 1985; Mattila et al., 1993). The levels of this metabolite in other animal-based foods are much lower (Mattila et al., 1995b,c).

In previous studies the antirachitic potency of egg yolk was shown to be dependent on the antirachitic intake of the hen producing it (Bethke et al., 1927; Guerrant et al., 1935). Guerrant et al. (1935), however, reported that the ability of the hen to transfer the antirachitic factor or factors from its diet to the egg is limited. More recently, Mattila et al. (1992) found remarkable variation in the cholecalciferol content of eggs produced by hens receiving different types of feed. The most probable explanation for this variation was the varying cholecalciferol content of the feeds used. Kawazoe et al. (1996, 1997) studied the effect of D_2-fortified shiitake mushroom diets on the transfer of vitamin D to egg yolk and found strong positive correlation. According to previous studies, it is quite clear that the cholecalciferol or ergocalciferol content of chicken feed affects the vitamin D contents of eggs; however, to understand the potency of this phenomenon, further research is needed. In addition, using new analytical techniques it is possible to monitor different vitamin D compounds (cholecalciferol and 25-hydroxycholecalciferol) rather than monitoring the presence of an indefinite antirachitic factor.

The aim of the present study was to elucidate how quickly and effectively cholecalciferol is transferred from chicken feed to egg yolk and how this supplemental vitamin D compound affects the 25-hydroxycholecalciferol content in egg yolk. Another aim was to examine the variation in vitamin D compounds in the egg yolks of individual hens. Some groups in the northern countries (especially the elderly) receive insufficient amounts of vitamin D from their diet (Lamberg-Allardt, 1984). Vitamin D-enriched eggs would increase the intake of this vitamin from food.

MATERIALS AND METHODS

The Feeding Experiment. A total of 54 laying hens (Lohman Whites) divided into three feeding groups of 18

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individuals were used in the experiment. At the beginning of the experiment the birds were 30 weeks of age, with 90% laying. The hens were kept in a windowless room in two-tier batteries with cages for 4 birds. The housing conditions (temperature, light) followed the directions used at the Agricultural Research Centre. Prior to the experiment all 54 hens were given feed containing 62.4 μg (2496 IU) cholecalciferol per kg for 1 week (balancing period).

Each of the three treatment groups received a different amount of cholecalciferol in its feed. Cholecalciferol levels were chosen so that the hens got adequate but not toxic levels of vitamin D; cholecalciferol contents in three different feeds were ca. 2 times lower, the same, and 3.5 times higher than in commercial feeds. The content of cholecalciferol in the feed of group 1 was 26.6 μg (1064 IU)/kg, as analyzed after manufacture. The analyzed contents of cholecalciferol in the feeds of the second and third groups were 62.4μg (2496 IU) and 216 μg (8640 IU)/kg, respectively. The feeds were otherwise similar, containing soybean meal 175 g/kg, barley 453 g/kg, oats 250 g/kg, rapeseed oil 20 g/kg, limestone 80 g/kg, dicalcium phosphate 15 g/kg, sodium chloride 3 g/kg, vitamin premix 2 g/kg, trace mineral premix 2 g/kg, and DL-methionine 0.5 g/kg. The basic diet contained crude protein, 155 g/kg, and metabolizable energy, 10.44 MJ/kg. The pelleted diets (diameter 4 mm) were manufactured in the feed mill of the Agricultural Research Centre. The vitamin premix contained all necessary vitamins, excluding cholecalciferol, which was added in a special premix. The content of supplemental vitamin A was 15 000 IU/kg and that of vitamin E 30 mg/kg. The birds had unlimited access to the feed and water. The feed intake of each treatment group was measured during the experimental period, which lasted 6 weeks. The daily feed intakes per bird were 120, 132, and 122 g for groups 1−3, respectively.

Egg samples were collected from each treatment group (a whole day's production; 15−18 eggs per group) at the end of the balancing period (0 week) and 4, 5, and 6 weeks after the beginning of the experiment and transported to the laboratory to be analyzed for the presence of cholecalciferol and 25-hydroxycholecalciferol. In addition, during the last week of the experiment, five additional eggs were collected from group 2 to examine the variation in cholecalciferol and 25-hydroxycholecalciferol content in individual egg yolks.

Pretreatment of the Egg and Feed Samples. In the laboratory the yolks and whites were separated and then pooled according to the feed used. Pooled yolks and egg whites were weighed, and the yolks were mixed, vacuum-packed in plastic bags, and stored at −70 °C until analysis. The five eggs collected for the individual variation test were separated, and the cholecalciferol and 25-hydroxycholecalciferol determinations were made from the yolks separately. The feed samples were homogenized, packed in plastic bags, and stored at −70 °C until analysis.

In-House Reference Sample. An in-house reference sample was prepared to test the day-to-day repeatability of the method. Sixty eggs were purchased from a retail store and separated, and the yolks were pooled and mixed to serve as the reference sample. The pooled yolks were packed in plastic bags in small portions and stored at −70 °C.

Cholecalciferol and 25-Hydroxycholecalciferol Determinations. Cholecalciferol and 25-hydroxycholecalciferol were determined in the egg yolks, using methods previously described (Mattila et al., 1992, 1993). These methods involve saponification, extraction, purification using solid-phase extraction (SPE) and semipreparative normal-phase HPLC, and quantification with reverse-phase HPLC using internal standard methods. Ergocalciferol served as an internal standard for cholecalciferol, and 25-hydroxyergocalciferol for 25-hydroxycholecalciferol. Cholecalciferol determinations in the feeds were performed according to Mattila et al. (1992) with a modification in the purification the procedure. After saponification, extraction, and evaporation the residue was diluted in 2 mL of *n*-hexane and then purified using semipreparative HPLC. The SPE purification procedure could thus be omitted in the case of feeds. The sample size of feeds and eggs was 10 g, except that 5 g samples were used when examining individual variation.

Table 1. Effect of Feed Cholecalciferol Content on the Cholecalciferol Content in Pooled Egg Yolks[a]

feeding period/ week	group 1 μg D_3 / 100 g	group 2 μg D_3/ 100 g	group 3 μg D_3/ 100 g
0	4.5	3.6	3.0
4	1.2	3.5	21
5	1.5	3.5	23
6	1.4	3.4	23

[a] Group 1: 26.6 μg cholecalciferol/kg feed. Group 2: 62.4 μg cholecalciferol/kg feed. Group 3: 216 μg cholecalciferol/kg feed.

Table 2. Effect of Feed Cholecalciferol Content on the 25-Hydroxycholecalciferol Content in Pooled Egg Yolks[a]

feeding period/ week	group 1 μg 25-OH-D_3/ 100 g	group 2 μg 25-OH-D_3/ 100 g	group 3 μg 25-OH-D_3/ 100 g
0	1.1	0.7	0.6
4	0.5	0.9	1.4
5	0.5	0.8	1.4
6	0.5	1.0	1.5

[a] Group 1: 26.6 μg cholecalciferol/kg feed. Group 2: 62.4 μg cholecalciferol/kg feed. Group 3: 216 μg cholecalciferol/kg feed.

For semipreparative purification the following apparatuses were used: a Waters model 510 pump (Waters Associates, Milford, MA), a Rheodyne 7125 injector (Rheodyne, Cotati, CA), a Merck-Hitachi L-4200 UV−vis detector (Hitachi, Tokyo, Japan), a Hewlett-Packard 3380A integrator (USA), and a μ-Porasil column (5 μm, 300 × 3.9 mm; Millipore Corp., Milford, MA). The analytical HPLC consisted of a Merck-Hitachi L-2000 pump (Hitachi), a Waters 486 UV-detector, a Waters 717 autosampler, a Waters TCM+ column oven, a Millennium 2010 chromatography manager (Waters), and a Vydac 201 TP54 column (5 μm, 250 × 4.6 mm; The Separation Group, Hesperia, CA).

Moisture Determinations. The moisture of egg yolk samples was determined by drying at 100 ± 2 °C to a constant weight (AOAC 952.08, modified; AOAC 1990).

Method Reliability Tests. Complete validation of the methods had been performed previously (Mattila et al., 1992, 1993); however, the linearity and repeatability of the detector response were also confirmed in the present study. In addition, the accuracy and repeatability of the method were continuously controlled by recovery tests and by determining the in-house reference sample. The recovery tests were performed by spiking the vitamin D compound under study into the samples prior to saponification. The recoveries were calculated using both internal and external standard methods. The mean recoveries of cholecalciferol were 108% and 74% ($n = 7$) when calculated using the internal and external methods, respectively. The corresponding recoveries of 25-hydroxycholecalciferol were 101% and 68% ($n = 6$). The repeatability of the method was examined by following the coefficient of variation of the in-house reference sample. The reference sample contained cholecalciferol, 2.8 μg/100 g, and 25-hydroxycholecalciferol, 1.1 μg/100 g, and the coefficients of variation (CV%) between days were 4.6% ($n = 7$) and 5.9% ($n = 6$), respectively.

RESULTS AND DISCUSSION

Results of the feeding experiment are presented in Tables 1 and 2. During the 1 week balancing period all hen groups received the same feed containing 62.4 μg cholecalciferol/kg. After the balancing period group 1 received lower, group 2 the same, and group 3 higher levels of cholecalciferol. Logically starting levels (0 week) of cholecalciferol and 25-hydroxycholecalciferol in the yolks of all groups were of the same magnitude, and after 4 weeks of feeding with the test feeds the levels

Table 3. Cholecalciferol (D_3), 25-Hydroxycholecalciferol (25-OH-D_3), and Dry Matter Contents of Individual Egg Yolks

egg yolk	D_3 μg/100 g	25-OH-D_3 μg/100 g	dry matter/%
1	3.4	1.0	50
2	3.5	0.9	50
3	3.7	0.8	49
4	4.3	1.5	49
5	3.0	0.7	50
mean	3.6	1.0	50
SD	0.48	0.31	0.55
CV%	13	31	1.1

decreased in group 1 yolks, were the same in group 2 yolks, and increased in group 3 yolks.

As seen in Tables 1 and 2, the cholecalciferol content of poultry feed correlated strongly with the cholecalciferol ($r = 0.995$) and 25-hydroxycholecalciferol ($r = 0.941$) content of egg yolk. When the feed cholecalciferol content was raised from 26.6 to 62.4 μg/kg, both the cholecalciferol and 25-hydroxycholecalciferol content in egg yolk doubled. When the feed cholecalciferol content was raised from 62.4 to 216 μg/kg (about a 3.5-fold increase), the cholecalciferol content in egg yolk increased about 7-fold and the 25-hydroxycholecalciferol content about 1.5-fold. Since 25-hydroxycholecalciferol is a metabolite of cholecalciferol, it was expected that levels of 25-hydroxycholcalciferol would not increase as strongly as the levels of cholecalciferol. As seen in Tables 1 and 2, the vitamin contents in yolks attained equilibrium very rapidly. The samples were taken after 4, 5, and 6 weeks, but equilibrium already appeared to have been attained after 4 weeks. Equilibrium may even have been reached more rapidly, but unfortunately, samples were not taken between 0 and 4 weeks.

Cholecalciferol and 25-hydroxycholecalciferol contents in the egg yolks of group 2 (3.4−3.6 and 0.7−1.0 μg/100 g, respectively) showed levels similar to those reported previously. Sivell et al. (1982) obtained 1.2 μg (48 IU) cholecalciferol/100 g for the battery of whole eggs, and Takeuchi et al. (1984) found 3.9 μg (155 IU) of cholecalciferol/100 g egg yolk. Mattila et al. (1992) analyzed two pooled samples representing eggs available through retail outlets. These yolks contained 4.0 (160 IU) and 5.6 μg (224 IU) cholecalciferol/100 g. Previously reported 25-hydroxycholecalciferol contents were 0.5−0.8 μg/100 g (Koshy and VanDerSlik, 1979), 0.95 μg/100 g (Koskinen and Valtonen, 1985), and 0.98 μg/100 g (Mattila et al., 1993). In the present study, group 2 received feed containing 62.4 μg cholecalciferol/kg. This concentration level is widely used in commercial chicken feeds; hence, it was not surprising that the levels of vitamin D compounds in the yolks of group 2 were similar to those previously reported.

Variations in cholecalciferol and 25-hydroxycholecalciferol content among individual eggs was examined in Group 2, because these eggs were assumed to be similar to those sold commercially to ordinary consumers. The CV% between cholecalciferol contents was 13% and between 25-hydroxycholecalciferol contents 31% (Table 3). In addition to physiological differences among hens, their differing appetites might have been the reason for this variation. Although moderate individual variation was present, its influence on vitamin D intake was assumed to be quite insignificant. In previous studies and in composition tables wide variation in the cholecalciferol content of egg yolk has been reported. Souci et al. (1986), Moller (1985), and Mattila et al. (1992) gave variations of 2.5 (100)−11.3 (452), 1.3 (52)−8.7 (348), and 1.4 (56)−15 μg (600 IU)/100 g, respectively. In the present study the effect of feed was very potent, and hence probably the cholecalciferol content of feed affects the cholecalciferol variations in the egg yolk most strongly.

According to the present study, the vitamin D content of eggs is easily increased by increasing the cholecalciferol content in feed. If the cholecalciferol content in feed is increased from 62.4 to 216 μg/kg, the eggs produced will contain over 7-fold more vitamin D. On a whole-egg basis (division of the results by 2.9) the edible part of the eggs in group 3 contained about 8 μg cholecalciferol/100 g and about 0.5 μg 25-hydroxycholecalciferol/100 g. According to Reeve et al. (1982), 25-hydroxycholecaciferol is 5 times more active than cholecalciferol. Hence, eating one egg (about 60 g) from this group per day will result in a vitamin D portion of about 6 μg, which adequately meets the daily intake recommendations for adults (5 μg per day; National Research Council, 1989). In northern countries where the relative lack of sunlight limits the intake of vitamin D, the possibility of producing vitamin D-enriched eggs is worth considering.

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