Feasibility of artificial light regimes to increase the vitamin D content in indoor-laid eggs

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ABSTRACT Vitamin D insufficiency is prevalent worldwide. Recently, we showed that exposure of laying hens to sunlight or artificial ultraviolet B (UVB) light is an efficient strategy to increase the vitamin D content in eggs. In the current study, using 2 different chicken genotypes and stocking densities, we addressed the question of whether different UVB-emitting regimes work under real indoor housing conditions in a floor system or in furnished cages. Here, we found a 3.7-fold increase in the egg vitamin D content in Lohmann Selected Leghorn hens and a 4.2-fold increase in Lohmann Brown hens after UVB exposure for 6 h/d. The data further reveal that UVB exposure under high stocking density is equally effective compared to that at low stocking density. The different light regimes were not associated with changes in the behavior of these animals. To conclude, artificial UVB-emitting light regimes are a practical strategy to increase the vitamin D content in indoor-laid eggs.

Key words: laying hen, UVB, vitamin D, bioaddition, egg

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INTRODUCTION

Data from population-based studies indicate that vitamin D insufficiency is prevalent in many parts of the world (Hilger et al., 2014). As seasonal and local variations in UVB light intensity limit the endogenous synthesis of vitamin D_3 and foods naturally rich in vitamin D are scarce, strategies to improve the vitamin D supply are becoming increasingly important. In contrast to supplements, the fortification of natural foods with vitamin D can cover the vitamin D supply of a wide population. On the other hand, the vitamin D fortification of common foods is a great challenge, particularly, if food matrices are inhomogeneous. A promising approach to increase the vitamin D content of natural food is through bioaddition, whereby food staples are produced in a way to obtain added value. Recently, our group was able to demonstrate that free range farming and exposure of hens to artificial ultraviolet B (**UVB**) light are efficient strategies for increasing the vitamin D content in egg yolk (Kühn et al., 2014). Under controlled and optimized experimental conditions, the vitamin D content of eggs could be increased 4- to 5-fold if hens were exposed to UVB light (Schutkowski et al., 2013; Kühn et al., 2015). We noted that this type of fortification strategy is safe for consumers because the time-dependent increase of the egg vitamin D content is nonlinear, and a maximal attainable vitamin D contents exists for the eggs (Kühn et al., 2015).

For practical implementation of artificial UVB light exposure as a novel approach to increase the vitamin D content of indoor-laid eggs, additional aspects have to be considered: (1) the novel vitamin D fortification approach must work under real housing conditions, while considering stocking density; (2) commonly used chicken genotypes must respond to UVB light exposure in such a way that their eggs become significantly enriched in vitamin D; and (3) animal welfare cannot be negatively affected by using artificial UVB-emitting lamps in the housing system. To address these aspects, we compared different light regimes and their capacity to increase the vitamin D content in eggs in 2 commonly used genotypes of laying hens (Lohmann Selected Leghorn (**LSL**) and Lohmann Brown (**LB**)) that were kept in a floor system. The animals' behavior and welfare were assessed by evaluating habitat preferences and feather damage as an indicator of feather pecking. In a second study, we investigated the impact of stocking density in a furnished cage system to improve the vitamin D content of the eggs by UVB light exposure.

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MATERIALS AND METHODS

Animals and Treatments

Two studies were conducted to elucidate the feasibility of artificial light regimes to increase the vitamin D content in eggs laid indoors. The experimental procedures were approved by the council of Saxony-Anhalt, Germany (approval No. 42502–3-765MLU).

The first study aimed to elucidate the effect of 3 different light regimes on the vitamin D content of eggs from 2 commercial genotypes of laying hens. To this end, 48 LSL and 48 LB laying hens with an initial age of 26 wk were randomly allocated to 4 groups, with each group including 12 LSL and 12 LB laying hens. Each group of 24 hens was housed in a floor system in pens with an area of 8.75 m^2, which corresponded to an average stocking density of 3 birds per m^2 in each pen. Prior to starting the experiment, all hens were kept under UVB-free conditions for at least 5 wk. The actual experimental period lasted 6 wk. According to a typical floor system, the pens were equipped with perches and areas for litter, nesting, manure, and food and with automatic feeders and nipple drinkers (Supplementary Figure S1). Each pen was equipped with two 120 cm long fluorescent tubes: one was installed beneath the perches and one in close proximity to the automatic feeders. The tubes were placed close to the ground to ensure irradiation of the legs, because this part of the body has been shown to contain extremely high concentrations of 7-dehydrocholesterol, which functions as precursor of vitamin D (Schutkowski et al., 2013). One pen was provided with 2 full-spectrum daylight lamps with a UVB intensity of 25 $\mu W/cm^2$ at a distance of 20 cm. Hens in this pen were exposed to the full-spectrum daylight for 6 h/d. Two pens were provided with UVB-emitting lamps (2 per pen) that had a UVB intensity of 49 $\mu W/cm^2$ at a distance of 20 cm. Hens were exposed to the UVB light for either 3 or 6 h/d. Hens in the fourth pen were not exposed to UVB light during the entire experiment and served as controls. The lamps were equipped with a light transmitting coverage to avoid thermal damage to the animals. The pens were shielded to avoid an unintended irradiation of hens from other pens. All pens were equipped with non-UVB-emitting lamps to provide a 15-h-light and 9-h-dark cycle. The daylight and UVB-emitting lamps were switched on only in the light cycle period. The interval of UVB or daylight exposure was 1 h of lighting followed by 1 h of non-UVB light exposure in the groups that were exposed for 6 h/d or 1-h lighting followed by 2 h of non-UVB light exposure in the group that was UVB-exposed for 3 h/d to ensure an equal UVB exposure throughout the day in all exposed groups. The hens were fed a commercial layer diet (Deuka, Könnern, Germany), with a vitamin D content of 2,500 IU/kg. All birds had free access to food and water.

For analytical purposes, the eggs were collected at baseline and after 3 and 6 wk of the experimental treatment. The eggs were weighed, and the yolk was separated, freeze-dried, and stored at –20°C until vitamin D analysis. At baseline and after 3 and 6 wk of the study, all hens were weighed, and the conditions of the feathers and skin were assessed. Additionally, all pens were video recorded during the first, third, and the last week of the experiment in order to assess habitat preferences in response to the light regime. To record the intraindividual day-to-day variation of the egg content of vitamin D, eggs from 2 birds of each genotype were analyzed daily over a period of 10 d.

The second study aimed to assess the influence of the stocking density on the vitamin D content of eggs after treatment with UVB light. The study was conducted on a commercial egg-producing farm. To this end, 420 LSL laying hens with an initial age of 28 wk were randomly allocated to 9 furnished cages, each with an area of 4.88 m^2. Six cages had a high stocking density of 12.3 hens/m^2 and contained 60 hens each, and 3 cages had a stocking density of 4.1 hens/m^2 and contained 20 hens per cage. The hens were housed in furnished cages that consisted of nesting areas, perches, feeders, and nipple drinkers. Cages were equipped with one 120 cm long UVB lamp (intensity of 49 $\mu W/cm^2$ at a distance of 20 cm) that was switched on for 6 h/d. The lamps were placed in the lower part of the pens to ensure irradiation of the legs. The stocking density was either 60 hens per cage or 20 hens per cage. A third group with a stocking density of 60 hens per cage was not exposed to UVB light during the entire experiment and served as a control. To avoid cage effects, each group consisted of 3 cages. Hens were fed a commercial layer diet (SAL, Sausedlitz, Germany) with a vitamin D content of 2,475 IU/kg. After an initiation period under UVB free conditions, the study was conducted for 6 wk. Egg sampling was performed at baseline and after 3 and 6 wk of the experiment. Eggs used for vitamin D analysis were weighed, and the yolk was separated, freeze-dried, and stored at –20°C until vitamin D analysis.

Analysis of Vitamin D Content in Egg Yolk

Freeze-dried yolk samples were treated as described elsewhere (Schutkowski et al., 2013) and were analyzed regarding their vitamin D_3 and 25-hydroxyvitamin D_3 ($25(OH)D_3$) contents by high-performance liquid chromatography-tandem mass spectrometry (**HPLC-MS/MS**) (Mattila et al., 1995; Higashi et al., 2008). In brief, prior to sample saponification under oxygen free conditions, deuterated vitamin D_3 (Sigma-Aldrich, Taufkirchen, Germany) and deuterated $25(OH)D_3$ (Chemaphor Chemical Services, Ottawa, Canada) were added as internal standards. Egg yolk samples were extracted with *n*-hexane, washed with ultrapure water, and evaporated under vacuum. The dried residues were dissolved in *n*-hexane/isopropanol (99/1, v/v) and further purified by use of normal-phase HPLC

(1100 Series, Agilent Technologies, Waldbronn, Germany) as described elsewhere (Mattila et al., 1995). Vitamin D_3 and 25(OH)D_3 and their corresponding internal standards were collected on the basis of their specific retention times. Both fractions were derivatized with 4-phenyl-1,2,4-triazoline-3,5-dione (**PTAD**). Yolk samples were injected into the HPLC-MS/MS (1200 Series, Agilent Technologies; QTRAP 5500, Sciex) which was equipped with a Hypersil ODS C18 column (5 μm, 2.0 × 150 mm^2, VDS Optilab, Berlin, Germany). The mobile phases consisted of (A) 50% aqueous acetonitrile, 5 mM ammonium formate, and 21 mM formic acid and (B) 100% acetonitrile and a flow rate of 576 μl/min with the following gradient: 0.00 min, 15.0% B; 3.10 min, 15.0% B; 4.00 min, 16.5% B; 5.00 min, 35.0% B; 8.00 min, 60.0% B; 18.0 min, 76.5% B; 20.0 min, 100% B; 23.0 min, 100% B; 24.0 min, 5.00% B; 25.0 min, 15.0% B; and 30.0 min, 15.0% B.

Ionization for mass spectrometric analyses was induced by positive electrospray ionization. Data were recorded in the multiple-reaction monitoring mode with the depicted ion transitions: vitamin D_3 560 > 298, deuterated vitamin D_3 563 > 301, 25(OH)D_3 576 > 298, deuterated 25(OH)D_3 582 > 298.

Assessment of Feather Damage

All birds in the first study were marked with leg rings and were individually scored for feather damage and skin injuries at baseline and after 3 and 6 wk of treatment with the different light regimes (Mahboub et al., 2004). The outcome of this recording was a dataset with several ordinal-scaled, animal-based, categorical characteristics. The scoring method comprised the assessment of feather and skin damages on 13 regions, including the cranial region (head and neck), dorsal region (back and rump), caudal region (tail and belly), lateral region (wing primaries, wing coverts, and legs), and ventral region (breast). Each of the 13 body areas was assigned to a score from 0 (no damage) to 6 (worst damage). A detailed description of the applied methods is given by Mielenz et al. (2010). As a consequence of the heterogeneous distribution of the body scores, the multinomial traits were transformed into binary traits by defining a threshold body integrity score. Accordingly, scores of 3 or below were defined as "mild alterations," and scores of 4 and higher as "more severe alterations."

Analysis of Habitat Preferences by Video Recording

In the first study, each pen was equipped with a video camera (Day & Night Digital Colour Camera, Monacor International, Bremen, Germany) that recorded the number of birds in the respective areas of the barn. Videos were recorded in the first and sixth week of the study. Possible changes in habitat preferences were assessed by an interval time sampling method. For that purpose, the mean number of laying hens staying in a defined area was counted every 10 min during the 15 h of lighting. The areas within a pen were classified according to the intensity of the UVB exposure predominant in the area (Supplementary Figure S1). The light-facing side of the feeders was an area of high UVB exposure; the shady side of the feeders and the perches were regions of a medium UVB exposure. The litter area, nests, and the area above the UVB lamp were free of UVB irradiation.

Statistical Analysis

Statistical analysis of vitamin D metabolites and egg parameters was performed using the SAS 9.4 software package (SAS Institute Inc., Cary, NC). The MIXED procedure was used for the egg and vitamin D traits. Least squares means (**LSM**) were estimated, and the differences between the LSM were tested for significance. Means were considered to be significantly different at $P < 0.05$. One of the following 2 models was used:

$$\begin{aligned} y_{ijkl} &= \mu + genotype_i + group_j + time_k \\ &\quad + (genotype \times group)_{ij} + (genotype \times time)_{ik} \\ &\quad + (group \times time)_{jk} + (genotype \times group \times time)_{ijk} \\ &\quad + e_{ijkl} \, (M1) \end{aligned}$$

$$\begin{aligned} y_{jkl} &= \mu + group_j + time_k + (group \times time)_{jk} \\ &\quad + e_{jk_l} \, (\mathrm{M2}) \end{aligned}$$

Model M1 was used to analyze the genotype, group, and time effects, including all 2-way and 3-way interactions for the first study. Model M2 was used to analyze the traits within the genotypes separately for the first study and the traits for the second study, with group and time as the main effects and group × time as the interaction.

The statistical analysis of the habitat preferences was performed using SAS 9.4 (SAS Institute Inc.). In the first step, the proportion of hens per group, genotype, and week for each area during times with or without UVB exposure was calculated. The procedure MIXED was used to estimate the LSM for the proportion of hens, and the differences in the LSM were tested for significance. Data were considered significantly different at $P < 0.05$. The studentized residuals of the linear model were tested for normality (procedure Univariate). The linear model included the fixed effects group, week, the hours with UVB lamps on or off, the area, and the interaction between these fixed effects. The repeated observations (between the weeks) were modeled using correlated residual effects. Different variance-covariance structures were tested. The best

structures were selected by the corrected Akaike information criteria (Akaike, 1974).

The statistical evaluation of feather damage and skin injury scoring was carried out within each genotype separately to get more comparable results due to corporal differences in the layer strains. An initial descriptive statistic was executed with the MEANS procedure to give a brief overview on the location and dispersion parameters and assess general model factors of influence. Furthermore, the multinomial data were transformed into a binomial trait by defining a threshold value (≤ 3) and were analyzed with a threshold model commonly used to evaluate repeated ordered categorical data from individual animals. Regarding this, we applied a generalized linear mixed model that included the animal as a random effect by using the SAS GLIMMIX procedure. The treatment or the date of the body scoring was included as a fixed effect in each genotype in the statistical model. For the test of significance, the odds ratios of the differences of the least square means were calculated. The visualization of the estimated probabilities was carried out for the different treatments, where the significance of the significant differences is notable.

RESULTS

Study 1–Efficacy of the Different Light Regimes in 2 Genotypes of Laying Hens to Increase the Vitamin D Content of Eggs

At first, the data were analyzed for significant differences between both genotypes. Afterwards, the data were analyzed separately for each genotype. Supplementary Table S1 summarizes the *P*-values for the main effects when comparing both genotypes.

As depicted, significant differences were revealed between both genotypes for vitamin D_3, 25(OH)D_3, and total vitamin D (sum of vitamin D_3 and 25(OH)D_3) in egg yolk. The vitamin D_3 content in egg yolk was higher in the LSL laying hens, whereas the LB laying hens had higher 25(OH)D_3 contents in the egg yolk. As the vitamin D_3 content in the egg yolk is much higher compared to the 25(OH)D_3 level, the LSL hens had the highest total vitamin D content in the egg yolk.

Figures 1A and 1B show that all UVB-emitting regimes were able to increase the vitamin D_3 content in the yolk of eggs from the LSL and LB hens. The exposure with UVB lamps for 6 h/d (Figure 1A and B) was the most efficient light regime for vitamin D_3 enrichment of eggs in both genotypes of laying hens. No difference was found between the UVB light exposure for 3 h and the full-spectrum light exposure for 6 h in increasing the vitamin D_3 content in egg yolk after 6 wk. The maximum increase in vitamin D_3 content of eggs was reached after 3 wk of intervention. After that, no further increase in vitamin D_3 was observed. In the groups exposed to UVB light for 6 h, a drop in the vitamin D_3 content of eggs was even observed from week 3 to week 6 (Figures 1A and 1B). Figures 1C and 1D demonstrate that the 25(OH)D_3 content in the egg yolk declined from baseline to week 3 and 6 in the control groups of the LSL and LB hens. Eggs from groups exposed to UVB-emitting lamps had higher 25(OH)D_3 contents than the nonexposed control groups. No difference in the 25(OH)D_3 contents of eggs was observed among the 3 treatment groups (Figures 1C and 1D). Figures 1E and 1F show the total vitamin D content, defined as the sum of vitamin D_3 and 25(OH)D_3, in egg yolk. As the vitamin D_3 content in egg yolk was largely higher than the content of 25(OH)D_3, the effects of the light regimes on the total vitamin D in eggs were comparable to those found for vitamin D_3. The exposure to UVB light for 6 h was the most effective strategy to increase vitamin D in eggs. In the LSL genotype, the effect of UVB light exposure for 3 h and the effect of full-spectrum light exposure were comparable (Figure 1E). In the LB genotype, the UVB light exposure for 3 h was more effective in increasing the total vitamin D in egg yolk than the full-spectrum light exposure for 6 h after 3 wk of intervention (Figure 1F).

To assess the day-to-day variation of the vitamin D_3 and 25(OH)D_3 content, eggs from 2 individual hens of both genotypes were collected over a period of 10 D (Figures 2A and 2B). The intraindividual variation ranged between 10 and 19%. For the vitamin D_3 content in egg yolk, this variation was higher in LSL compared to LB hens, but an inverse relationship was observed for the 25(OH)D_3 content.

Study 1–Influence of the Different Light Regimes in two Genotypes of Laying Hens on Egg Weight and Egg Shell Thickness

As depicted in Supplementary Figure S2, LSL hens from the control group showed a marginally higher egg and egg yolk weight compared to the other groups. Egg shell thickness was not affected by the UVB light exposure. No influence of the UVB light exposure was revealed for either egg weight, egg yolk weight, or egg shell thickness in hens from the LB genotype.

Study 1–Feather and skin conditions of the laying hens

To evaluate the impact of the light regimes on feather and skin damages, hens were scored at baseline, as well as 3 and 6 wk after starting the intervention. As depicted in Figure 3, the body integrity of the LSL and LB hens that were exposed to UVB light was not different from hens from the control group for the majority of the body regions. The probabilities of the scorings in the LB are generally observably higher than in the LSL as a consequence of differences in the exterior appearance of the body regions and the same scoring methodology for each of them, depending on the ratable

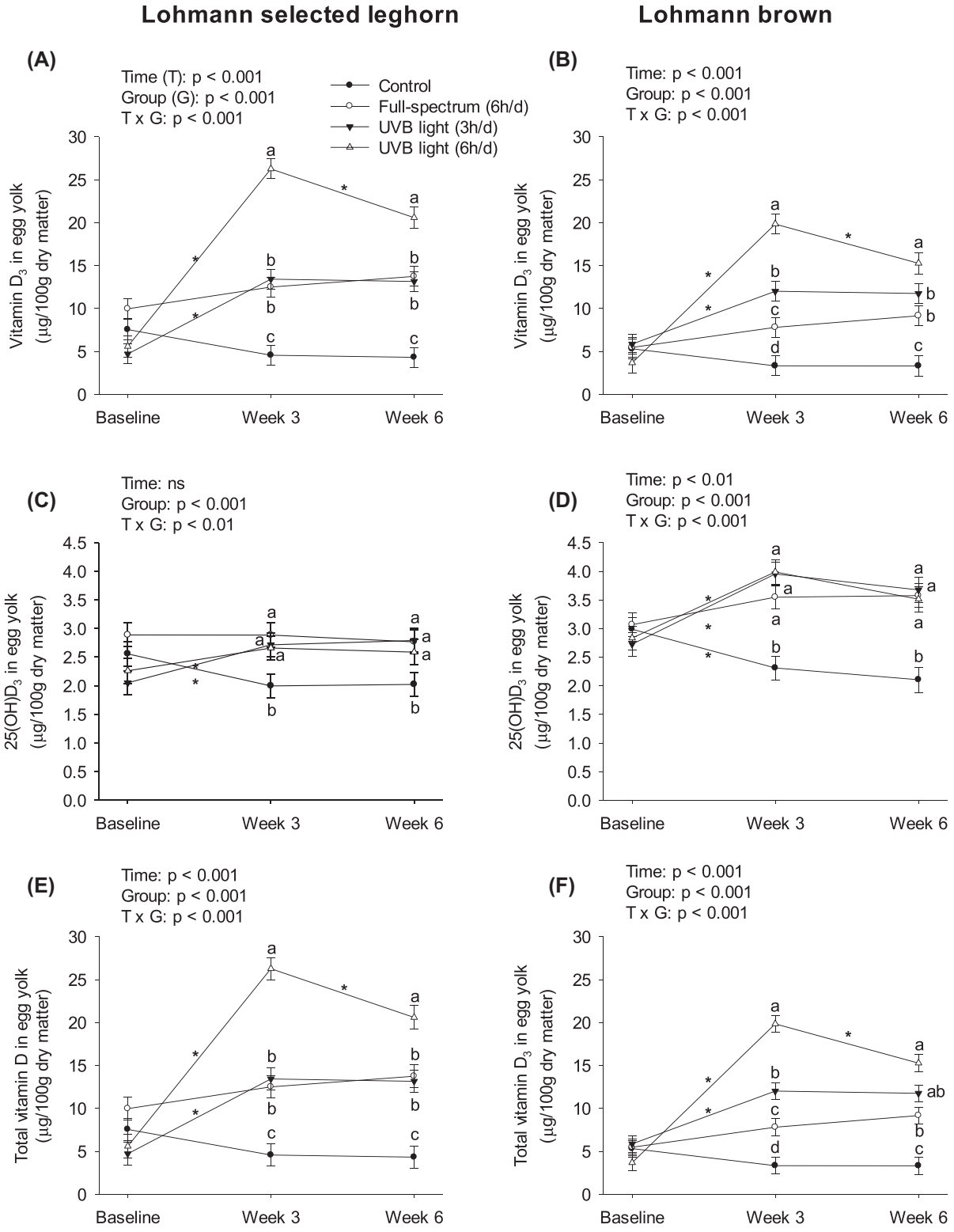

Figure 1. Concentrations of vitamin D metabolites in egg yolk of hens exposed to different UVB light regimes. (A) Vitamin D_3 concentration in egg yolk from Lohmann Selected Leghorn (LSL) hens, (B) vitamin D_3 concentration in egg yolk from Lohmann Brown (LB) hens, (C) 25(OH)D3 concentration in egg yolk from LSL hens, (D) 25(OH)D_3 concentration in egg yolk from LB hens, (E) total vitamin D concentration in egg yolk from LSL hens, (F) total vitamin D concentration in egg yolk from LB hens. Data are presented as least squares means (LSM) ± standard error (SE) of LSM. When statistical analysis revealed a significant interaction between group x time, the individual means of the groups were compared by post hoc test. Different capital letters indicate differences among the 4 groups at one time point. Asterisks indicate differences between 2 time points within one group.

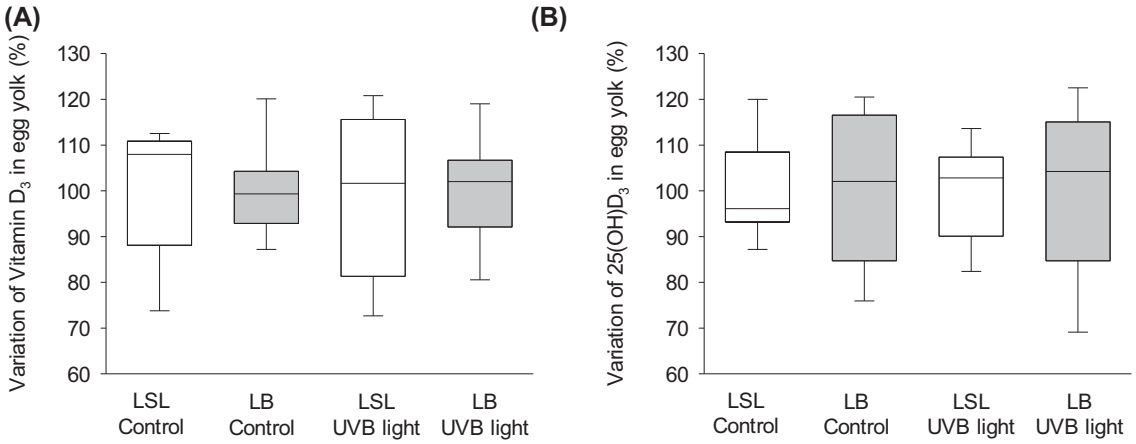

Figure 2. Variations of the concentrations of vitamin D metabolites in egg yolk of hens that were exposed to different UVB light regimes. (A) Variation of vitamin D_3 in egg yolk, (B) variation of 25(OH)D_3 in egg yolk. Data are presented as least squares means (LSM) ± standard deviation.

or countable traits. Thus, the light regimes did not have detrimental effects on the feathers. Supplementary Figure S3 shows that, irrespective of the treatment and the genotype, the score values increased from baseline to week 6 of the study.

Study 1–Functional Area Preferences of the Laying Hens

Functional area preferences of the hens in response to the light regimes were assessed by recording the number of hens in functional areas of the pen that differed in their level of UVB light exposure (Supplementary Figure S1). Figure 4 illustrates the percentage of hens from each group in the functional areas during the times in which the UVB-emitting lamps were switched off and on at baseline (Figures 4A–4C) and at week 6 (Figures 4D–4F) relative to the control group. Data show that the habitat preferences of the hens did not differ significantly between times in which the UVB-emitting lamps were switched off or on. In addition, the control and all treatment groups showed a similar behavior within the pen at baseline and after 6 wk. Approximately, one-half of the hens were located in the area without UVB exposure (nest and litter) and the other half stayed in the areas with medium or high UVB exposure.

Study 2

Irrespective of the stocking density, the vitamin D_3 and total vitamin D content of eggs from hens exposed to UVB-emitting lamps increased from baseline to week 6 of the experiment. The maximum increase of vitamin D in eggs had already been observed after 3 wk of UVB exposure (Figures 5A and 5C). Interestingly, no significant difference in the 25(OH)D_3 content of the eggs was observed between the UVB-exposed and non-exposed hens after 3 wk of the experiment (Figure 5B), but a significant difference was found after 6 wk. After 6 wk, 25(OH)D_3 concentrations were increased in both groups treated with UVB light. Notably, the 25(OH)D_3 content was highest in the group with the highest stocking density.

DISCUSSION

The current studies aimed to test the feasibility and efficacy of different UVB-emitting light regimes to increase the vitamin D content of indoor-laid eggs under simulated housing conditions. We were able to demonstrate that all light regimes used in the studies had the potential to significantly improve the vitamin D content in indoor-laid eggs under actual practical housing conditions of laying hens. Among the light regimes tested, the UVB light exposure for 6 h/d was most efficient for increasing the vitamin D content in indoor-laid eggs and could increase the vitamin D content almost 4-fold. Thus, intake of an egg from the LSL hens provides 1.9 μg, whereas an egg produced by LB hens provides 1.4 μg vitamin D. Cashman et al. (2012) postulated that 25(OH)D_3 is 5 times more effective in raising circulating 25(OH)D_3 levels than vitamin D_3. Those authors had proposed that 25(OH)D_3 has to be weighted when calculating the total vitamin D activity of eggs (vitamin D_3 + (5 × 25(OH)D_3)). The fortified egg would therefore provide the consumer with 2.7 μg vitamin D, irrespective of the hen genotype. In contrast, the control egg contains 1.3 μg of active vitamin D metabolites. In a recent study conducted by our group, we were able to increase the vitamin D content of eggs from hens exposed to UVB light to 3.9 μg per egg when the 5-fold higher biological activity of 25(OH)D_3 compared to vitamin D_3 was considered. However, such high contents of vitamin D in eggs could be reached if hens are housed individually and the UVB lamp installed in an optimal distance to each animal. Although we did not reach these high vitamin D concentrations in eggs in the current study, we assume that quadrupling the vitamin D content of commercially available

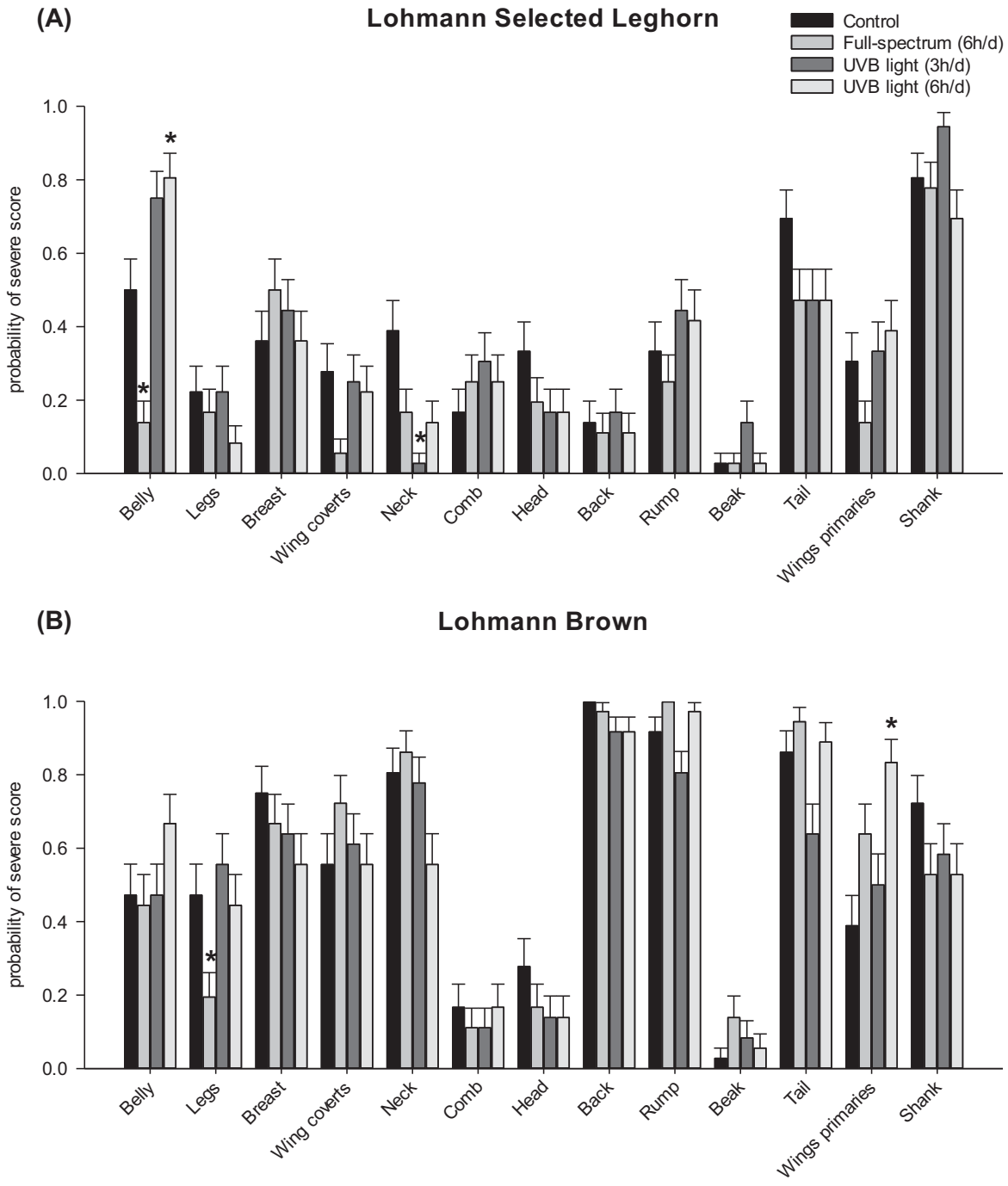

Figure 3. Effect of different UVB light regimes on feather damage of the laying hens. (A) Probability for Lohmann Selected Leghorn hens, (B) probability for Lohmann Brown hens. Data are presented as back-transformed least squares means (LSM) ± standard error (SE) of LSM. Dunnett's test was used to compare treatments and controls. Significant differences between treatment groups and controls are marked with an asterisk ($P < 0.05$).

indoor-laid eggs may also contribute significantly to improving the vitamin D status of individuals. In Europe, the mean dietary intake of vitamin D varies from 1.1 to 8.2 μg/d (2016). The EFSA panel and the American Institute of Medicine considers a daily vitamin D intake of 15 μg as recommendable (Institute of Medicine 2011; EFSA 2016). Thus, the intake of a fortified egg would provide the consumer with 13% (or 18% when considering the 5-fold higher biological activity of 25(OH)D_3) of the recommended daily vitamin D intake. In a systematic review, Autier et al. estimated that a daily intake of 1 μg vitamin D_3 increases the serum 25(OH)D_3 level by approximately 1 ng/mL (2.5 nmol/L) (Autier et al., 2012). This means that a fortified egg would be able to increase the serum concentrations of 25(OH)D by at least 2 ng/mL or higher if the 25(OH)D_3

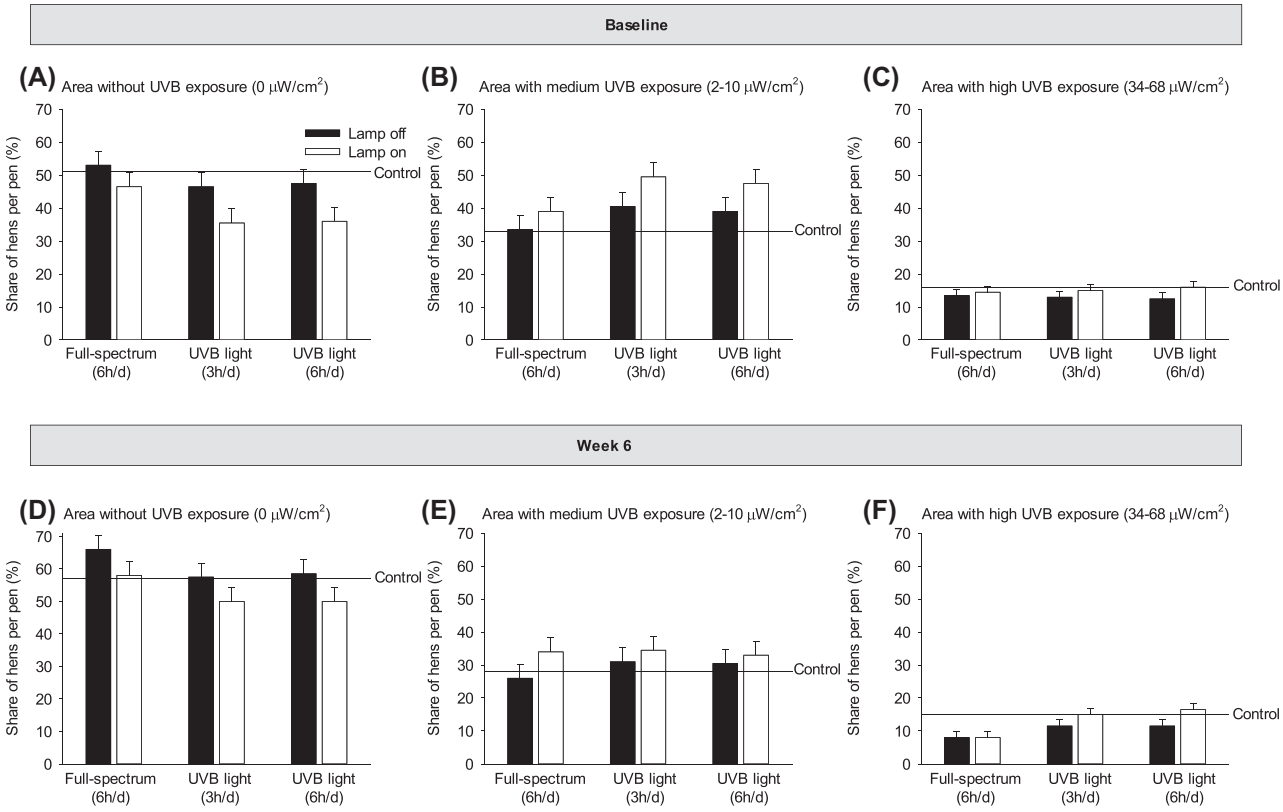

Figure 4. Functional area preferences of hens that were exposed to different UVB light regimes. Proportion of hens in areas with different UVB intensities in the pen during hours with or without UVB exposure in the pen at the start of the experiment and after 6 wk. Functional area preferences in (A) the area without UVB exposure at start, (B) in the area with medium UVB exposure at start, (C) in the area with high UVB exposure at start, (D) in the area without UVB exposure at week 6, (E) in the area with medium UVB exposure at week 6, and (F) in the area with high UVB exposure at week 6. Data are presented as least square means (LSM) ± standard error (SE) of LSM.

content of the eggs is being considered. The risk of vitamin D intoxication for the consumer is very small as it would be necessary to eat 38 eggs per day to exceed the recommended safe upper intake level of 100 μg/d (EFSA, 2012). Because egg yolk contains considerable amounts of cholesterol, nutrition societies recommend only a moderate intake of eggs (Spence et al., 2010).

In Ireland, a human intervention study was conducted that aimed to compare the influence of a daily intake of vitamin D enriched eggs vs. 2 or fewer commercial eggs per week on the serum 25(OH)D_3 level. The eggs were enriched with vitamin D by feeding hens with 3,000 IU vitamin D_3/kg diet. The study demonstrated that the daily intake of an egg was sufficient to prevent the decline of serum 25(OH)D_3 levels during winter, whereas the intake of 2 or fewer commercial egg per week failed to prevent this decline (Hayes et al., 2016).

In the current study, we used 2 genotypes of laying hens that are commonly used for egg production. In both genotypes, the light regimes were able to improve the vitamin D content in eggs.

Under practical livestock conditions, importantly the implementation of any novel housing equipment should not have adverse effects on animal welfare and behavior. In contrast to humans, birds are able to visually perceive UV light, in particular UVA (Bowmaker et al., 1997). Previous data show that birds change their behavior, including social behavior, foraging, and reproduction in response to UVB exposure (Pohl, 1992; Bennett and Cuthill, 1994; Lewis and Gous, 2009). To assess animal welfare and behavior, we investigated the habitat preferences, feather damage, and the performance (egg weight, egg shell thickness). Findings from the present study indicate that the treatments did not have adverse effects on feather damage and egg production parameters and did not change the area preferences indicative of UVB avoidance behavior. Both lamps used in the studies emit UVA and UVB light. Thus, we assume that light regimens to enrich eggs with vitamin D do not have adverse effects on the behavior and performance of the animals. The obvious differences between the 2 laying strains essentially resulted from the use of scoring methods of countable traits and the different exterior appearances of the strains. As supported by the results, we cannot conclude a poorer suitability for one of the strains. Previous data from a study in which turkeys were reared under conditions of an artificial UVB source showed that the birds preferred the UVB-exposed areas compared to areas without additional UVB light (Moinard and Sherwin, 1999). Because the physiology, behavior, and welfare of laying hens are assumed to be highly affected by the light conditions, including the wavelength and intensity of the

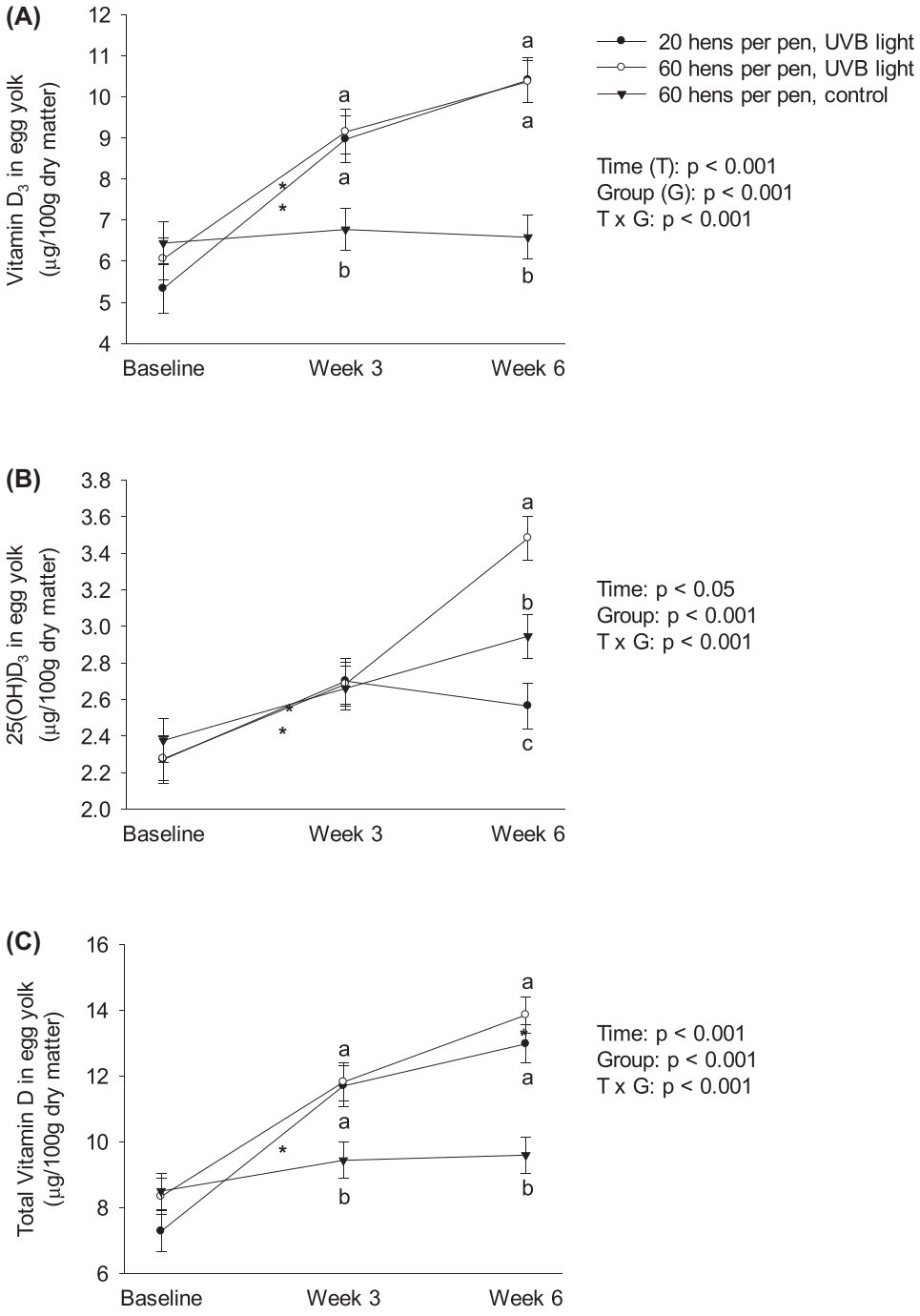

Figure 5. Concentrations of vitamin D metabolites in egg yolk of hens that were housed at either high or low stocking densities and were exposed to UVB light. (A) Vitamin D_3 concentration in egg yolk, (B) 25(OH)D_3 concentration in egg yolk, (C) total vitamin D concentration in egg yolk. Data are presented as least square means (LSM) ± standard error (SE) of LSM. When statistical analysis revealed a significant interaction between group x time, individual means of the groups were compared by the post hoc test. Different capital letters indicate differences between the 4 groups at one time point. Asterisks indicate differences between 2 time points within one group.

light (Manser, 1996), we assessed the extent of feather pecking in the different groups of hens as a biomarker of stress. Feather pecking is considered one of the most serious problems in laying hens, as it increases the risk of cannibalism and thereby increases mortality (Kjaer and Sørensen, 2002). Excessive exposure to light has been shown to be one factor that encourages feather pecking (Kjaer and Sørensen, 2002). Laying hens have UV-reflecting markings on their plumage that are visible only in UV and are used to identify each other (Sherwin and Devereux, 1999; Lewis, 2010). Under UV exposure, these markings change in their reflectance, which may thereby affect behavior and feather pecking (Sherwin and Devereux, 1999). In a study with turkeys, pecking was significantly reduced in birds exposed to UVB light compared to birds that received no UVB

(Lewis et al., 2000). The current data confirm that 6-h UVB light exposure has no detrimental effects on feather pecking conditions. Furthermore, our data indicate that intact or mildly damaged body regions are more predominant at the time of placement, and severely damaged body regions are more likely to be seen beginning with week 6.

The second study revealed that the stocking density has no adverse effects on the efficacy of our bioaddition approach. Thus, a stocking density that typically can be found in a furnished cage system allows an enrichment of eggs after UVB exposure, although the effectiveness of the UVB exposure to increase the vitamin D content in eggs from the furnished cages was lower in comparison to the first study.

SUPPLEMENTARY DATA

Supplementary data are available at *Poultry Science* online.

Supplementary Figure S1. Schematic representation of the furnished cages that consisted of nesting areas, perches, feeders, and nipple drinkers. The positions of the 2 UVB lamps are indicated in the figure. Areas without UVB exposure are indicated in black. Areas with medium UVB exposure are gray, whereas areas with high UVB exposure are white with black dots.

Supplementary Figure S2. Weight of egg and egg yolk and eggshell thickness of eggs from of hens exposed to different UVB light regimes. (A) Weight of eggs from Lohmann Selected Leghorn (LSL) hens, (B) weight of eggs from Lohmann Brown (LB) hens, (C) egg shell thickness of eggs from LSL hens, (D) egg shell thickness of eggs from LB hens, (E) egg yolk weight of eggs from LSL hens, (F) egg yolk weight of eggs from LB hens. Data are presented as least squares means (LSM) ± standard error (SE) of LSM.

Supplementary Figure S3. Time dependence of the probability of a severe body score for various parts of the bodies of hens exposed to different UVB light regimes. (A) Probability for Lohmann Selected Leghorn hens, (B) probability for Lohmann Brown hens. Data are presented as back-transformed least squares means (LSM) ± standard error (SE) of LSM.

Supplementary Table S1. Effect of different light regimes on vitamin D metabolites in egg yolk. Overview over all *P*-values for effects and their interaction.

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