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Effect of Short-Term UVB Exposure on Vitamin D Concentration of Eggs and Vitamin D Status of Laying Hens

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ABSTRACT: Vitamin D deficiency in humans is widespread, and only a few food items are important natural sources of vitamin D. This study investigated the effect of UVB exposure of laying hens on the vitamin D content in egg yolk. In a two-factorial design, hens fed a vitamin D-deficient (−D) or -adequate (+D) diet were nonexposed or exposed to UVB light over a period of 4 weeks. UVB exposure of the −D group caused nearly normal egg production rate and egg shell quality; exposure of the +D group did not further improve these parameters. UVB exposure tended to improve the concentration of plasma 25-hydroxycholecalciferol ($25(OH)D_3$), but had no effect on 1,25-dihydroxycholecalciferol in plasma or on cholecalciferol and $25(OH)D_3$ in egg yolk. The present study shows that a short-term exposure of laying hens to UVB light is not an appropriate way to improve the vitamin D content of egg yolk.

KEYWORDS: *egg yolk, vitamin D, UVB exposure, laying hen*

INTRODUCTION

Vitamin D deficiency constitutes a largely unrecognized and widespread public health problem. It has been suggested that 1 billion people worldwide have vitamin D deficiency or insufficiency.[1,2] Humans get vitamin D from sunlight exposure and from their foods or dietary supplements. Seasonal variations of UVB light exposure, increasing indoor activities, stationary care in nursing homes, and the frequent use of sunscreens to reduce the risk of skin cancer are the main causes for the low synthesis of cutaneous vitamin D. Thus, an increasing number of people depend on dietary sources of vitamin D. On the other hand, vitamin D is found naturally in only a few foods. Besides fish and milk, eggs, particularly egg yolks, are considered to be the important food source of vitamin D.[3]

It is well established that the cholecalciferol and 25-hydroxycholecalciferol ($25(OH)D_3$) contents in egg yolk correlate positively with the cholecalciferol content in poultry feed.[4] However, maximum limits for cholecalciferol supplementation in animal feeds are specified worldwide. In Europe, the maximum amount of supplemented cholecalciferol specified by the Council of the European Communities (Council Directive 70/524/EEC) is 3000 IU/kg feed for laying hens. This means that beyond this limit further diet-induced increases of vitamin D content in eggs are not feasible. Another possibility to improve vitamin D content in eggs could be UVB exposure of laying hens. Only a few data implicate the dominant influence of solar radiation on the levels of vitamin D in animal products. Previous investigations show temporal variation in vitamin D in bovine milk that ranges between 0.06 IU/g milk fat in winter and 0.23 IU/g milk fat in summer.[5] Corresponding data about the effect of UVB exposure of laying hens on the vitamin D content of eggs are not available.

The aim of the present study was to investigate whether treatment of laying hens with UVB light could increase the vitamin D content of their eggs. The effect of UVB exposure was elucidated in hens that received no or maximum amounts of vitamin D with their feeds. The main issue of this study focused on the analysis of cholecalciferol and $25(OH)D_3$, the major vitamin D metabolites in egg yolk.[6] Besides that, egg production rate, egg shell quality, egg yolk lipids, and plasma levels of $25(OH)D_3$, 1,25-dihydroxycholecalciferol ($1,25-(OH)_2D_3$), and calcium were determined in response to experimental treatments.

MATERIALS AND METHODS

Animals and Treatment. An experiment was conducted with 40 Lohmann White layers with an age of 36 weeks and an average body weight of 1.93 ± 0.18 kg. The hens were fed 3 weeks before starting the experiment with standard feed that contained 2500 IU cholecalciferol/kg feed. The hens were then allotted to four groups of 10 each. Two groups of hens received a vitamin D-adequate diet with 3000 IU cholecalciferol (Molekula Ltd., Dorset, U.K.)/kg diet (+D); the other two groups were fed a vitamin D-deficient diet (0 IU cholecalciferol/kg diet) (−D), respectively. The groups were either nonexposed (−UVB) or exposed (+UVB) to UVB light. Except for vitamin D, the hens received a nutritionally adequate diet consisting of (in g/kg diet) wheat (470), extracted soybean meal (220), corn (100), barley (68.2), calcium carbonate (85), soybean oil (30), dicalcium phosphate (13), vitamin and mineral premix (10), sodium chloride (2), and DL-methionine (1.8). This diet contained 11.6 MJ metabolizable energy and 173 g crude protein per kg, as determined by the official German VDLUFA methods.[7]

The hens were kept one bird per cage in an environmentally controlled room at 18 °C. The room was lit for 14 h daily at an intensity of 20−30 lx. Feed and water (via nipple drinkers) were available ad libitum. The experiment was conducted over a 4 week

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period. All procedures followed established guidelines for the care and handling of animals and were approved by the veterinary council of Saxony-Anhalt. Body weight, food intake, laying performance, egg weight, and shell quality were recorded weekly.

UVB Treatment. UVB-treated hens were exposed to UVB emitting lamps for 4 × 15 min daily controlled by a timer turning on the light at 9:00 a.m., 12:00 p.m., 3:00 p.m., and 6:00 p.m. The 120 cm long UVB lamps (40 W, Terra Repti Glo 10.0, Hagen, Germany) were placed at a distance of 50 cm above the hens' heads, which present a small nonfeathered area. The lamps emitted UVB light in ranges of 280−310 nm. A UVB meter was used to monitor UV radiation levels to which the hens were exposed (accuracy, ± 8%). The UVB irradiation dosage measured at a distance of 50 cm was 15 $\mu W/cm^2$. This dosage was similar to that used for reptiles to ensure an efficient synthesis of vitamin D.[8] A UVB opaque board was placed between the UVB-treated and nonexposed groups to block incidental radiation.

Sample Collection. To determine egg weight and shell quality, eggs from each hen were collected at the beginning of the experiment and after weeks 1, 2, 3, and 4 of the experimental period. Egg yolks for analysis of cholecalciferol, $25(OH)D_3$ and lipids and blood for analysis of $25(OH)D_3$, $1,25(OH)_2D_3$, calcium, and lipids were collected from each hen at the beginning and at the end of the experiment. Blood was collected in heparinized tubes; plasma was separated by centrifugation at 1500*g* for 10 min at 4 °C. Egg yolk and plasma were stored at −20 °C until analysis.

Analysis of $25(OH)D_3$ and $1,25(OH)_2D_3$ Concentrations in Plasma. Plasma concentration of $25(OH)D_3$ was analyzed by coupled liquid chromatography−mass spectrometry (LC-MS) using a MassChrom kit (Chromsystems, Munich, Germany) for the determination of $25(OH)D_3$ and $25(OH)D_2$. In brief, 50 μL of plasma samples was mixed with 12.5 μL of precipitation reagent and 100 μL of a solution of deuterated $25(OH)D_3$ ($25(OH)D_3$-d_6) that was used as internal standard. Prior to transfer of the samples into autosampler vials, the sample mixtures were incubated for 10 min at 4 °C and centrifuged for 10 min at 15000*g*. The final supernatants were used for separation and quantification of 25(OH)D by an API 2000 LC-MS system (Applied Biosystems, Darmstadt, Germany) using APCI and MRM mode coupled to an Agilent 1100 HPLC (Agilent, Waldbronn, Germany) equipped with a trap and analytical column (MassChrom kit, Chromsystems). The mass transitions used were (*m*/*z*) 395.2/269.1, 383.4/211.2, and 389.5/211.2 for $25(OH)D_2$, $25(OH)D_3$, and $25(OH)D_3$-d_6, respectively. Detection limits were 6.7 and 5.2 ng/mL for $25(OH)D_3$ and $25(OH)D_2$, respectively. Between-run precision data were calculated from two control sera (n = 4 for each). For $25(OH)D_3$, the CVs were 6.7% for the control serum at low concentration (16.7 ng/mL) and 3.7% for the control serum at high concentration (38.4 ng/mL).

The plasma concentration of $1,25(OH)_2D_3$ was determined by use of a commercial enzyme immunoassay (IDS, Boldon, U.K.). Prior to analysis, $1,25(OH)_2D_3$ was purified by immunoextraction according to the manufacturer's protocol.

Analysis of Calcium in Plasma. For determination of the calcium concentration in plasma, a colorimetric assay (Analyticon Biotechnologies AG, Lichtenfels, Germany) was used. Prior to analysis, the plasma was diluted 1:4 with 0.9% NaCl to avoid interferences with triglycerides in plasma.

Analysis of Egg Shell Thickness and Stability. Shell thickness was measured with a micrometer screw capable of 0.01 mm accuracy. For that purpose, shell fragments from the equatorial region were collected, and organic material, including shell membranes, was removed. Egg shell stability was determined by a highly sensitive electronically controlled breaking strength tester (Messtechnik Gutsch, Nauendorf, Germany) and was expressed in newtons (N).

Analysis of Vitamin D_3 and $25(OH)D_3$ Concentrations in Egg Yolk. Concentrations of cholecalciferol and $25(OH)D_3$ in egg yolk were analyzed by LC-MS. The detailed protocol will be published elsewhere. In brief, homogenized egg yolk was spiked with deuterated internal standards (D_3-d_3 6,19,19 and $25(OH)D_3$-d_3 6,19,19) and saponified under nitrogen. Vitamin D metabolites were separated and concentrated by ion-exchange solid phase extraction. Material was derivatized with 4-phenyl-1,2,4-triazoline-3,5-dione to improve sensitivity and then subjected to LC-MS. Identification and quantification were performed on an API 4000 Q-trap mass spectrometer (Applied Biosystems) using MRM mode. For separation, the probe of the mass spectrometer was directly connected to a Jasco HPLC system (autosampler, AS-2057 plus; quarternary gradient pump, PU-2080 plus; Gross-Umstadt, Germany) equipped with an Eurospher 100-5 C18-column (Knauer, Berlin, Germany).

Analysis of Cholesterol and Triglycerides in Plasma and Egg Yolk. Lipids from egg yolk were extracted with a mixture of *n*-hexane and isopropanol (3:2, v/v). For determination of the concentrations of lipids in egg yolk, aliquots of the lipid extracts were dried and the lipids dissolved using Triton X-100/chloroform (1:1, w/w).[9] Concentrations of cholesterol and triglycerides in plasma and egg yolk were analyzed using enzymatic reagent kits (DiaSys Diagnostic Systems, Holzheim, Germany). Prior to plasma triglyceride analysis, plasma samples were diluted 1:10 with 0.9% NaCl.

Statistical Analysis. Data were analyzed by General Linear Model ANOVA, including the factors dietary vitamin D, UVB exposure, and the interaction of these factors using the Minitab Statistical software (Minitab, State College, PA). In the case of significant effects (P < 0.05), the means of the four groups were compared by Fisher's multiple-range test. Means were considered to be significantly different at P < 0.05. Values in the text are given as the mean ± SD.

RESULTS

Food Intake and Final Body Weight. Daily food intake was significantly lower in the −D/−UVB group than in the other treatment groups (−D/−UVB, 95.7 ± 6.5 g/day; +D/−UVB, 113.3 ± 12.7 g/day; −D/+UVB, 107.4 ± 7.4 g/day; +D/+UVB, 100.7 ± 15.9 g/day; P < 0.05). Groups that received vitamin D with their diets and the −D group exposed to UVB light did not differ in food intake. Hens from the +D/+UVB group had higher final body weights than hens from the other groups (−D/−UVB, 1880 ± 48 g; +D/−UVB, 1839 ± 175 g; −D/+UVB, 1942 ± 159 g; +D/+UVB, 2154 ± 303 g; P < 0.05). The final body weights of hens from the −D/−UVB, +D/−UVB, and −D/+UVB groups did not differ.

Laying Performance. Within the first two weeks of the experiment, egg production rate (number of eggs per hen and week) was not influenced by dietary vitamin D and UVB exposure, respectively (Figure 1A). During the third and fourth weeks of the experiment, numbers of eggs produced per hen and week were significantly influenced by dietary vitamin D and UVB exposure, and there was an interaction between these two factors (Figure 1A). In the third week, hens from the −D/−UVB group produced markedly fewer eggs than hens from the other three groups; no differences in egg production were observed between the +D groups and the −D/+UVB group. In the fourth week, the −D/−UVB group had still the lowest egg production rate, yet lower than in the third week. In the fourth week, also the egg production rate of hens from the −D/+UVB group were slightly affected compared to the +D/−UVB group. Hens that received vitamin D with their diets had the highest egg production rates, whereby additional UVB exposure did not further improve the rate of egg production in these animals. During the first 3 weeks of the experiment, mean egg weights were not different between the treatment groups. In the fourth week, eggs from the −D/−UVB group had lower weights than those from the other groups (Figure 1B). Egg weights from the +D/−UVB, −D/+UVB, and +D/+UVB groups were not different.

Egg Shell Quality. Within the first 2 weeks of the experiment, egg shell stability and egg shell thickness were not influenced by dietary vitamin D or UVB exposure, respectively

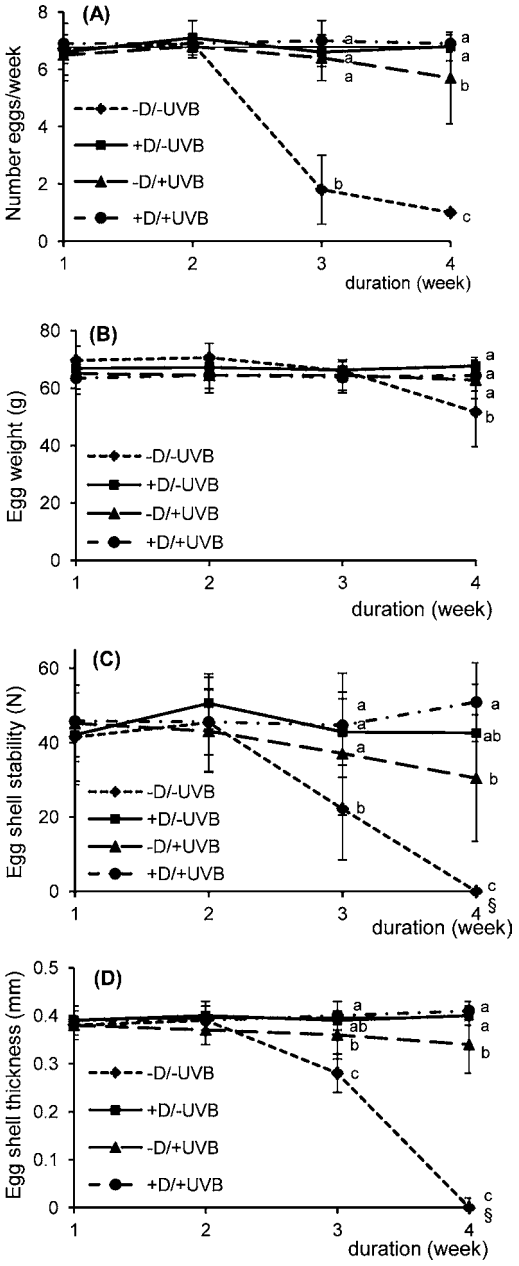

Figure 1. Effect of dietary vitamin D (D) and UVB exposure (UVB) on (A) laying performance of hens, (B) egg weight, (C) egg shell stability, and (D) egg shell thickness during the experimental period of 4 weeks. Values are the mean ± SD, n = 10. Data were analyzed by two-way ANOVA. Classification factors were vitamin D in the diet, UVB exposure, and the interaction between both factors. (A) Effect of (week 3) vitamin D, $P < 0.001$; UVB, $P < 0.001$; and vitamin D × UVB, $P < 0.001$; (week 4) vitamin D, $P < 0.001$; UVB, $P < 0.001$; and vitamin D × UVB, $P < 0.001$. (B) Effect of (week 4) vitamin D, $P < 0.001$; and vitamin D × UVB, $P < 0.001$. (C) Effect of (week 3) vitamin D, $P < 0.01$; and UVB, $P < 0.10$; (week 4) vitamin D, $P < 0.05$; UVB, $P < 0.05$; and vitamin D × UVB, $P < 0.10$. (D) Effect of (week 3) vitamin D, $P < 0.001$; UVB, $P < 0.01$; and vitamin D × UVB, $P < 0.10$; (week 4) vitamin D, $P < 0.05$; UVB, $P < 0.05$; and vitamin D × UVB, $P < 0.05$. Individual means of the treatment groups were compared by Fisher's multiple-comparison test. Values with no common letters are significantly different ($P < 0.05$). §The majority of the few existing eggs had no shell.

(Figure 1C,D). During the third week of the experiment, egg shell stability and thickness were significantly influenced by dietary vitamin D. Eggs produced from hens of the −D/−UVB group had a lower shell stability and a lower shell thickness than those from hens of the other three groups (Figure 1C,D); no differences in egg shell quality were observed between the +D groups and the −D/+UVB group. In the fourth week, eggs from the −D/−UVB group had the lowest shell stability and thickness, followed by eggs from hens of the −D/+UVB group. Eggs from hens that received vitamin D with their diets had the highest shell stability and thickness (Figure 1C,D). Additional UVB exposure of hens from the +D group did not further improve egg shell quality.

Plasma Concentrations of $25(OH)D_3$, $1,25(OH)_2D_3$, and Calcium. None of the plasma samples had detectable concentrations of $25(OH)D_2$ (data not shown). The plasma concentration of $25(OH)D_3$ at the end of the experiment period was significantly influenced by dietary vitamin D, whereas UVB exposure tended only to alter concentrations of circulating $25(OH)D_3$ (Figure 2A). Hens of the +D groups had markedly higher plasma concentrations of $25(OH)D_3$ than hens of the −D groups ($P < 0.001$, Figure 2A). UVB exposure slightly improved plasma concentrations of $25(OH)D_3$ in both

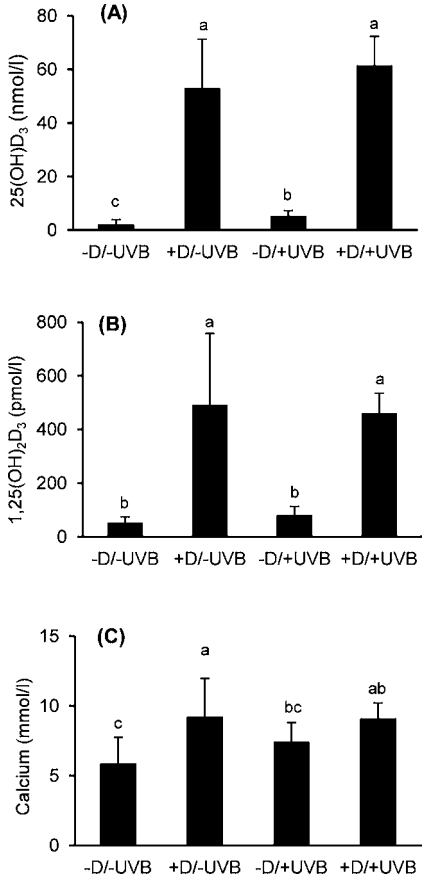

Figure 2. Final plasma concentrations of (A) $25(OH)D_3$, (B) $1,25(OH)_2D_3$, and (C) calcium of hens in response to dietary vitamin D (D) and UVB exposure (UVB). Values are the mean ± SD, n = 10. Data were analyzed by two-way ANOVA. Classification factors were vitamin D in the diet, UVB exposure, and the interaction between both factors. (A) Effect of vitamin D, $P < 0.001$; and UVB, $P < 0.10$. (B) Effect of vitamin D, $P < 0.001$. (C) Effect of vitamin D, $P < 0.001$. Individual means of the treatment groups were compared by Fisher's multiple-comparison test. Bars with no common letters are significantly different ($P < 0.05$).

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groups of hens nonsupplemented or supplemented with vitamin D ($P < 0.10$).

Plasma concentrations of $1,25(OH)_2D_3$ and calcium were significantly influenced by dietary vitamin D but not by UVB exposure. Hens of the +D groups had higher plasma concentrations of $1,25(OH)_2D_3$ and calcium than hens of the −D groups (Figure 2B,C).

Concentrations of Vitamin D_3 and $25(OH)D_3$ in Egg Yolk. Concentrations of vitamin D_3 and $25(OH)D_3$ in egg yolk were strongly influenced by dietary vitamin D, but not by UVB exposure (Figure 3). Irrespective of the UVB treatment, egg

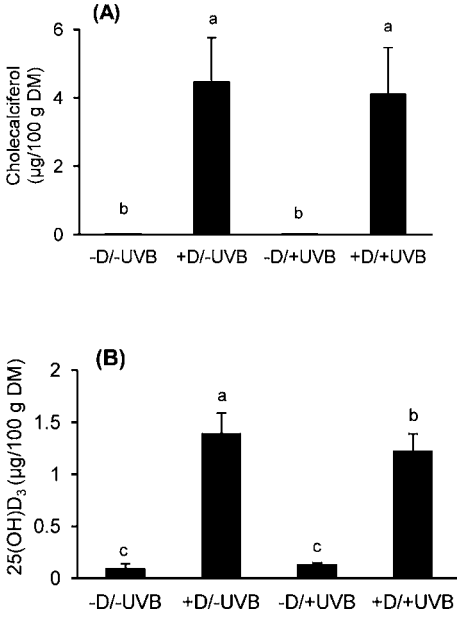

Figure 3. Final concentrations of (A) cholecalciferol and (B) $25(OH)D_3$ in egg yolk of hens in response to dietary vitamin D (D) and UVB exposure (UVB). Values are the mean ± SD, $n = 10$. Data were analyzed by two-way ANOVA. Classification factors were vitamin D in the diet, UVB exposure, and the interaction between both factors. (A) Effect of vitamin D, $P < 0.001$. (B) Effect of vitamin D, $P < 0.001$; and vitamin D × UVB, $P < 0.05$. Individual means of the treatment groups were compared by Fisher's multiple-comparison test. Bars with no common letters are significantly different ($P < 0.05$). DM, dry matter.

yolks of hens from the −D groups had no detectable concentration of vitamin D_3, whereas egg yolks of hens from the +D groups contained >4 µg/100 g of vitamin D_3 (Figure 3A). Egg yolks of hens from the −D groups were further characterized by a markedly reduced concentration of $25(OH)D_3$ compared to the +D groups (Figure 3B). Figure 4 shows the changes in concentrations of vitamin D_3 and $25(OH)D_3$ in egg yolks of the treatment groups within the 4 weeks of experimental treatment. The vitamin D_3 concentration in egg yolk strongly decreased in the −D groups, whereas it remained nearly at the same level in those of the +D groups. UVB exposure did not influence the changes of vitamin D_3 in egg yolks.

Changes of $25(OH)D_3$ concentrations in egg yolk were influenced by dietary vitamin D and UVB exposure (Figure 4B). In the +D groups the concentration of $25(OH)D_3$ in egg yolk had not changed within the experimental period. In hens from the −D groups the concentrations of $25(OH)D_3$ in egg yolk decreased. However, in the −D/+UVB group the decline

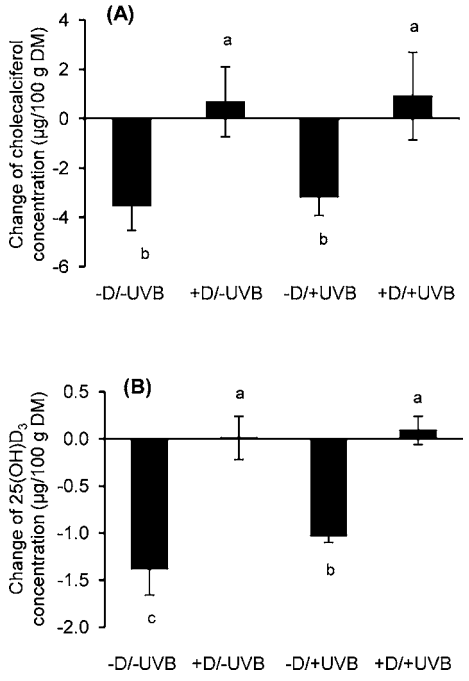

Figure 4. Changes of (A) cholecalciferol and (B) $25(OH)D_3$ concentrations in egg yolk of hens in response to dietary vitamin D (D) and UVB exposure (UVB) over a period of 4 weeks. Values are the mean ± SD, $n = 10$. Data were analyzed by two-way ANOVA. Classification factors were vitamin D in the diet, UVB exposure, and the interaction between both factors. (A) Effect of vitamin D, $P < 0.001$. (B) Effect of vitamin D, $P < 0.001$; and UVB, $P < 0.05$. Individual means of the treatment groups were compared by Fisher's multiple-comparison test. Bars with no common letters are significantly different ($P < 0.05$). DM, dry matter.

of $25(OH)D_3$ was not as strong as in the −D/−UVB group (Figure 4B).

Concentrations of Cholesterol and Triglycerides in Plasma and Egg Yolk. Plasma concentrations of cholesterol and triglycerides were influenced neither by dietary vitamin D nor by UVB exposure (plasma cholesterol, −D/−UVB, 4.79 ± 2.79 mmol/L; +D/−UVB, 5.60 ± 4.14 mmol/L; −D/+UVB, 3.71 ± 1.17 mmol/L; +D/+UVB, 5.04 ± 2.29 mmol/L; plasma triglycerides, −D/−UVB, 19.3 ± 15.5 mmol/L; +D/−UVB, 27.3 ± 23.8 mmol/L; −D/+UVB, 20.1 ± 9.1 mmol/L; +D/+UVB, 28.0 ± 9.6 mmol/L). In contrast, the concentration of cholesterol in egg yolk was influenced by dietary vitamin D and UVB exposure, respectively, and there was an interaction between these two factors. Egg yolks from hens of the −D/−UVB group had 22% higher cholesterol concentrations than egg yolks from hens of the +D groups (Figure 5). UVB-treated hens from the −D group had lower concentrations of egg yolk cholesterol than the −D/−UVB group, but higher cholesterol levels than the +D groups (Figure 5). The cholesterol concentration in egg yolk was negatively correlated with the circulating plasma concentrations of $25(OH)D_3$ ($R^2 = 0.363$, $P < 0.001$). Triglyceride concentration of egg yolk was not influenced by dietary vitamin D or UVB exposure, respectively (−D/−UVB, 416 ± 32 mg/g; +D/−UVB, 422 ± 15 mg/g; −D/+UVB, 444 ± 30 mg/g; +D/+UVB, 430 ± 16 mg/g).

DISCUSSION

This study aimed to investigate whether UVB exposure of laying hens is capable of improving their vitamin D status and

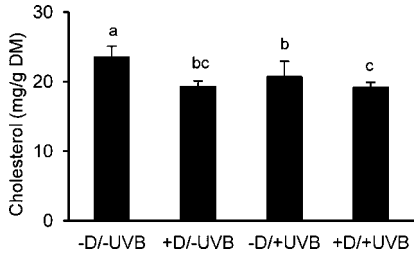

Figure 5. Final concentration of cholesterol in egg yolk of hens in response to dietary vitamin D (D) and UVB exposure (UVB). Values are the mean ± SD, $n = 10$. Data were analyzed by two-way ANOVA. Classification factors were vitamin D in the diet, UVB exposure, and the interaction between both factors. Effect of vitamin D, $P < 0.001$; UVB, $P < 0.01$; and vitamin D × UVB, $P < 0.01$. Individual means of the treatment groups were compared by Fisher's multiple-comparison test. Bars with no common letters are significantly different ($P < 0.05$). DM, dry matter.

hence the vitamin D content of their egg yolks. The results of this study demonstrate that short-term exposure of laying hens to moderate UVB levels improved egg production rate, egg shell stability, and egg shell thickness and also slightly increased 25(OH)D_3 concentrations in plasma of hens nonsupplemented with dietary vitamin D, but did not modulate the vitamin D_3 content of egg yolk. The present findings show clearly that dietary vitamin D but not the short-term exposure to moderate UVB light is a key factor in modulating the vitamin D content of eggs.

In this study, vitamin D-supplemented groups received diets with 3000 IU cholecalciferol/kg, which is the quantitative limit of vitamin D in chicken feed. The DM concentrations of vitamin D_3 and 25(OH)D_3 analyzed in the egg yolks of the vitamin D-supplemented groups ranged between 4.1 and 4.6 μg/100 g and between 1.2 and 1.4 μg/100 g, respectively, and showed levels similar to those reported previously. Takeuchi et al.[10] found 3.9 μg of cholecalciferol/100 g egg yolk, and Mattila et al.,[11] who analyzed two pooled samples of eggs from retail outlets, found cholecalciferol concentrations in egg yolk between 4.0 and 5.6 μg/100 g. Previously reported 25(OH)D contents in egg yolk ranged between 0.95 μg/100 g[12] and 0.98 μg/100 g.[6] It is remarkable that major amounts of vitamin D in egg yolk are present as unmetabolized cholecalciferol, not as 25(OH)D_3. In 1973, Edelstein et al.[13] found that there are two cholecalciferol-binding proteins in domestic fowls: one binds 25(OH)D_3, whereas the other binds mainly cholecalciferol. It is suggested that the selective mechanism incorporating cholecalciferol into yolk gives the chick embryo the opportunity to control its own 25(OH)D_3 supply through having to produce it itself from the cholecalciferol precursor.[14]

The failing effect of UVB exposure on the vitamin D status of the laying hens is in contrast to the results found in UVB-exposed cattle. In a current study, plasma concentrations of 25(OH)D in cows responded in strict correlation to the average surface area that was exposed to UVB, despite hair coverage.[15] Other findings show that Angus cattle that were on pasture from the time of calving until August had plasma concentration of 25(OH)D of 75 ng/mL, accompanied by 25(OH)D levels of 25 ng/g in muscle tissue,[16] whereas cattle housed in shelters had vitamin D concentrations in muscle tissue that ranged between 0.5 and 1.68 ng/g.[17,18]

There are two major factors that could explain the failing effect of UVB light on vitamin D concentration in egg yolk, although egg production rate and egg shell quality indicate the efficacy of UVB exposure to enhance endogenous vitamin D synthesis. These factors concern the UVB dosage and the position of the UVB lamps. The capacity of chicken skin to generate vitamin D from 7-dehydrocholesterol has long been known.[19] In 1994, Tian et al.[19] found that the leg skin of birds had 30 times greater concentrations of 7-dehydrocholesterol than that of the back. In that study UVB irradiation of chicken legs led to a 4-fold increase in the circulating plasma concentration of vitamin D_3. Under natural conditions birds frequently sun in open, unobstructed areas by lying to the side and spreading their feathers. It can be assumed that this behavior could possibly contribute to endogenous vitamin D synthesis. We therefore assume that the weak effect of UVB exposure on 25(OH)D_3 plasma concentration, vitamin D_3, and 25(OH)D_3 concentrations in egg yolk could be due to low UVB radiation levels near the legs because we installed the UVB lamps 50 cm above the hens' heads. A second factor that could be responsible for the weak effect of UVB exposure on vitamin D status in our study is the UVB dosage. In central Europe, a maximum UVB irradiance (approximately 150 μW/cm^2) can be observed at noon in early July.[20] Thus, an advanced study by use of UVB dosages close to conditions of natural sunlight in summer and UVB radiation that allows an exposure of all nonfeathered body parts of the chicken, including legs, will provide more evidence as to whether UVB exposure is capable of increasing vitamin D concentration in egg yolk.

Another interesting result of this study was the observed negative correlation ($r = -0.602$) between plasma concentrations of 25(OH)D_3 and cholesterol concentration in egg yolk. It has been known for a long time that most of the egg yolk cholesterol originates from liver and plasma, respectively,[21] although plasma cholesterol concentration does not relate to cholesterol levels in egg yolk.[22] According to these findings, analyzed changes of cholesterol concentration in egg yolk in our study were also not accompanied by corresponding changes of plasma cholesterol concentrations. Although nutrition, genetic, and pharmacological agents are capable of influencing cholesterol deposition in egg yolk,[23] there are only a few well-researched nutrition factors that show significant effects on egg yolk cholesterol. Among them, polyunsaturated fatty acids[24] and plant sterols[25] are capable of lowering egg yolk cholesterol concentrations. However, there is only a single trial in which egg cholesterol was determined in response to different levels of dietary vitamin D.[26] In that study egg cholesterol concentration followed no consistent pattern. Currently, we do not have an explanation for the interrelation between vitamin D status and cholesterol in egg yolk.

In conclusion, this study shows that treatment of vitamin D-deficient laying hens with UVB light for 1 h per day improved laying performance and egg shell quality, but was not capable of improving the vitamin D content in egg yolk. A point to be clarified is whether a UVB irradiation in the vicinity of leg skin is more efficient in improving vitamin D content of egg yolk than a UVB irradiation from the top.

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■ ABBREVIATIONS USED

$1,25(OH)_2D_3$, 1,25-dihydroxycholecalciferol; $25(OH)D_3$, 25-hydroxycholecalciferol; 25(OH)D-*d*, deuterated 25(OH)D; APCI, atmospheric pressure chemical ionization; DM, dry matter; HPLC, high-performance liquid chromatography; LC-MS, coupled liquid chromatography−mass spectrometry; MRM, multiple-reaction monitoring.

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