UVB Exposure of Farm Animals: Study on a Food-Based Strategy to Bridge the Gap between Current Vitamin D Intakes and Dietary Targets

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Abstract

Vitamin D deficiency is a global health problem. This study aimed to investigate the efficacy of ultraviolet (UV) B radiation for improving vitamin D₃ content of eggs and meat. In a two-factorial design hens that received diets with 0 (+D₃) or 3,000 IU (+UVB/D₃) vitamin D₃/kg were non-exposed (-UVB) or exposed to UVB radiation (+UVB) for 3 h daily over 4 weeks. Data show that UVB radiation was very effective in raising the vitamin D₃ content of egg yolk and meat. Egg yolk from +UVB/−D₃ hens had a higher vitamin D₃ content (17.5±7.2 µg/100 g dry matter (DM)) than those from the −UVB/+D₃ group (5.2±2.4 µg/100 g DM, p<0.01). Vitamin D₃ content in egg yolk of vitamin D₃-supplemented hens could be further increased by UVB radiation (32.4±10.9 µg/100 g DM). The content of 25-hydroxyvitamin D₃ (25(OH)D₃) in the egg yolk also increased in response to UVB, although less pronounced than vitamin D₂. Meat revealed about 4-fold higher vitamin D₃ contents in response to UVB than to dietary vitamin D₃ (p<0.001). In conclusion, exposure of hens to UVB is an efficient approach to provide consumers with vitamin D₃-enriched foods from animal sources.

Introduction

Vitamin D₃ deficiency is a global health problem that has considerable impact on health [1,2,3]. It is suggested that up to 50% of young adults suffer from vitamin D insufficiency worldwide [4]. Vitamin D₃ promotes calcium and phosphate absorption in the intestine, decreases the clearance of these minerals from the kidney and is needed for bone mineralization and bone growth [5,6]. In the last years, more attention has been paid to vitamin D₃ due to its multiple health benefits. More than 220 genes are identified that significantly changed in expression in response to vitamin D₃ [3], particularly those that are involved in cell proliferation, cell differentiation, and immune function [5,7,8], and vitamin D₃ deficiency is associated with several diseases such as cancer and autoimmune disorders [3,7,9]. Thus, the maintenance of an adequate vitamin D₃ status seems to provide a great preventive health potential. The main source (80–90%) of vitamin D₃ is the endogenous synthesis of vitamin D₂ in the skin by exposure to natural sunlight, whereas nutrition contributes to only 10–20% of the vitamin D₂ supply [6,10]. Failing outdoor activities, seasonal variations, air pollution, pigmented skin, and the use of sunscreens affect the efficacy of UVB radiation for cutaneous vitamin D₂ synthesis. Therefore, an increasing number of people depend on dietary sources of vitamin D₃ to prevent vitamin D₃ deficiency or inadequacy. With the exception of fatty fish species, such as salmon and mackerel, and fish liver oils [11], most natural foods contain very low amounts of vitamin D₃ and are not capable of improving vitamin D₃ status or fulfilling the recommendations for diet intake of vitamin D₃. Based on a report of the U.S. Institute of Medicine, vitamin D₃ is recommended in daily amounts of 15 µg for people younger than 71 years in the USA and Canada [12]. Recommendations for vitamin D₃ intake in different European countries range between 5 and 20 µg daily for adult men and women [13]. In most of the European countries, the recommended amounts of vitamin D₃ were not met by the intake of natural foods [14]. Therefore, food-based strategies need to be developed to improve vitamin D₃ status. In the United States and in Canada, a series of industrial produced foods were fortified with vitamin D [15]. In Europe, vitamin D fortification of food is highly regulated and critically discussed. A novel approach to enrich foods with specific nutrients is the “bio-addition”; thereby, foods are fortified through the addition of nutrients to animal feed during livestock farming production, or manipulation of post-harvest food processes. Eggs are widely and regularly consumed, and offer an interesting target for vitamin D₃ fortification. However, with respect to vitamin D, it is not allowed to fortify animal feed with vitamin D beyond a defined maximum. We therefore came up with the idea that UVB exposure of farm animals such as laying hens might become a promising option to further improve the vitamin D content of foods from animal sources.

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origin. In an initial experiment we could show that chickens whose upper part of their body was exposed to UVB did not produce vitamin D-enriched eggs [16]. Current analysis from our research group showed that most of the 7-dehydrocholesterol (7-DHC), the pre-cursor and limiting factor for vitamin D₃ synthesis, was located in the unfeathered skin of the chicken legs. Based on this finding, we hypothesized that an UVB exposure which ensured irradiation of the whole chicken body, including legs, should increase the vitamin D content of eggs and meat. In order to assess the effectiveness of a whole body irradiation of chickens in producing vitamin D-enriched eggs and meat, we analyzed the vitamin D content of eggs and meat in response to UVB treatment of chickens that were fed either a vitamin D₃-deficient diet or a diet that contained the maximum permissible amount of dietary vitamin D. Besides vitamin D₃ metabolites in plasma, eggs, and meat, the laying performance, and also egg shell quality and bone stability were analyzed. We further investigated the folate status of the animals to rule out pronounced side effects of the UVB treatment, since solar radiation is supposed to affect the folic acid levels [17,18,19].

Materials and Methods

Comparative Analysis of 7-DHC Concentrations in Different Skin Areas of Chickens

To obtain information about the amounts and distribution of cutaneous 7-DHC in chickens, we analyzed the 7-DHC concentrations in skin of comb, wattles, unfeathered and feathered legs, and wing of 8 vitamin D₃-adequately supplied Lohmann layers with an age of 21 weeks. Prior to skin sample preparation featherers were plucked and the skin was dissected free from underlying muscle and fat. Skin samples (approximately 2 × 2 cm) were then snap frozen in liquid nitrogen and stored at −80°C until 7-DHC analysis. Sample treatment and analysis of the 7-DHC concentration in skin is described below.

Animals and Treatment

The experiment was conducted with 36 Lohmann layers with an initial age of 27 weeks and an average body weight of 1777 g (±141 g). Before starting the experiment, hens were fed a standard diet containing 2,500 IU vitamin D₃/kg for 2 weeks. Then, the hens were randomly assigned into four groups of 9 hens each. The hens were individually housed in an environmentally controlled room at 16°C and light (30 lx) from 6:00 a.m. to 8:00 p.m. All hens were fed a diet that consisted of (g/kg diet) wheat (470), extracted soy bean meal (220), corn (100), barley (68.2), calcium carbonate (85), soybean oil (30), dicalcium phosphate (13), vitamin and mineral mix (10), sodium chloride (2) and DL methionine (1.8). Except vitamin D₃, vitamins and minerals were added according to the recommendations of the GfE [20]. From the 36 layers, 18 hens received a diet without any vitamin D₃ (0 IU vitamin D₃/kg; vitamin D₃-deficient diet, -D₃), the other 18 hens were fed a diet supplemented with 3,000 IU vitamin D₃ (Molekula, Gillingham, U.K.) per kg diet (vitamin D₃-adequate diet, +D₃). The diets were calculated on the basis of GfE recommendations for laying hens and contained 11.6 MJ/kg [20]. All diets were fed over a period of 4 weeks. Feed and water from nipple drinkers were available ad libitum during the whole experiment. The experimental procedure was performed according to the established guidelines for care and handling of laboratory animals and was approved by the council of Saxony-Anhalt, Germany (No. 42502-3-656 MLU). The hens were weighted once a week. Food intake, laying performance, egg weight and shell quality were monitored weekly.

UVB Treatment

Two groups of hens (-D₃/+UVB and +D₃/+UVB) were exposed to UVB for 3 h daily (from 8:30 to 8:30, from 11:00 to 12:00, from 14:00 to 15:00 and from 16:30 to 17:00). The 10 cm long, 23 W UVB lamps (Hobby UV Kompakt Desert 8% UVB, Dohse Aquaristik KG, Gelsdorf, Germany) with equipped heat protection (Dohse Aquaristik KG) were placed near the cage doors to ensure optimal UVB exposure of the hens’ legs. The lamps emitted UVB in ranges of 280 to 310 nm. The UVB radiation dosage at a distance of 20 cm was 76 μW/cm² (according to the manufacturer’s specification). This UVB irradiation intensity corresponds to that of natural sunlight during summer in the Middle Europe (50° latitude) [21]. An UVB opaque board was placed between the UVB-treated and the non-exposed groups to block incidental irradiation. The UVB lamps had no influence on the temperature inside the cages.

Sample Collection

Blood samples for analysis of vitamin D metabolites, minerals, and folate from each hen were taken at the beginning and at the end of the experiment. The blood was collected in heparinized tubes (Sarstedt, Numbrecht, Germany) and centrifuged at 1,100 g for 10 min at 4°C. Plasma samples were stored at −80°C pending analysis. To determine egg weight and shell quality, eggs from each hen were collected at the beginning and after week 1, week 2, week 3, and week 4 of the experiment. Egg yolk for analysis of vitamin D₃ and 25(OH)D₃ was collected from eggs of each hen at the beginning and at the end of the experiment. At the end of the experimental period, the hens were killed by decapitation. Tibialis longus muscle of each hen was removed for quantification of vitamin D₃ and 25(OH)D₃, tibiotarsus was excised to measure bone stability, and liver was removed for analysis of folate. All samples were stored at −80°C pending further analysis.

Analysis of 25(OH)D₃ and 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) in Plasma

The plasma concentration of 25(OH)D₃ was determined by coupled liquid chromatography-mass spectrometry (LC-MS/MS) according to Higashi et al. [22]. In brief, plasma samples were mixed with deuterated 25(OH)D₃, which was solved in acetonitrile, (Chemaphor Incorporation, Ottawa, Canada) as an internal standard and extracted with n-hexane. To the dried residue, 4-phenyl-1,2,4-triazolin-3,5-dione (solved in acetonitrile) was added for derivatization. Subsequently to the addition of ethanol and salivation in the mobile phase, the samples were analyzed by HPLC (Agilent 1100, Agilent Technologies, Waldbronn, Germany) equipped with Hypersil ODS-column 100 × 2 mm², 5 μm (Agilent Technologies), coupled to a MS system (API 2000, Applied Biosystems, Darmstadt, Germany). The detection limit for 25(OH)D₃ was 3.7 nmol/l. Between run precision data were calculated from 2 control sera. The coefficient of variation for 25(OH)D₃ was 3.0%.

The plasma concentration of 1,25(OH)₂D₃ was determined using a commercially available ELISA kit (IDS, Boldon, U.K.) according to the manufacturer’s protocol.

Analysis of Calcium and Inorganic Phosphate in Plasma

Calcium in plasma samples was quantified by a colorimetric assay. The test system was based on the formation of a calcium-o-kresolphalein complex ([Analytic Biotecnologies AG, Lichtenfels, Germany]). Prior to analysis, plasma was diluted 1:4 with 0.9% NaCl to avoid interferences with triglycerides in plasma.
The plasma concentration of inorganic phosphate was measured spectrophotometrically according to the manufacturer’s protocol (Analyticon Biotechnologies AG). The test system was based on the measurement of ammonium molybdate which forms a complex with inorganic phosphate.

Analysis of 7-DHC in Skin, and Vitamin D₃, 25(OH)D₃ in Egg Yolk and Meat

Vitamin D₃, 25(OH)D₃, and 7-DHC were determined by LC-MS/MS. In brief, samples were homogenized, deuterated internal standard (D₃-d₁₅, 25(OH)D₃-d₁₈, and ergosterol, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) was added and hydrolyzed under alkaline conditions and oxygen exclusion. Samples were extracted with n-hexane and hexane phase was washed with ultrapure water. The vitamin D metabolites were fractionated using HPLC (Agilent 1100 HPLC, Agilent Technologies) according to Mattila et al. [23]. Further analytical steps were in accordance to those described for 25(OH)D₃ in plasma. The detection limit was 0.17 ng/100 g for vitamin D₃, and 0.1 ng/100 g for 25(OH)D₃. The coefficient of variation for vitamin D₃ was 3.9%, and 4.6% for 25(OH)D₃.

Analysis of Egg Shell Thickness and Stability

Thickness of each egg shell was measured by use of a micrometer screw capable of 0.01 mm accuracy. Thickness of three fragments from the equatorial region of each egg shell was averaged. Prior to analysis, shell membranes were removed. The stability of egg shells were determined by an electronically controlled breaking strength tester (Messotechnik Gutsch, Naundorf, Germany). Values were expressed in Newton (N).

Analysis of Tibiotarsus Stability

Three-point bending tests were performed to determine the fracture loads. The specimens were tested using a Zwick Z1050 electro-mechanical testing machine (Zwick GmbH & Co KG, Ulm, Germany). The loading rate was set to 80 mm/min, the span (distance between the supports) to 80 mm and the radius of the cylindrical supports and the cylindrical loading blocks to 5 mm. The specimens were carried out at 23±2°C and a relative humidity of 50%.

Analysis of Folate in Plasma and Liver

Folate in plasma and liver samples was quantified using a microbiological test kit containing Lactobacillus rhamnosus coated microtiter plates according to the manufacturer’s protocol (R-Biopharm AG, Darmstadt, Germany). Prior to analysis, liver samples were homogenized and enzymatically hydrolyzed using pancreatin (R-Biopharm AG, Darmstadt Germany).

Statistical Analysis

Values are expressed as mean±SD. Statistical analyses were performed using SPSS 20 (IBM, Armonk, NY, USA). Two-way ANOVA was used to compare the effects of UVB irradiation (-UVB vs. +UVB), dietary vitamin D₃ (vitamin D₃-deficient diet vs. vitamin D₃-adequate diet), and their interaction. When two-way ANOVA revealed a significant interaction between UVB and vitamin D₃, a post-hoc comparison was performed. In case of variance homogeneity, means of the four groups were compared by Tukey’s test, or in case of variance heterogeneity by Games-Howell test. Significances of differences between basal and final means were tested by the paired t-test. Means were considered significantly different at p<0.05. Values under the detection limit are represented by randomly assigned values.

Results

Concentration of 7-DHC Varies Strongly in the Different Skin Areas of Hens

7-DHC is the limiting factor for vitamin D₃ synthesis. To figure out which part of the chicken skin contains most of the vitamin D₃ precursor molecule and should be inevitably exposed to UVB irradiation to increase vitamin D synthesis, we determined the concentrations of 7-DHC in 5 different skin samples of 6 laying hens by LC-MS/MS. Figure 1 shows large differences in 7-DHC contents between the chosen skin parts. The cutaneous area of the unfeathered legs contained the highest 7-DHC levels, which were on average 190-fold higher than that of the comb. The lowest 7-DHC concentrations were observed in the feathered parts of the skin such as wings and feathered legs. These findings prompted us to mount the UVB lamps in the experimental housing system in a lateral position to ensure an adequate UVB irradiation of skin in the leg area.

UVB Radiation and Dietary Vitamin D₃ did not Influence Food Intake and Body Weight

None of the hens showed behavioral peculiarities or symptoms of erythema in response to UVB radiation. Two-way ANOVA did not reveal main and interactive effects of UVB exposure and dietary vitamin D₃ on daily food intake (-D₃/−UVB group, 114.1±12.8 g; +D₃/−UVB group, 118.6±10.0 g; -D₃/+UVB group, 121.9±7.3 g; +D₃/+UVB group, 117.3±8.1 g; mean±SD) and body weight (-D₃/−UVB group, 1734±151 g; +D₃/−UVB group, 1797±187 g; -D₃/+UVB group, 1952±196 g; +D₃/+UVB group, 1810±97 g; mean±SD; Table S1).

Effects of UVB Exposure on Plasma Concentrations of 25(OH)D₃ and 1,25(OH)₂D₃ of hens on a Vitamin D₃-deficient and Vitamin D₃-adequate Diet

To examine the vitamin D status of hens in response to UVB radiation and dietary vitamin D₃, the plasma concentrations of 25(OH)D₃ and 1,25(OH)₂D₃ were analyzed. Figure 2A and 2B show that the plasma concentration of 25(OH)D₃ increased much more in response to UVB radiation and dietary vitamin D₃ than the plasma level of 1,25(OH)₂D₃. Two-way ANOVA data reveal a
strong interactive effect of UVB exposure and dietary vitamin D3 on the plasma concentration of 25(OH)D3 (p<0.001, Figure 2A, Table S1), and independent effects of UVB exposure (p<0.01) and dietary vitamin D3 (p<0.001) without treatment factor interaction on the circulating plasma level of 1,25(OH)2D3 (Figure 2B, Table S1). By comparison of the 25(OH)D3 plasma levels in response to the treatment factors it was noticeable that UVB treatment was capable of increasing the 25(OH)D3 plasma levels in the group of hens on a vitamin D3-deficient diet (p<0.001) but not in the hens that received the vitamin D3-adequate diet (Figure 2A). Supplementation of dietary vitamin D3 markedly increased the plasma level of 25(OH)D3 in the group which was non-exposed to UVB radiation but not in the group exposed to UVB radiation (p<0.001, Figure 2A). Figure 2B shows that hens on a vitamin D3-deficient diet that were non-exposed to UVB had the lowest plasma level of 1,25(OH)2D3 compared to the other groups. Two-way ANOVA data revealed that both treatment factors contributed to increase the 1,25(OH)2D3 plasma concentration (p<0.01, Table S1).

**Plasma Concentrations of Calcium and Inorganic Phosphate were not Affected by UVB Exposure and Dietary Vitamin D3**

Despite strong differences in vitamin D status, two-way ANOVA did not reveal any significant effects of UVB exposure or dietary vitamin D3 on plasma concentrations of calcium (+UVB/+D3 group, 6.79±1.66 nmol/l; -UVB/+D3 group, 8.11±1.65 nmol/l; +UVB/-D3 group, 7.50±1.96 nmol/l; +UVB/+D3 group, 8.22±1.52 nmol/l; mean ± SD) and inorganic phosphate (+UVB/-D3 group, 1.73±0.37 nmol/l; -UVB/+D3 group, 1.89±0.39 nmol/l; +UVB/-D3 group, 1.76±0.20 nmol/l; +UVB/+D3 group, 1.98±0.39 nmol/l; mean ± SD) (Table S1).

**Effects of UVB Exposure on Vitamin D3 and 25(OH)D3 in Egg Yolk of Hens on a Vitamin D3-deficient and Vitamin D3-adequate Diet**

Figures 3A and 3C show the changes (final - basal) and final contents of vitamin D3 in egg yolk in response to UVB exposure of hens on a vitamin D3-deficient and vitamin D3-adequate diet. Two-way ANOVA revealed significant effects of UVB radiation (p<0.001), dietary vitamin D3 (p<0.001) and an interaction between these two factors (p<0.05) on the vitamin D3 content of the egg yolk (Table S1). The findings demonstrate that both treatment factors were capable of increasing the vitamin D3 content in eggs, even though the UVB irradiation was more effective than the dietary vitamin D3 supplementation. Importantly, we found that dietary vitamin D3 could increase the vitamin D3 content of egg yolk stronger in the group exposed to UVB than in the group non-exposed to UVB radiation. Thus, by far the highest content of vitamin D3 in eggs could be obtained with a combination of UVB exposure and dietary vitamin D3. Figures 3B and 3D show the changes (final - basal) and final 25(OH)D3 contents in egg yolk in response to the vitamin D3 and UVB treatment. An interactive effect of vitamin D3 and UVB exposure on 25(OH)D3 changes (p<0.001) and the final 25(OH)D3 (p<0.001) content of egg yolk was confirmed by two-way ANOVA (Table S1). Main effects for UVB exposure (p<0.001) and dietary vitamin D3 (p<0.001) were also significant (Table S1). As expected, the 25(OH)D3 content of eggs decreased compared to baseline if hens on a vitamin D3-deficient diet were non-exposed to UVB radiation (Figure 3B, p<0.001). UVB irradiation and dietary vitamin D3 improved the 25(OH)D3 content in egg yolk (two-way ANOVA, p<0.001, Figure 3D, Table S1), although the UVB irradiation was marginally more effective than the dietary vitamin D3 in increasing the 25(OH)D3 content in egg yolk. Interestingly, dietary vitamin D3 particularly increased the 25(OH)D3 contents of egg yolk in UVB-non-exposed hens on the vitamin D3-deficient diet, and to a minor extent in UVB-exposed animals (Figure 3D). Nevertheless, as shown for the vitamin D3 content of eggs, the highest 25(OH)D3 contents in eggs resulted from a combination of UVB radiation and dietary vitamin

![Figure 2](image-url)
D3. In all treatment groups, egg white did not show any detectable contents of vitamin D2 (detection limit 0.17 μg/100 g) and 25(OH)D2 (0.1 μg/100 g) (data not shown).

Effects of UVB Exposure on Vitamin D3 and 25(OH)D3 in Fibularis Longus Muscle of Hens on a Vitamin D3-deficient and Vitamin D3-adequate Diet

Irrespective of the vitamin D3 in the diet, hens non-exposed to UVB radiation had no detectable vitamin D3 contents in their muscles. Supplementation with dietary vitamin D3 and also UVB exposure slightly increased the muscle contents of 25(OH)D3, whereby the UVB-exposed groups reached higher contents in their muscles than the group fed the vitamin D3-adequate diet (p<0.05).

Effect of UVB Exposure on Laying Performance, Egg Weight and Egg Shell Quality of Hens on a Vitamin D3-deficient and Vitamin D3-adequate Diet

Mean egg production rate (number of eggs per hen and week) was not significantly influenced by the treatment factors, although hens from the -UVB/-D3 group showed a slight drop in egg production within the last experimental week compared to the other group (-UVB/+D3 group, 6.0±1.6 eggs/week; -UVB/+D3 group, 7.8±0.8 eggs/week; +UVB/-D3 group, 6.9±0.3 eggs/week; +UVB/+D3 group, 7.8±0.6 eggs/week) (Table S1). Two-way ANOVA data show that the mean egg weights at defined times within the 4-week period of the experiment were not significantly influenced by UVB exposure and dietary vitamin D3.
Effect of UVB Exposure on Tibiotarsus Stability and Folate Status of Hens on a Vitamin D3-deficient and Vitamin D3-adequate Diet

Hens non-exposed to UVB radiation that received the vitamin D3-deficient diet revealed a lower mechanical stability of tibiotarsus than hens from the other groups (Figure 6, \( p < 0.01 \)). Two-way ANOVA data revealed an interactive effect of dietary vitamin D3 and UVB exposure on tibiotarsus stability (\( p < 0.01 \), Table S1). In order to investigate possible effects of UVB radiation on folate status, the concentrations of folate in plasma and liver of the hens were determined. Neither the concentration of folate in plasma (-UVB/-D3 group, 49.8±18.6 nmol/l; -UVB/+D3 group, 45.3±7.4 nmol/l; +UVB/-D3 group, 56.8±21.5 nmol/l; +UVB/+D3 group, 41.5±10.4 nmol/l; mean ± SD), nor that in liver (-UVB/-D3 group, 10.5±1.1 µg/g; +UVB/+D3 group, 9.8±2.5 µg/g; +UVB/-D3 group, 10.1±1.7 µg/g; +UVB/+D3 group, 10.5±1.4 µg/g; mean ± SD) was influenced by dietary vitamin D3 and UVB exposure, respectively. This was confirmed by the two-way ANOVA data (Table S1).

Discussion

Results from the current study reveal UVB exposure of hens as an appropriate way and a highly effective approach to increase the vitamin D content mainly in eggs and also in meat. Data further show that an exposure to UVB is capable of raising the vitamin D content in egg yolk and muscle much stronger than feeding hens with diets that contain maximum permissible dosages of vitamin D3. UVB radiation was still effective in increasing the vitamin D content of eggs and meat even in the group that received 3,000 IU vitamin D3/kg feed. Previous studies that aimed to increase the vitamin D3 content in eggs are not feasible. Our findings confirmed by the two-way ANOVA data (Table S1).

Figure 4. UVB exposure increases vitamin D3 content in skeletal muscle. (A) Data in the top panel represent mean ± SD (n=9) of vitamin D3 content in fibularis longus muscle of non-treated (-UVB) or UVB-treated (+UVB) hens that were fed either a vitamin D3-deficient (-D3) or vitamin D3-adequate diet (+D3), respectively. Values below the detection limit of 0.17 µg/100g for vitamin D3 are represented by randomly assigned values (#). The detection limit is marked by a dotted line (---). UVB exposure, but not dietary vitamin D3 was capable of increasing the vitamin D3 content in muscle to values above the detection limit. (B) Data in the bottom panel represent mean ± SD (n=9) of 25(OH)D3 content in fibularis longus muscle of non-treated (-UVB) or UVB-treated (+UVB) hens that were fed either a vitamin D3-deficient (-D3) or vitamin D3-adequate diet (+D3), respectively. Values below the detection limit of 0.1 µg/100g for 25(OH)D3 are represented by randomly assigned values (#). The detection limit is marked by a dotted line (---). Individual means of the groups were compared by post-hoc test. Asterisks within one diet group (-D3 and +D3) indicate a significant difference between -UVB and +UVB groups, \( p < 0.05 \).

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respectively (Figure 5A, Table S1). Data demonstrate higher egg weights at the end of the 4-week experiment compared to baseline in the groups that were UVB exposed and/or received vitamin D3 with their diets (\( p < 0.05 \), paired t-test), but not in the -UVB/-D3 group (Figure 5A).

Figures 5B and 5C show the egg shell thickness and the egg shell stability in response to UVB radiation and dietary vitamin D3 during the 4-week experiment. From the beginning of the second experimental week, egg shell thickness was significantly influenced by dietary vitamin D3 (\( p < 0.05 \), two-way ANOVA, Figure 5B), and there was a tendency of an interaction effect on egg shell thickness at the end of the experiment (\( p = 0.053 \), two-way ANOVA). Although the -UVB/-D3 group showed a trend toward lower egg shell thickness after 3 and 4 weeks of the experiment, paired t-test data did not reveal differences compared to baseline. During the experimental period an increasing influence of UVB and dietary vitamin D3 on egg shell stability became evident. At the end of the experiment, two-way ANOVA analysis revealed significant main effects of UVB radiation (\( p < 0.01 \)) and dietary vitamin D3 (\( p < 0.05 \)) and a significant interaction between these both factors (\( p < 0.05 \)) on egg shell stability (Figure 5C). Compared to baseline, the stability of eggs from the -UVB/-D3 group was constantly dropping during the experimental period, and reached a minimum after 4 weeks which was significantly lower compared to baseline (\( p < 0.05 \), paired t-test, Figure 5C).
of 7 g yolk dry matter, an egg from an UVB exposed hen on a vitamin D₃-adequate diet would provide on average 2.5 μg vitamin D (vitamin D₃ + 25(OH)D₃) compared to eggs from non-exposed hens on the same diet which contained 0.55 μg. Vitamin D₃ analysis reveals that meat from the +D₃/+UVB group contained 0.7 μg vitamin D/100 g compared to meat of the +D₃/−UVB group that contained 0.2 μg/100 g.

In 2008, Ko et al. already established UVB radiation as a method to increase the vitamin D₂ content in sliced shiitake and white button mushrooms [27]. In that study, an UVB radiation
dose of 75 kJ/m² increased the vitamin D₂ contents in gill of shiitake mushrooms from less than 500 µg/100 g in the non-exposed mushrooms up to 6,000 µg/100 g in the radiated mushrooms. Sliced button mushrooms exposed to UVB doses of 30 kJ/m² revealed a vitamin D₂ content of 3,500 µg/100 g. Although irradiation of mushrooms seems to be highly efficient in vitamin D₂ fortification, it should be taken into consideration that the final vitamin D contents per gram food were extremely high and probably of hazardous nature. In contrast to mushrooms, the efficiency of vitamin D₃ synthesis in the skin of animals is not only influenced by UV intensity but also by skin pigmentation and the thickness of hair coat, feathers or horns. Scales. Currently, there are only few published data that investigated the effectiveness of solar and UV irradiation in raising the levels of vitamin D₃ in plasma, egg yolk and muscle. 25(OH)D₃ is primarily synthesized in liver by 25-hydroxylation of vitamin D₃ from endogenous synthesis or diet. The hepatic 25-hydroxylation is not strictly feedback regulated and therefore mainly reflects vitamin D₃ status [32]. This relationship was confirmed by the observation that the concentrations of 25(OH)D₃ in plasma, egg yolk and muscle increased significantly with dietary vitamin D₃ and also UVB exposure. Domestic fowl synthesizes two vitamin D₃-binding proteins, one that binds 25(OH)D₃ and the other which mainly binds vitamin D₃ [19]. It is suggested that the selective mechanism that incorporates vitamin D₃ into yolk gives the chick embryo the opportunity to control its own 25(OH)D₃ supply [33]. UVB irradiation of farm animals seems to provide a safe approach to increase vitamin D₃ without running the risk of vitamin D₃ overdose. In the case of intense UV irradiation or if animals are exposed to excessive or prolonged exposure to sun, previtamin D₃ and vitamin D₃ photoisomerizes to biologically inactive tachysterol and lumisterol, which are desquamated with keratinocytes during normal skin turnover [6,33,34]. Health and performance data further indicate no symptoms of erythema, behavioral disorders or an impaired folate status in consequence of the applied UV treatment. The folate concentrations in plasma and liver of the hens were analyzed since UV irradiation is known to be capable of degrading folate in human blood and skin [35,36]. UVA radiation (315–400 nm) is suggested to be mainly responsible for this effect because it has a greater dermal penetration depth, and can degrade the biological form of folate, 5-methyltetrahydrofolate (5MTHF), in dermal circulation by generation of reactive oxygen species [37,38,39,40]. Other mechanisms such as the direct degradation of folate in the blood by UVB may also contribute to impact folate status [35,37]. In contrast, UVB radiation (280–315 nm) is unable to penetrate into the dermal circulation and has therefore presumably a lower potential to impact blood levels of folate [35,38,39]. Plasma and liver folate data of the current experiment confirm no adverse effect of UVB radiation on folate status, although it should be considered that the period of UVB exposure was relatively short.

This study further reveals that UVB irradiation is capable of optimizing laying performance, egg shell quality, and bone stability in hens that received no vitamin D₃ with their diet. Although hens from the +D₃/+UVB group had significantly higher plasma levels of 25(OH)D₃ and 1,25(OH)₂D₃ then hens from the +D₃/-UVB group, laying performance, egg weight, egg shell thickness, and egg shell stability could not be further improved by the additional treatment with UVB radiation. This is in accordance with previous data that did not show any additional effect of UV radiation on laying performance and egg shell quality in breeders supplemented with sufficient amount of vitamin D₃ [41].

Conclusions

In conclusion, the current study shows that UVB exposure of chickens that ensures irradiation of the whole body, including legs, is highly effective in increasing the vitamin D concentration in eggs, and also meat. We therefore consider UVB treatment of farmed animals as an effective and novel approach for “bio-addition” of foods with vitamin D. Considering the option that free-ranged chickens are still exposed to natural sun light, free-range husbandry could become a cheap alternative to the artificial UVB irradiation to produce vitamin D₃ fortified eggs.
Supporting Information

Table S1  Two-way analysis of variance table for the chicken and egg data.

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Author Contributions

Conceived and designed the experiments: GIS. Performed the experiments: AS, JK, FH, TT. Contributed reagents/materials/analysis tools: AK, TT. Wrote the paper: AS, JK, GIS.