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Effect of Different Vitamin D Supplementations in Poultry Feed on Vitamin D Content of Eggs and Chicken Meat

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ABSTRACT: According to a new European Union regulation, vitamin D_3 can be partially or totally substituted with 25-hydroxyvitamin D_3 (25-OH-D_3) in hens' feed. The purpose of this study was to clarify how this regulation has affected the vitamin D content of commercial eggs and chicken meat. Another aim was to investigate how effectively 25-OH-D_3 is transferred from the hens' diet to egg yolk by analyzing eggs from farms using known commercial feeds and by conducting an animal study. Vitamin D determinations were made by HPLC methods. The vitamin D_3 contents of two commercial egg yolk pools were 4.9 ± 0.14 and 4.0 ± 0.10 $\mu g/100$ g, and the 25-OH-D_3 contents were 1.3 ± 0.19 and 1.0 ± 0.07 $\mu g/100$ g. The chicken meat pools contained 0.2−0.3 μg of vitamin $D_3/100$ g, whereas the content of 25-OH-D_3 was ≤ 0.2 $\mu g/100$ g. These results are comparable to earlier data. The animal and farm studies showed that 25-OH-D_3 was effectively transferred from the hens' diet to yolk. However, because the relative activity between 25-OH-D_3 and vitamin D_3 is unknown, it remains questionable whether the use of 25-OH-D_3 in hens' feed is beneficial to human vitamin D intake from eggs.

KEYWORDS: egg, hen, chicken, meat, vitamin D, vitamin D_3, 25-hydroxyvitamin D_3

INTRODUCTION

Important vitamin D compounds in man include cholecalciferol (vitamin D_3), ergocalciferol (vitamin D_2), and their hydroxylated metabolites. During the summer period, vitamin D_3 is produced in the skin by ultraviolet irradiation of 7-dehydrocholesterol. In addition, vitamin D compounds are absorbed from the diet in the intestinal track. Vitamins D_2 and D_3 are biologically inert and must first be metabolized to 25-hydroxyvitamin D (25-OH-D) in the liver and then to 1,25-dihydroxyvitamin D in the kidney before function (see, e.g., refs 1 and 2). Oral intake of vitamin D is predominant in the winter due to the lack of sunlight.

The best-known function of vitamin D is its antirachitic property. In young children, vitamin D deficiency causes poor mineralization of bones, leading to rickets. In adults, vitamin D deficiency increases the risk of osteoporosis and fractures, and the poor mineralization of newly laid down bone matrix in adult bone results in osteomalacia. Moreover, muscle weakness due to vitamin D deficiency raises the risk of falling and fractures. Vitamin D also has several noncalcemic functions in the body. There is increasing scientific evidence that implicates vitamin D deficiency with a heightened risk of type I diabetes, multiple sclerosis, rheumatoid arthritis, hypertension, cardiovascular heart disease, and many common cancers (see, e.g., refs 3−7).

Numerous studies have shown that a low vitamin D status is a global problem (see, e.g., refs 3 and 8). Current Nordic nutrition recommendations for dietary vitamin D intake vary from 7.5 to 10 μg/day depending on age and physiological condition.[9] In Finland, the authorities recommend daily supplements from 7.5 to 20 μg depending on age. Vitamin D is naturally present in only a limited number of foods, such as fish and egg yolk,[10,11] which contain vitamin D_3. Egg yolk also has significant quantities of 25-OH-D_3.[12] Meat and milk contain small amounts of vitamin D_3 and its 25-hydroxylated metabolite.[13] It has been suggested that the activity of 25-OH-D is 1−5 times that of native vitamin D_3.[8,14] High amounts of vitamin D_2 have also been found in some wild mushrooms.[15] In some countries it is customary to fortify foods with vitamin D.

Eggs are an interesting source of vitamin D, because their vitamin D content can be easily increased by raising the amount of vitamin D_3 in hens' diets.[16−18] However, vitamins belong to feed additives, the usage of which is regulated by the European Commission ((EC) No. 1831/2003). Until 2006, vitamin D supplementation was only permissible using vitamin D_3, but the addition of 25-OH-D_3 has been permitted since. According to this new regulation (EC No. 887/2009), the maximum content of the combination of 25-OH-D_3 with vitamin D_3 per kilogram of complete feedstuff is ≤ 0.125 mg for chickens for fattening and ≤ 0.080 mg for other poultry.

During 1992−1995, the vitamin D contents of Finnish commercial eggs were analyzed and the results entered into the Finnish food composition data bank (www.fineli.fi). Because of the new EC regulation, these data are now outdated. As mentioned, it is well-known that the egg vitamin D content can be raised by adding more vitamin D_3 to the feed, whereas little is known about the effect of 25-OH-D_3 supplementation. The aim of this study was to analyze the vitamin D content of commercial eggs and chicken meat and to update the Finnish food composition tables in this respect. A further aim was to study how effectively 25-OH-D_3 is transferred from hens' diets to their eggs. These data are needed to evaluate the intake of vitamin D from eggs in different feeding practices.

MATERIALS AND METHODS

The study was divided into three parts: (1) analysis of the average contents of vitamin D_3 and 25-OH-D_3 in commercial eggs and chickens,

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Table 1. Composition of Experimental Diets

	treatment			
	1 vitamin D_3, 43 μg/kg	2 vitamin D_3, 31 μg/kg; 25-OH-D_3, 30 μg/kg	3 25-OH-D_3, 55 μg/kg	4 25-OH-D_3, 122 μg/kg
ingredient (g/kg)				
barley	246.8	246.8	246.8	246.8
wheat	299.9	299.9	299.9	299.9
oat	150.0	150.0	150.0	150.0
soybean meal	160.0	160.0	160.0	160.0
rapeseed oil	30.0	30.0	30.0	30.0
monocalcium phosphate	14.0	14.0	14.0	14.0
limestone	92.0	92.0	92.0	92.0
salt	4.00	4.00	4.00	4.00
trace mineral premix[a]	2.00	2.00	2.00	2.00
vitamin premix[b]	0.28	0.28	0.28	0.28
DL-methionine	1.10	1.10	1.10	1.10
calculated composition (g/kg)				
crude protein	165.4			
calcium	36.9			
available phosphorus	3.75			
total phosphorus	6.20			
lysine	7.77			
methionine	3.64			
cysteine	3.13			
threonine	5.94			
analyzed composition (g/kg DM, unless stated differently)				
dry matter (g/kg)	885.4	897.2	889.6	893.4
crude protein	182.2	175.8	174.1	175.9
ash	100.0	144.6	136.8	139.4

[a] Content/kg feed: calcium, 0.6 g; iron, 29 mg; copper, 8 mg; manganese, 10 mg; zinc, 65 mg; iodine, 0.5 mg; selenium, 0.2 mg. [b] Content/kg feed: vitamin A, 12500 IU (retinol); vitamin E, 60 IU (α-tocopherol); menadione, 3.22 mg; thiamin, 2.94 mg; riboflavin, 0.48 mg; pyridoxine, 3.96 mg; cyanocobalamin, 0.025 mg; biotin, 0.16 mg; folic acid, 1.6 mg; niacin, 49.75 mg; calciumpantothenate, 12.25 mg.

(2) analysis of eggs from farms that use known commercial feeds containing vitamin D_3 or a mixture of vitamin D_3 and 25-OH-D_3, and (3) conduct of an animal study to investigate how 25-OH-D_3 is transferred from hens' diets to their eggs.

Average Vitamin D_3 and 25-OH-D_3 Contents of Commercial Eggs and Chickens. For the study, 58 and 53 eggs were provided from the largest Finnish egg wholesaler in the spring and fall of 2010, respectively. Each egg at both time points came from a different farm. The egg yolks and egg whites were separated in the laboratory, after which the yolks were pooled. The yolks were then mixed, vacuum-packed in plastic bags, and stored at −20 °C until analysis. The yolk percentage was 32 and 33% in the spring and fall egg samples, respectively.

Chicken meat samples (shredded breast and shredded marinated leg and thigh) were purchased in Forssa, Finland, from three different retail stores representing different food retail chains. There were three to four subsamples (300−400 g) of both meat types from each retail store. The subsamples were pooled in the laboratory according to meat type by weighing 100 g of each subsample into the pool and homogenizing the pool with a household homogenizer. The samples were packed and stored similarly to the yolk samples.

Vitamin D_3 and 25-OH-D_3 Contents of Eggs Collected from Farms. The other egg samples for this study were provided from five farms, 10 eggs from each farm. All of the farms used known commercial feeds. Two farms used feed containing only vitamin D_3 (2800 IU (70 μg)/kg, according to the feed manufacturer), and their birds were 28−47 weeks old. Three of the farms used commercial feed containing both vitamin D_3 (1500 IU (37.5 μg)/kg) and 25-OH-D_3 (0.030 mg/kg). One of these three farms had young hens (19−28 weeks) as well as two old hens (over 48 weeks). In the laboratory, the yolks were pooled by farm and stored until analysis as described above. The vitamin D contents of the commercial feeds were not analyzed.

Effect of 25-OH-D_3-Enriched Hens' Diet on Egg 25-OH-D_3 Content. Altogether 144 Lohmann Selected Leghorn hens, aged 54 weeks at the beginning of the experiment, were used for the animal study. The hens were housed in three-hen cages, each cage having a depth of 41 cm, a width of 48 cm, a rear height of 55 cm, and a front height of 60 cm. A row of six cages shared a feed trough and constituted an experimental unit. There were two experimental units per treatment. The experiment period was 6 weeks. The hens received one of four dietary treatments (Table 1), supposedly supplemented as follows: (1) 3000 IU (75 μg) of vitamin D_3/kg of feed, (2) 1500 IU (37.5 μg) of vitamin D_3 and 40 μg of 25-OH-D_3/kg of feed; (3) 80 μg of 25-OH-D_3/kg of feed; and (4) 160 μg of 25-OH-D_3/kg of feed. The analyzed contents, however, were somewhat lower (Table 1). The vitamin D levels were chosen to meet EU regulations, except for group 4, which received a higher dose of vitamin D as compared to regulations. A chain feeder ran once a day to provide the feeds to the hens. To ensure ad libitum feed access, the hens were offered approximately twice the amount of feed they were expected to consume. Leftover feed was automatically collected into a separate container for each experimental unit and then reused for the same experimental unit. Nipple drinker lines supplied water to the birds ad libitum.

The number and total weight of the eggs were recorded daily. Mean laying rate, mean egg weight, and total egg mass produced were calculated for the 6 week experiment period. Feed refusals were subtracted from the feed offered to yield feed consumption. The hens were weighed at 56 weeks of age and at the end of the experiment. Mortality was recorded daily, and cumulative mortalities were calculated at the end of the experiment.

Egg quality was assessed at 60 weeks of age, after 41 days on the experimental diets. Egg weight, albumen height, specific gravity, and shell-breaking strength of eight eggs per replicate were measured. The shell-breaking force was determined as the compressive fracture force using a Canadian eggshell tester (OTAL Precision Co. Ltd., Ottawa, ON, Canada; Hamilton, 1982). The specific gravity of the eggs was assessed based on Archimedes's principle: the mass of water (22 °C) displaced by the egg was weighed by placing the egg in a wire basket, supported from the outside, into a water bowl on a scale. Specific gravity was then calculated as the quotient of the egg mass and the mass of water displaced by the egg. Thick albumen height was measured with a digital tripod micrometer (York Electronic Centre, Technical Services and Supplies Ltd., York, U.K.) and converted to Haugh units.

At the end of the study (6 weeks), egg samples were collected from each treatment group (a whole day's production; 32−35 eggs per group) and transported to the laboratory to be analyzed for the presence of vitamin D_3 and 25-OH-D_3. The eggs were separated, and the yolks were pooled according to the treatment group to be stored until analysis as described above.

Analysis of Vitamin D_3 and 25-OH-D_3. Vitamin D_3 and 25-OH-D_3 contents in egg yolk were determined using methods described previously.[11,12] These involved saponification, extraction, purification by solid-phase extraction (SPE) and semipreparative normal-phase HPLC, and quantification by reverse-phase HPLC using internal standard methods. Vitamin D_2 served as an internal standard for vitamin D_3 and 25-OH-D_2 for 25-OH-D_3. Fractions containing vitamin D_2 plus vitamin D_3 as well as 25-OH-D_2 plus 25-OH-D_3 were separated from yolk samples during SPE purification and were separately purified and quantified by HPLC.[11,12] Semipreparative purification was performed with a Perkin-Elmer series 200 pump, autosampler, and UV−vis detector and a μ-Porasil column (5 μm, 300 × 3.9 mm; Millipore Corp., Milford, MA) with a silica guard column. The mobile phases for vitamin D and 25-OH-D fractions were 1.2% 2-propanol in hexane and 3% 2-propanol in hexane, respectively, both flowing at a rate of 1 mL/min. The employed analytical HPLC system consisted of an HP 1090 M series II high-performance liquid chromatograph equipped with an HP1090 series II diode array and a Vydac 201 TP54 column (5 μm, 250 × 4.6 mm; The Separation Group, Hesperia, CA) with a guard column. The HPLC pumps, autosampler, and diode array system were monitored and controlled, and the analytical data were evaluated using the HP 3D Chem Station computer program. The other analytical conditions were the same as earlier described.[11,12]

The sample size for chicken meat was 10 g, and the same internal standards were added as to yolks. Also, the same analytical steps were involved, except that SPE purification was excluded and the vitamin fractions were separated by normal-phase semipreparative HPLC using the same apparatus as for yolks and the protocol described earlier.[13] The analytical HPLC apparatus was also the same as for yolks. For chicken meat, two Vydac columns were connected with a thin capillary tube. The mobile phases consisted of 6% water in methanol and 15% water in methanol for the vitamin D and 25-OH-D fractions, respectively, flowing at a rate of 1 mL/min. The injection volume was 100 μL. Due to interfering compounds, the fraction containing 25-OH-D_2 plus 25-OH-D_3 was re-collected from a marinated chicken sample using a collection time of from 2 min before the retention time of 25-OH-D_3 to 2 min after that of 25-OH-D_2. The fraction was evaporated under nitrogen and dissolved into 100 μL of the mobile phase and reanalyzed. This time the injection volume was 80 μL.

Vitamin D content in the feed was determined according to a modified method of Mattila et al.[13] Vitamin D compounds were separated by semipreparative normal-phase HPLC.[13] The quantification of vitamin D_2 and vitamin D_3 as well as 25-OH-D_2 and 25-OH-D_3 fractions was performed similarly as for yolks[11,12] using apparatus described above. The sample size for feeds was 10 g.

Method Reliability Tests of the Vitamin D Analyses. Although a complete validation of the methods had been performed previously,[11−13] the linearity and repeatability of the detector response were confirmed in the present study. Recovery tests were performed by spiking the vitamin D compound under study into the samples prior to saponification. Recoveries were calculated using both internal and external standard methods. The mean recoveries of vitamin D_3 and 25-OH-D_3 were 94 and 93% ($n = 6$), respectively, when calculated using the internal standard method. Recovery of the internal standard was calculated for every replicate and found to vary from 48 to 98% for vitamin D_2 and from 42 to 72% for 25-OH-D_2, being highest for feeds and lowest for egg yolks. The samples were also analyzed in the absence of an internal standard to ensure that other vitamin D compounds or interfering compounds did not coelute with the internal standard peak.

Statistical Analyses. Statistical analyses of laying hen performance and studies on egg quality were performed with the GLM procedure of SAS (SAS Institute Inc., Cary, NC). The production variables were subjected to ANOVA using the following model: $Y_{ij} = \mu + t_i + \varepsilon_{ij}$, where Y_{ij} = observation, μ = general mean, t_i = effect of treatment ($i = 1, ..., 4$), and ε_{ij} = error term. Comparisons between the control treatment (43 μg of vitamin D_3/kg) and the other treatments were made with Dunnett's t test. The residuals were plotted against fitted values to ascertain the normality of the experimental data. Tables 4 and 5 show the original least-squares means and standard errors (SE). Each vitamin D analysis was done at least in triplicate, and the results are expressed as means and standard deviations (SD). Part of the data on vitamin D contents was analyzed by Student's t test. Differences of $p < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Average Contents of Vitamin D_3 and 25-OH-D_3 in Commercial Eggs and Chicken Meat. The determinations of vitamin D compounds were made from egg yolk, because practically all of the vitamin D in eggs is accumulated in the yolk, whereas egg white contains only traces of vitamin D.[19−21] A commercial egg yolk pool was collected at two different time points to study the variation in vitamin D content. In the first pool (spring 2010), the content of vitamin D_3 was 4.9 ± 0.14 μg/100 g and that of 25-OH-D_3, 1.3 ± 0.19 μg/100 g. The second pool (fall 2010) contained slightly less of both vitamin D_3 and 25-OH-D_3: 4.0 ± 0.10 and 1.0 ± 0.07 μg/100 g, respectively. To convert these contents to a whole egg basis, dividing the spring and fall results by 3.13 and 3.03 will provide fairly good estimations (see Materials and Methods). The mean results for vitamin D_3 and 25-OH-D_3 would thus be 1.4 μg/100 and 0.38 μg/100 g whole egg, respectively.

Similar results were obtained in 1992 for vitamin D_3 using the same protocol and analytical method. At that time, the results for two pooled samples were 4.0 and 5.6 μg/100 g.[11] In 1993, the average 25-OH-D_3 content in pooled egg yolk was reported as

Table 2. Vitamin D_3 and 25-OH-D_3 Contents in Egg Yolks Gathered from Farms

farm[a]	vitamin D_3 (μg/100 g)	25-OH-D_3 (μg/100 g)
1	2.7 ± 0.16	2.5 ± 0.13
2	2.7 ± 0.15	2.0 ± 0.10
3	4.0 ± 0.13	2.2 ± 0.25
4	6.6 ± 0.20	1.2 ± 0.015
5	6.8 ± 0.29	1.2 ± 0.19

[a] Farms: 1, 1500 IU vitamin D_3 and 30 μg 25-OH-D_3/kg feed (19−28-week-old birds); 2 and 3, 1500 IU vitamin D_3 and 30 μg 25-OH-D_3/kg feed (>48-week-old birds); 4 and 5, 2800 IU vitamin D_3/kg feed (28−47-week-old birds).

Table 3. Effect of Feed Vitamin D Composition and Contents on the Vitamin D_3 and 25-OH-D_3 Contents of Egg Yolk

treatment[a]	vitamin D_3 (μg/100 g yolk)	25-OH-D_3 (μg/100 g yolk)
1	3.4 ± 0.14	0.71 ± 0.10
2	1.4 ± 0.19	1.5 ± 0.06
3	≤0.2	2.1 ± 0.09
4	≤0.2	4.3 ± 0.14

[a] Treatments: 1, 43 μg of vitamin D_3/kg of feed; 2, 31 μg of vitamin D_3 and 30 μg of 25-OH-D_3/kg of feed; 3, 55 μg of 25-OH-D_3/kg of feed; 4, 122 μg of 25-OH-D_3/kg of feed.

0.98 ± 0.069 μg/100 g.[12] This content is exactly the same as that obtained for the second yolk pool in the present study. The result from the first pool, however, was 30% higher. These findings indicate that the new EU regulation concerning vitamin D supplementation in poultry feed has so far not had any major effect on the vitamin D_3 and 25-OH-D_3 contents of egg yolk in Finland. Because the new regulation allows the addition of 25-OH-D_3 to feed as a substitute for vitamin D_3, the vitamin D_3 levels in egg yolk might have been expected to decrease and those of 25-OH-D_3 to increase more pronouncedly than was observed.

The corresponding contents in chicken meat were also comparable to earlier data produced in a similar way. According to Mattila et al.,[13] the vitamin D_3 content in chicken meat was 0.29 μg/100 g and that of 25-OH-D_3 0.25 μg/100 g. In the present study, the vitamin D_3 content in the leg and thigh meat was 0.3 μg/100 g and that in the fillet, 0.2 μg/100 g. The 25-OH-D_3 contents were ≤0.2 μg/100 g in both meat types. It should be noted that the meat content of marinated leg and thigh was 70%, so the true vitamin D content is 30% higher.

Very few other studies have been published concerning the vitamin D content in eggs or chicken meat. Sondergaard and Leerbeck[20] and Takeuchi et al.[21] found very similar vitamin D_3 contents in egg yolk as obtained in the present study: approximately 4 μg/100 g. According to Sivell et al.,[22] the vitamin D_3 contents in battery and free-range whole eggs varied from 0.5 to 2.1 μg/100 g. Koshy and VanDerSlik[23] reported 25-OH-D_3 contents of 0.5−0.8 μg/100 g egg yolk. Mawer and Gomes[24] determined the presence of different vitamin D compounds in chicken and beef samples using HPLC and/or biospecific methods. They found undeterminable contents of vitamin D_3, whereas the contents of 25-OH-D_3 ranged from 0.32 to 0.42 μg/100 g.

To be able to calculate the total vitamin D content in poultry products, the relative activity between 25-OH-D_3 and vitamin D_3 has to be known. However, the activity conversion factor for 25-OH-D_3 remains a matter of dispute.[14] Most of the studies conducted to assess this factor were performed 30−40 years ago in deficient rats, giving estimated values of between 1.4 and 5.[14,25] Jacobsen et al.[25] were the first to investigate the relative activity between vitamin D_3 and 25-OH-D_3 in healthy mammals, using pigs as a model for man. The followed effects included the contents of 25-OH-D_3 in plasma and of vitamin D_3 and 25-OH-D_3 in the pork loin and liver. Their study suggests that 25-OH-D_3 should be regarded as having the same activity as vitamin D_3 in food databases. According to the (EC) Nutrition and Health Claims Regulation,[26] a "source" claim can be made for foodstuffs that meet at least 15% of the recommended daily amount (RDA) per 100 g, whereas a "rich in" claim applies when nutrient levels exceed 30% of the RDA. Whole eggs contained 1.4 μg of vitamin D_3 and 0.38 μg of 25-OH-D_3/100 g, and the Nordic nutrition recommendations for dietary vitamin D intake vary from 7.5 to 10 μg/day. Accordingly, the egg can be regarded as a source of or rich in vitamin D, depending on the relative activity of 25-OH-D_3.

Vitamin D_3 and 25-OH-D_3 Contents in Eggs Collected from Farms. The effect of various feeding practices on the vitamin D_3 and 25-OH-D_3 contents of egg yolks is clearly seen in Table 2. Vitamin D_3 content was lower and 25-OH-D_3 content higher in the egg yolks from farms using feeds in which half of vitamin D_3 was substituted with 25-OH-D_3 ($p < 0.01$).

The results of this part of the study indicate that the average vitamin D content in commercial eggs would probably be altered if all egg producers were to use feeds containing 25-OH-D_3. It is, however, questionable whether the use of 25-OH-D_3 in hens' feed is beneficial for vitamin D intake from eggs. The critical factor here is the vitamin D activity of 25-OH-D_3 in humans. If its activity is 1−4 times that of vitamin D_3, then the vitamin D content of egg yolk would probably remain approximately the same or even decrease if half of the vitamin D_3 in feed were substituted with 25-OH-D_3. If the activity of 25-OH-D_3 is 5 times that of vitamin D_3, the vitamin D content would then probably be somewhat higher in yolks. In the light of a recent study,[25] an activity conversion factor that high is improbable. Nevertheless, when the results under Average Contents of Vitamin D_3 and 25-OH-D_3 in Commercial Eggs and Chicken Meat are compared with the results of this section, it can be assumed that most egg producers today probably still use feeds containing only vitamin D_3.

Effect of 25-OH-D_3-Enriched Diet on 25-OH-D_3 Content. The aim of the animal study was to clarify how efficiently 25-OH-D_3 transfers from the hens' diet to egg yolk. The experiment included four different treatments. The vitamin D contents in three of the diets met the EU regulations, and in one diet the 25-OH-D_3 content was somewhat higher. The first treatment group received a diet containing only vitamin D_3 (control group), the second diet contained nearly equal amounts of vitamin D_3 and 25-OH-D_3, and the third and fourth groups contained only 25-OH-D_3.

Table 3 indicates that 25-OH-D_3 was transferred from the hens' diet to egg yolk in a linear manner. The pooled yolk from the control group, which received only vitamin D_3 from the diet (43 μg/kg), contained 0.71 μg of 25-OH-D_3 and 3.4 μg of vitamin D_3/100 g. The pooled yolk of the treatment group fed solely on 25-OH-D_3 supplemented feed (122 μg/kg feed) contained 4.3 μg of 25-OH-D_3/100 g yolk, whereas the vitamin D_3 content was ≤0.2 μg/100 g. As mentioned in the Introduction, a new EU regulation states that the maximum content of the combination of 25-OH-D_3 with vitamin D_3 per kilogram of complete feedstuff for laying hens is ≤80 μg. Our data demonstrate that if this

Table 4. Effects of Various Levels of Dietary Vitamin D_3 and 25-OH-D_3 on the Performance of Laying Hens

treatment[a]	egg production (%)	egg weight (g)	egg production (g/day per hen)	feed consumption (g/day per hen)	feed conversion ratio (kg feed/kg eggs)
1 (control)	91.7	65.9	60.4	118.0	1.96
2	91.9	65.0	59.7	110.2	1.85
3	91.1	65.9	60.0	110.2	1.84
4	91.9	64.9	59.6	113.1	1.90
standard error	2.012	0.432	1.285	3.9561	0.090
p value[b]					
control vs 2	1.000	0.421	0.959	0.456	0.721
control vs 3	0.994	1.000	0.993	0.453	0.673
control vs 4	1.000	0.346	0.942	0.725	0.920

[a] Treatments: 1, control, 43 μg of vitamin D_3/kg; 2, 31 μg of vitamin D_3 and 30 μg of 25-OH-D3/kg; 3, 55 μg of 25-OH-D3/kg; 4, 122 μg of 25-OH-D3/kg. [b] *p* values from Dunnett's *t* test for H_0: mean = control.

Table 5. Effects of Various Levels of Dietary Vitamin D_3 and 25-OH-D_3 on the Eggshell and Albumen Quality

treatment[a]	egg specific wt (g/mL)	shell-breaking strength (kp)	Haugh units
1 (control)	1.086	3.6	85.0
2	1.087	3.6	85.1
3	1.087	3.5	82.6
4	1.087	3.7	83.4
standard error	0.0017	0.146	0.801
p value[b]			
control vs 2	0.916	1.000	1.000
control vs 3	0.843	0.798	0.206
control vs 4	0.878	0.902	0.424

[a] Treatments: 1, control, 43 μg of vitamin D_3/kg; 2, 31 μg of vitamin D_3 and 30 μg of 25-OH-D3/kg; 3, 55 μg of 25-OH-D3/kg; 4, 122 μg of 25-OH-D3/kg. [b] *p* values from Dunnett's *t* test for H_0: mean = control.

Table 6. Effects of Various Levels of Dietary Vitamin D_3 and 25-OH-D_3 on the Live Weight of Laying Hens

treatment[a]	live weight (g) at 56 weeks of age	live weight (g) at 60 weeks of age
1 (control)	1806	1813
2	1834	1842
3	1814	1819
4	1754	1756
standard error	13.920	17.599
P value[b]		
control vs 2	0.462	0.578
control vs 3	0.958	0.990
control vs 4	0.123	0.180

[a] Treatments: 1, control, 43 μg of vitamin D_3/kg; 2, 31 μg of vitamin D_3 and 30 μg of 25-OH-D3/kg; 3, 55 μg of 25-OH-D3/kg; 4, 122 μg of 25-OH-D3/kg. [b] *p* values from Dunnett's *t* test for H_0: mean = control.

maximum content is used by supplementing the hens' diet with 25-OH-D_3 and excluding vitamin D_3, the egg yolk would contain solely 25-OH-D_3, probably <4 μg/100 g (Table 3). Whether this is beneficial for human nutrition depends strongly on the activity of 25-OH-D_3 in relation to vitamin D_3.

The EU approved 25-OH-D_3 as a substitute for vitamin D_3 in poultry feeds because there was sufficient evidence that 25-OH-D_3 is at least as effective as vitamin D_3 in optimizing the performance of chickens for fattening, laying hens, and turkeys.[27] In this study, 25-OH-D_3 proved as effective as vitamin D_3 and did not show any advantage over vitamin D_3 in supporting the hens' performance or the eggshell quality. The experimental diets had no effects on either egg production, egg weight, feed consumption, or feed conversion ratio (Table 4) as compared to the control diet. Neither were there any significant effects of the dietary treatments on albumen quality (Haugh units), shell breaking strength, or specific gravity of the eggs (Table 5). The dietary treatments had no significant effects on the hens' body weight (Table 6). Indeed, very variable results have been reported earlier for the superiority of 25-OH-D_3 over vitamin D_3 for egg shell quality and production performance of laying hens.[28]

Although 25-OH-D_3 may be more potent in its vitamin D activity for poultry than vitamin D_3, its higher potency depends on and varies with the assessed criterion and the applied dosage. Studies have shown that some skeletal characteristics in laying hens can be improved with 25-OH-D_3, but the results are inconsistent (reviewed by Fleming[29]). According to Mattila et al.,[18] elevated levels of vitamin D_3 also show positive functions on bone strength in laying hens. The force needed to fracture the tibia of laying hens increased when the feed was moderately supplemented with vitamin D_3 (5100−5800 IU/kg of feed) as compared to the control diet (2600−3000 IU of vitamin D_3/kg). Leg and gait disorders continue to pose a considerable problem for the broiler industry. Vitamin D metabolites have been found effective in preventing tibial dyschondroplasia, but also a high vitamin D status of young chicks, induced by feeding high concentrations of vitamin D_3, can alleviate or prevent tibial dyschondroplasia (reviewed by Fleming[29]). On the grounds of the above-mentioned studies, it seems probable that elevated levels of vitamin D_3 in hens' feed instead of 25-OH-D_3 substitution would be beneficial for poultry.

The vitamin D status of humans is far from optimal in many countries around the world. Unfortunately, there are only a few foods that contain significant amounts of vitamin D. From a public health point of view, it would be beneficial to increase the potential sources of vitamin D by fortifying specific products that are commonly consumed over a whole population. Eggs are such products, and it is also easy to fortify their vitamin D_3 content. In fact, according to Mattila et al.,[16−18] the vitamin D_3 content of eggs can be easily raised by increasing the content of vitamin D_3 in hens' diets. By doubling the vitamin D_3 content in feeds as compared to the regulations, it is possible to produce eggs with at least double the vitamin D_3 content (approximately 2−3 μg/egg). The content of 25-OH-D_3 would increase somewhat as well.[16] Hens would also benefit from the extra vitamin D_3.[18] Nevertheless, vitamin D_3 is more suitable than 25-OH-D_3 for egg vitamin D fortification due to economic and safety reasons. Vitamin D can be toxic in high doses, and 25-OH-D_3 is more toxic than vitamin D_3. Yarger et al.[30] suggested on the basis of body weight and renal calcifications that 25-OH-D_3 is 5−10 times more toxic for chickens for fattening than vitamin D_3. In addition, the fact that the biological activity of diet-borne 25-OH-D_3 is not known makes it less safe than vitamin D_3.

In conclusion, like vitamin D_3, also 25-OH-D_3 is transferred from hens' feed to egg yolk with a clear dose response. However, if 25-OH-D_3 is the only vitamin D source in the hens' diet, the eggs would contain no vitamin D_3 at all. The benefits for human nutrition of 25-OH-D_3 supplementation in feeds depends on its vitamin D activity relative to vitamin D_3, and this remains unknown so far. However, our results imply that the vitamin D contents of commercial eggs and chicken meat have not been changed as a consequence of a recent EU regulation (EC No. 887/2009), which allows substituting vitamin D_3 in poultry feed partly or totally with 25-OH-D_3. Hence, most egg and broiler producers probably still use feeds containing solely vitamin D_3.

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