JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2003,** *51,* 283−287 **283**

Effect of Cholecalciferol-Enriched Hen Feed on Egg Quality

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Eggs are one of the most important sources of vitamin D in the human diet, and their vitamin D content can be further increased by adding more vitamin D to hen feed. To investigate this issue more closely, we performed two feeding experiments. In both, zero egg samples were collected while the hens were fed regular feeds with a vitamin D content of 1720 or 4280 IU/kg. In experiment 1, egg samples were collected 2, 4, 7, 9, 11, 13, 16, 23, and 30 days after beginning the high-cholecalciferol (11 200 IU/kg) feeding period. In experiment 2, samples were collected 2, 4, 6, 8, 13, 28, 56, 84, 112, 140, and 168 days after beginning the high-cholecalciferol (12 000 IU/kg) diet. The egg samples were then assayed for their cholecalciferol content, and some samples, also for the presence of 25-hydroxycholecalciferol by an HPLC method. Further, the vitamin D-fortified eggs were compared with the controls by a sensory evaluation, by conducting fatty acid and functional analyses (emulsion capacity, gel forming capacity, foaming properties) and by measuring eggshell strength. Because vitamin D can be toxic in high doses, we also performed histopathological tests on the hens at the end of experiment 2. The top cholecalciferol contents in egg yolk (ca. 30 μg/100 g) were reached 8−13 days from starting the high-cholecalciferol diet. After 112 days feeding the cholecalciferol content gradually decreased to ca. 22 μg/100 g. When added to eggs as described above, vitamin D did not affect their sensory or functional properties or their fatty acid composition. Moreover, the cholecalciferol levels used in this study appeared not to affect eggshell strength or to be harmful for hens.

KEYWORDS: Vitamin D; cholecalciferol; egg yolk; hens; feed enrichment

INTRODUCTION

Vitamin D is crucial for normal bone formation and has been linked to the prevention and treatment of postmenopausal osteoporosis (*1*). It can be produced in the skin by the action of sunlight or absorbed from the diet in the intestinal tract. The intake of vitamin D from food is especially important in northern latitudes. However, its content in foods is generally low, and in winter many population groups in Northern and Middle Europe are at risk of hypovitaminosis (*2*−*6*).

Besides being important for humans and other mammals, vitamin D is also one of the key dietary factors for hens. In fowls, vitamin D is responsible for normal growth, egg production, shell quality, and reproduction. The essentiality of vitamin D in hen feed is particularly significant for profitable egg and meat production in the modern poultry industry where the birds are raised indoors (*7*, *8*). According to official recommendations (*9*) the nutrient requirement for normal egg production and shell quality is 500 IU of cholecalciferol/kg feed.

Eggs are among the few potent natural sources of vitamin D for humans. According to our previous study (*10*), their vitamin D content can be further increased by supplementing hen feed with additional vitamin D. We found that it was possible to produce eggs containing 7-fold more cholecalciferol than so-called normal eggs by increasing the cholecalciferol content of the feed from the regular level (62.4 μg (2496 IU)/kg) to a level ca. 3.5 times higher (216 μg (8640 IU)/kg). Moreover, Kawatzoe et al. (*11*, *12*) investigated the effect of supplementation with vitamin D from different sources on the transfer of vitamin D to egg yolk and found a strong positive correlation.

An EU directive currently prohibits the addition of more than 3000 IU/kg cholecalciferol into hen feed, and comprehensive research is needed before this directive can be changed. Because there are only a few published reports on the transfer of vitamin D from feed to egg yolk, our aim in this study was to investigate this matter in more detail. We conducted two experiments of different durations to elucidate how quickly, effectively, and repeatably cholecalciferol is transferred from hen feed to egg yolk. Further, we studied how a raised vitamin D level in feeding affected the sensory properties, emulsion capacity, gel forming capacity, foaming properties, eggshell strength, and fatty acid

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10.1021/jf020743z CCC: $25.00
Published on Web 11/28/2002

content of the eggs. And finally, since vitamin D can be toxic in high doses, we performed histopathological analyses as well.

MATERIALS AND METHODS

Feeding Experiments. Two high-cholecalciferol feeding experiments were performed, the former one (experiment 1) lasting 30 days and the latter (experiment 2) 168 days. Altogether, 36 laying hens (Lohmann White) were used in both experiments, half of them on control and half on experimental treatment. At the beginning of experiments 1 and 2, the birds were 56 and 38 weeks of age, respectively. The hens were kept in a windowless room in 2-tier batteries with cages for three birds each. The housing conditions (temperature, light) were in accordance with the breeder's instructions. Prior to experiments 1 and 2, all the hens were given regular feeds. Analyzed contents of these feeds were 43 μg (1720 IU) and 107 μg (4280 IU) of cholecalciferol/kg, respectively.

When the feeding experiments were begun, the cholecalciferol content of the feed of the high-cholecalciferol group in experiment 1 was raised to 280 μg (11 200 IU) and in experiment 2 to 300 μg (12 000 IU) per kg. The basic diet in experiment 1 contained protein concentrate (161.5 g/kg), barley (500 g/kg), oats (248.5 g/kg), and limestone (90 g/kg). The basic diet in experiment 2 contained soybean meal (195 g/kg), barley (250 g/kg), wheat (150 g/kg), oats (250 g/kg), rape seed oil (50 g/kg), limestone (85 g/kg), salt (4 g/kg), vitamin premix (2 g/kg), trace mineral premix (2 g/kg), and DL-methionine (0.5 g/kg). The crude protein contents of basic diets 1 and 2 were 191.7 and 184.8 g/kg of DM, and the metabolizable energy contents, 10.44 and 11.35 MJ/kg, respectively. The pelleted diets (diameter 4 mm) had been manufactured at the feed mill of MTT Agrifood Research Finland. The vitamin premix contained all the necessary vitamins, with the exception of cholecalciferol. 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 both feed and water. Feed intake and egg production were measured over 4-week periods. The mean daily feed intakes per bird were 118 and 120 g in experiments 1 and 2, respectively. The hens' egg production level was similar in the control and high-cholecalciferol treatment groups in both experiments: mean egg production per day was 56.9 in experiment 1 and 60.5 g in experiment 2.

Egg samples were taken in both experiments from a whole day's egg production (10−18 eggs). Zero samples were collected while the hens were eating regular feeds (1720 or 4280 IU/kg). Then, in experiment 1, samples were collected 2, 4, 7, 9, 11, 13, 16, 23, and 30 days from the beginning of the high-cholecalciferol (11 200 IU/kg) feeding period. In experiment 2, eggs were collected 2, 4, 6, 8, 13, 28, 56, 84, 112, 140, and 168 days from starting the high-cholecalciferol (12 000 IU/kg) diet. After collection, the eggs were taken to the laboratory, and the yolks were separated and pooled according to their collection day and respective experiment. The yolks were mixed, vacuum-packed in plastic bags, and stored at −18 °C until the cholecalciferol and 25-hydroxycholecalciferol analyses. Cholecalciferol was analyzed from all feed and egg yolk samples. 25-Hydroxycholecalciferol was determined from the egg yolk samples of days 0, 9, and 23 in experiment 1. The feed samples were homogenized, vacuum-packed in plastic bags, and stored at −18 °C to await analysis.

To perform a sensory evaluation and fatty acid analysis and to determine the eggs' functional properties (foaming index, emulsion activity, emulsion stability, gel forming capacity (hardness)), eggs were collected from control and high-cholecalciferol groups. In feeding experiment 1, eggs were collected for these analyses after a 4-week feeding period and, in experiment 2, after 8, 16, and 24 weeks. The analyses were performed from five pooled whole eggs (emulsion activity, emulsion stability), from pools of four albumens and four whole eggs (foaming index), from a pool of four albumens (gel forming capacity), or from a pool of five egg yolks (fatty acids). In feeding experiment 2, also eggshell strength was measured from the eggs of control and high-cholecalciferol groups after 0, 11, and 24 weeks. Analyses were performed from 15 eggs.

Analyses. *Cholecalciferol and 25-Hydroxycholcalciferol*. The cholecalciferol and 25-hydroxycholecalciferol contents were determined from egg yolk using methods described previously (*13*, *14*). These involved 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. The amount of added ergocalciferol depended on the expected content of cholecalciferol in the egg yolk sample. In the case of 25-hydroxycholecalciferol, an external standard method with recovery corrections was used. Cholecalciferol determinations in the feeds were performed according to Mattila et al. (*13*) with a modification in the purification procedure. After saponification, extraction, and evaporation, the residue was dissolved 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 sizes of the feeds and eggs were 2−10 and 10 g, respectively. Semipreparative purification was carried out using a Perkin-Elmer series 200, Norwalk, CT, chromatograph with a μ-Porasil column (5 μm, 300 × 3.9 mm; Millipore Corp., Milford, MA). In the analytical step the same apparatus was applied with a Vydac 201 TP54 column (5 μm, 250 × 4.6 mm; The Separation Group, Hesperia, CA). All analyses were performed in duplicate or in triplicate.

The complete validation of the cholecalciferol and 25-hydroxycholecalciferol methods used in this study had already been performed previously (*13*, *14*); however, the linearity and repeatability of the detector response were confirmed also in the present study. Moreover, the accuracy of the method was controlled continuously by recovery tests. These tests were performed by spiking the vitamin D compound under study into the samples prior to saponification. The recoveries for cholecalciferol, calculated using an internal standard method, varied between 93 and 112% (n = 11). For 25-hydroxycholecalciferol the recovery was 56% (n = 2), as calculated by the external standard method.

Sensory Evaluation, Fatty Acid Content, and Functional Properties. The determinations of functional properties (foaming index, emulsion activity, emulsion stability, gel forming capacity (hardness)) and the fatty acid content of whole eggs, yolks, or albumens (see above) as well as a sensory evaluation (multicomparison test with 10 panelists) were performed as described by Rokka et al. (*15*). The functional and sensory properties were measured from fresh eggs and from eggs stored for 3 weeks (at 12 °C in 60% humidity). Prior to sensory evaluation, the eggs were boiled for 10 min and then cooled to room temperature, after which the yolks were immediately evaluated for odor, taste, and general acceptance.

Histopathological Tests. At the end of experiment 2, pathological tests were performed for five random-sampled birds from the high-cholecalciferol and the control groups. The nutritional status, skeletal condition, and calcification of soft tissues (kidney, liver, heart muscle, lung) of the birds were evaluated macroscopically. In addition, the conditions of the liver and kidneys were determined histologically.

Eggshell Strength. Eggshell strength was measured as compressive fracture force with a Canadian Egg Shell Tester (OTAL Precion Co. Limited, Ontario, Canada).

RESULTS AND DISCUSSION

It has been shown that the cholecalciferol content of eggs can easily be raised by adding more cholecalciferol to hen feed. Moreover, the content of cholecalciferol in egg yolk has been found to be proportional to the level of added cholecalciferol (*10*−*12*). One of the aims of this study was to investigate how quickly cholecalciferol is transferred from feed to egg yolk and how stable the value remains once the top content has been reached. Cholecalciferol content of the egg yolks of the control group was not followed, because according to our previous study there was only minor variation when the hens were on regular diet (*10*).

As shown in **Figure 1**, the addition of cholecalciferol into hen feed effectively increased the cholecalciferol content of egg yolks in this study. The magnitude of this increase was quite similar in both experiments, and the vitamin content in yolk reached equilibrium very rapidly. The cholecalciferol content

Table 1. Main Fatty Acid Composition (in Mean %) of Egg Yolks in the Control and Vitamin D-Enriched Diets ($n = 2$)

	feeding expt 1		feeding expt 2					
	after 4 months of feeding		after 2 months of feeding		after 4 months of feeding		after 6 months of feeding	
fatty acid	control	vit D	control	vit D	control	vit D	control	vit D
C16:0	25.7	23.1	23.2	23.4	23.0	23.0	21.4	22.9
C16:1/C17:0	2.7	1.5	1.7	1.7	1.6	1.8	1.6	1.8
C18:0	9.5	9.3	9.2	8.3	8.8	8.2	8.7	8.3
C18:1	42.7	46.7	45.9	46.2	45.4	47.2	46.1	48.6
C18:2 ω6	11.2	12.4	12.8	13.3	14.1	12.5	14.5	11.2
C18:3 ω3	0.9	1.4	1.6	1.5	1.4	1.6	1.5	1.5
C20:4 ω6	1.5	1.4	1.3	1.6	1.3	1.3	1.6	1.1
C22:5 ω3	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.1
C22:6 ω3	1.1	1.3	1.4	1.5	1.2	1.4	1.2	1.4
ω3 tot.	2.3	2.8	3.1	3.1	2.8	3.1	2.9	3.0
ω6 tot.	12.7	13.8	14.1	14.9	15.4	13.8	16.2	12.3
ω6/ω3	5.6	4.9	4.6	4.9	5.4	4.5	5.6	4.1

Table 2. Mean Values of Foaming Index, Emulsion Activity, Emulsion Stability, and Gel Forming Capacity (Hardness/N) of Eggs Produced by Using Control and Vitamin D-Enriched Diets[a]

		feeding expt 1		feeding expt 2					
		after 4 months of feeding		after 2 months of feeding		after 4 months of feeding		after 6 months of feeding	
		control	vit D	control	vit D	control	vit D	control	vit D
				Foaming Index ($n = 2$)					
albumen	fresh	1150	900	800	900	900	900	900	1050
	stored[b]	800	900	900	850	950	900	1000	900
whole egg	fresh	433	467	633	567	548	581	550	567
	stored[b]	483	517	567	550	567	533	529	533
				Emulsion Activity ($n = 2$)					
whole egg	fresh	67	68	68	67	68	67	68	67
	stored[b]	64	65	66	65	64	65	64	65
				Emulsion Stability ($n = 2$)					
whole egg	fresh	54	43	67	58	68	61	62	66
	stored[b]	24	37	64	59	61	64	19	17
				Gel-Forming Capacity ($n = 4$)					
albumen	fresh	5	5						
	stored[b]	8	7						

[a] n = number of analyzed replicates of the pooled samples. [b] 3 weeks at 12 °C and 60% relative humidity.

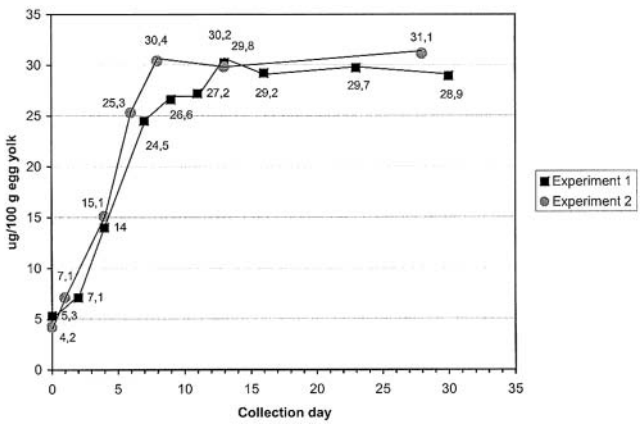

Figure 1. Effect of high-cholecalciferol feed on the cholecalciferol content of egg yolk during a 1-month feeding period (experiment 1, 11 200 IU cholecalciferol/kg of feed; experiment 2, 12 000 IU cholecalciferol/kg of feed).

in the zero samples was 5.3 and 4.2 μg/100 g of egg yolk in experiments 1 and 2, respectively, and after 8−13 days of feeding it rose proportionally to ca. 30 μg/100 g of egg yolk in both experiments. Thereafter, the content of cholecalciferol in egg yolk remained relatively stable in both experiments (**Figure 1**). In experiment 2, which was longer in duration, the cholecalciferol content was analyzed also after 56, 84, 112, 140, and 168 days of feeding. The respective values were 28.8, 28.5, 26.6, 24.5, and 21.7 μg cholecalciferol/100 g of egg yolk. Hence, there was a gradual reduction in the cholecalciferol content of the egg yolks after 112 days. This phenomenon was not necessarily due to an impaired ability of the hen to transfer cholecalciferol from the feed to the egg, however. Other reasons for the decreasing cholecalciferol content in egg yolk might be the instability of cholecalciferol in the feed or the unhomogeneity of the feed. Unhomogeneity might also be the reason for the varing cholecalciferol contents in the regular feeds that were fed prior to experiments 1 and 2.

Moreover, we analyzed the 25-hydroxycholecalciferol content in the egg yolk samples from days 0, 9, and 23 of experiment 1. The values were 1.1, 1.9, and 1.7 μg/100 g of egg yolk, respectively. These figures confirm our previous results (*10*) according to which there is also a slight rise in the content of 25-hydroxyholecalciferol when hens are kept on a high-cholecalciferol diet. This increase was of the same magnitude as that reported in our earlier study (*10*), taking into account that the feed in the present study contained somewhat more cholecalciferol.

To study whether the age of the bird affects its ability to transfer cholecalciferol into egg yolk, we used hens of different ages in experiments 1 and 2. The hens were 56 weeks old at

Table 3. Results of Sensory Analysis of Vitamin D-Enriched Egg Yolks As Compared with Control Egg Yolks ($n = 10$)[a]

		feeding expt 1						feeding expt 2					
		odor		taste		general acceptance		odor		taste		general acceptance	
feeding lasts		mean	Sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
1 month	fresh	0.3	0.8	0.2	1.1	0.1	1.0						
	stored[b]	0	0.6	0.1	0.9	0.1	0.9						
2 months	fresh							–0.2	1.1	–0.3	1.2	–0.1	1.1
	stored[b]							0.3	0.8	0	1.2	0.2	0.8
4 months	fresh							0	0.5	–0.1	0.9	–0.1	0.8
	stored[b]							0.5	0.7	0.1	0.6	–0.2	0.6

[a] Scale used: –2 (clearly worse than control); –1 (slightly worse than control); 0 (similar to control); +1 (slightly better than control); +2 (clearly better than control). [b] 3 weeks at 12 °C in 60% humidity.

the beginning of experiment 1 and 38 weeks of age in experiment 2. As seen in **Figure 1** there was not much age-related difference in the transfer of cholecalciferol from feed to egg yolk.

Table 1 shows the main fatty acid composition of egg yolk in our study. No major dissimilarities could be observed between the high-cholecalciferol and control groups in experiments 1 and 2. The ratio of ω6 and ω3 fatty acids was slightly better in the vitamin D-enriched yolks than in the controls. Only slight differences were found in functional and sensory properties (odor, taste, and general acceptance) between the control and vitamin D-enriched eggs (**Tables 2** and **3**). There was rather wide variation in emulsion stability between the eggs stored for different lengths of time and from different experiments (**Table 2**) but only small variation between the control and vitamin D groups. The strength of the eggshell was also very similar in control and high-cholecalciferol groups. Mean fracture forces of 3.5 ± 0.26, 3.8 ± 0.58, and 3.6 ± 0.59 kp (control group) and 3.6 ± 0.34, 3.8 ± 0.76, and 3.7 ± 0.36 kp (high-cholecalciferol group) were obtained for 0, 11, and 24 week samples, respectively. The above results indicate that enriching hen eggs with vitamin D does not affect the other properties of eggs. Vitamin D-enriched eggs turned out to be acceptable both nutritionally, technologically, and sensorily.

Although vitamin D is essential for hens, it can be toxic in high doses. Morrisey et al. (*16*) studied the toxicity of cholecalciferol in chicks by adding 0, 10, 100, 1000, 10 000, or 100 000 μg/kg of feed. After the feeding period they performed a histopathological examination of the chicks' renal tissue which appeared to be a sensitive means of detecting vitamin D toxicity. Renal tubular calcification was observed at 10 000 μg (400 000 IU) cholecalciferol/kg of feed. This lesion was absent in all chicks fed cholecalciferol at 1000 μg/kg of feed. That level was thus set as the maximum safe dietary level of cholecalciferol. The levels used in the present study were clearly lower, ca. 300 μg (12 000 IU)/kg of feed. Hence, it was improbable that any hypervitaminosis would occur. Hens receiving the high-cholecalciferol diet and the control group had similar bone quality, skeletal condition, and no calcification of soft tissues. In the histological tests the conditions of liver and kidney in both groups were found to be similar: no necrosis or calcification of kidney tubules were observed.

ACKNOWLEDGMENT

The authors wish to thank Maire Seutu and Satu Örling for their technical assistance. We are grateful to Kaarina Karppinen, Tapani Ratilainen, and Ritva Muotila for the care of the experimental animals.

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Received for review July 9, 2002. Revised manuscript received October 1, 2002. Accepted October 2, 2002. The Ministry of Agriculture and Forestry funded this work.

JF020743Z