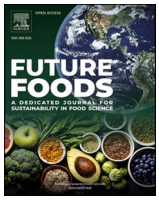
Future Foods 12 (2025) 100692

Contents lists available at ScienceDirect

Future Foods

journal homepage: www.elsevier.com/locate/fufo



Feeding black soldier fly (*Hermetia illucens*) larvae with mushrooms and then exposing them to UVB produces larvae extremely rich in vitamins D_2 and D_3

Lisa Morand-Laffargue [a], Florence Charles [b], Charlotte Sabran [a], Patrick Borel [a,*]

[a] C2VN, INRAE, Aix Marseille Univ, INSERM, Marseille, France
[b] Avignon Université, INRAE, UMR SQPOV F-84000, Avignon, France

ARTICLE INFO

Keywords:
Edible insects
Cholecalciferol
Ergocalciferol
Livestock diet
Supplementation

ABSTRACT

Black soldier fly larvae (BSFL) are a sustainable source of protein for feed but they can also be a sustainable source of some phytochemicals due to their ability to bioaccumulate them. Knowing that ergosterol in mushrooms can be chemically converted to vitamin D_2 (VD_2) under ultraviolet B (UVB) exposure, the aim was to investigate whether BSFL can bioaccumulate ergosterol and whether larvae ergosterol can be converted into VD_2 under UVB exposure.

Method: BSFL were reared on mushrooms for 2 weeks and then exposed to UVB (2.7 $mW/cm^2/s$ for up to 5 h). Ergosterol, VD_2 and vitamin D_3 (VD_3) were quantified by HPLC in mushrooms and larvae.

Main results: larvae non-exposed to UVB did not contain any detectable amount of VD_2 or VD_3. Larvae reared on mushrooms bioaccumulated ergosterol (702 ± 95 mg/Kg FW in larvae vs 444 ± 56 mg/Kg in mushrooms). Larvae reared on mushrooms and then exposed to UVB for 1 h contained high concentrations of VD_2 but also of VD_3 (46 ± 2 and 10.9 ± 0.5 mg/Kg FW, respectively).

Conclusions: BSFL can bioaccumulate ergosterol. UVB exposure of ergosterol-enriched BSFL results in VD_2 but also in VD_3 rich BSFL. The maximal VD concentration obtained in BSFL was about 23 times higher than cod liver oil, the food known to be richest in VD. These results suggest that VD-enriched BSFL could be used to supplement the nutrition of certain livestock with VD, or be valued as a functional ingredient. This procedure has been patented (PCT/EP2024/074854).

1. Introduction

Vitamin D (VD) is essential for humans and many animals, including major livestock and pets. It comes either from food rich in this vitamin, e.g. oily fish, or from skin synthesis under the action of UVB light. Two different forms of VD, which have nearly equivalent VD activity (Holick et al., 2008; Thacher et al., 2010), are synthetized in nature. VD_3, or cholecalciferol, is the form synthesized by humans and many animals in the skin under the action of UVB rays. Its precursor is 7-dehydrocholesterol (7-DHC). But it is also possible to synthesize VD_2, or ergocalciferol, from ergosterol, a molecule found mainly in mushrooms (Mattila et al., 2002). The transformation of 7-DHC and of ergosterol in respectively VD_3 and VD_2, is carried out by a chemical process induced by UVB light which does not require the intervention of a specific enzyme.

A significant proportion of the population does not have sufficient intake of this vitamin (Ovesen et al., 2003; Tangpricha et al., 2002), either through lack of exposure to the sun or through too little consumption of foods rich in this vitamin. In humans, to compensate for an insufficient intake of VD, for example in people who expose themselves very little to the sun, e.g. elderly people in institutions or inhabitants of the Nordic countries, it is common to recommend supplements (Brustad et al., 2003; Itkonen et al., 2021; Pludowski et al., 2018; Wicherts et al., 2011), whether in the form of high doses of purified VD every 3 or 4 months (Kearns et al., 2014), or in the form of cod liver oil (Brustad et al., 2003). In farmed animals, particularly those raised indoors, such as battery-raised hens, it is necessary to supplement the diet with VD to

Abbreviations: black soldier fly larvae, (BSFL); VD2, (Vitamin D2); VD3, (Vitamin D3); ultraviolet B, (UVB); fresh weight, (FW); 7-dehydrocholesterol, (7-DHC).

* Corresponding author: UMR C2VN "Center for CardioVascular and Nutrition Research of Marseille", Faculté de Médecine, 27 boulevard Jean Moulin, 13005, Marseille, France.

E-mail address: patrick.borel@univ-amu.fr (P. Borel).

https://doi.org/10.1016/j.fufo.2025.100692
Received 14 March 2025; Received in revised form 21 May 2025; Accepted 20 June 2025
Available online 21 June 2025

compensate for the lack of exposure to UV rays and the low content of VD from certain basic diets (Duffy et al., 2017; Ogbonna et al., 2023). VD supplementation of the diet is therefore common and necessary, whether in human or animal nutrition.

The incorporation of edible insects into our food chain, whether directly or indirectly via their incorporation into livestock feed, is recommended by many government agencies, e.g. FAO and EFSA (EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA Panel) et al., 2023; Van Huis et al., 2013), as it presents many environmental benefits, e.g. less greenhouse gas production, less water consumption (Van Huis et al., 2013). Insects are primarily seen as an alternative source of protein, but they can also provide significant amounts of micronutrients. We have thus shown that the black soldier fly larvae (BSFL) can bioaccumulate such high concentrations of provitamin A and vitamin E that the incorporation of usual quantities of this insect into the diet of many livestock is sufficient to provide them with their daily needs in these vitamins (Borel et al., 2021; Morand-Laffargue, Vairo, et al., 2023). However, although VD has been detected in certain species of insects and the exposure of these insects to UVB has made it possible to slightly increase its concentration (Oonincx et al., 2018), it remains too low for insects to be a significant source of this vitamin when we incorporate usual quantities of insects in the feeding of livestock or even for reasonable human consumption. Indeed, one would need to consume around 90 g of mealworms (*Tenebrio molitor)* exposed 64 h to high UVB irradiance as described in the article by Oonincx et al. (Oonincx et al., 2018) to cover our daily needs of 15 μg of VD. It is also not possible to bioaccumulate VD_3 in insects from foods rich in this vitamin, such as oily fish, as foods of animal origin are not authorized as feed for insects intended for human consumption.

Knowing that we have known for a long time how to enrich mushrooms with VD_2 by exposing them to UVB (Roberts et al., 2008; Rondanelli et al., 2023), and that we have shown that insects can bioaccumulate lipid molecules such as carotenoids and vitamin E (Borel et al., 2021; Morand-Laffargue, Creton, et al., 2023, 2023), we decided to try to obtain VD_2-rich BSFL by loading them with ergosterol before exposing them to UVB. This article describes the surprising results that were obtained and which led to the filing of a patent describing a procedure for obtaining BSFL, and potentially other insect species, extremely rich in VD_2 but also in VD_3.

2. Material and methods

2.1. Chemicals

Ethanol, *n*-hexane and HPLC grade acetonitrile, methanol and water were purchased from Carlo Erba reagents (Peypin, France). Standards (HPLC purity > 95 %), i.e. ergosterol, ergocalciferol (VD_2), cholecalciferol (VD_3) as well as retinyl acetate (used as internal standard for the calculation of the extraction yields) were from Sigma-Aldrich (Saint-Quentin-Fallavier, France).

2.2. Experimental foods

Whole white mushrooms (*Agaricus bisporus*) used as substrates for BSFL rearing, were purchased from a local supermarket.

2.3. BSFL farming

The rearing protocol was conducted at BioMiMetiC (Boulbon, France). The objective was to obtain between 20 and 100 g of larvae for each group. Thus, 4 kg of mushrooms were used as rearing substrates for each group and were sown with 200 mg of eggs. The rearing procedure was as follows: first, eggs collected from an oviposition support were placed in a rearing room settled on a temperature of 29 ± 1 °C and a relative humidity of 65 ± 5 %. Twenty-four hours later, mushrooms were cut into small pieces and placed in the rearing trays (clear plastic boxes 267 × 380 × 150 mm high). Once the mushrooms were prepared, hatching devices were placed on them, allowing eggs to hatch about 3 cm above the mushrooms. Eggs were from black soldier fly colony maintained by BioMiMetiC and were laid less than 24 h before being used in the experiment. The rearing boxes containing the mushrooms and eggs were placed in the rearing room. Larvae hatched from the eggs were reared for 13 days at these same parameters, under artificial light/dark cycles of 14:10 (h:h). At the end of the rearing period, the larvae were separated from the remaining mushrooms and frass using a sieve (2 × 2 mm). Larvae were then washed with tap water, dried by wiping them with absorbent paper, and transferred to an empty plastic box. For experiments aimed at testing the effect of fasting the larvae on their VD content, larvae were kept for 4 days in the empty box to empty their digestive tract of any remaining substrate (Larouche, 2019). After this period, larvae were separated from their exuviae and droppings, washed again with tap water and dried using absorbent paper before being frozen at −80 °C.

2.4. UVB exposure

A UVB lamp (DSP UVB tube, OSRAM HNL, 24 W) (Osram Gmbh, München, Germany) was heated for 30 min before exposing the larvae or mushrooms to UVB light. The power of the UVB lamp, measured using a radiometer, was 2.75 $mW/cm^2/s$. The dose of exposure to UVB was then calculated by multiplying the lamp power by the exposure time in seconds. The larvae were either exposed to UVB while alive before or after fasting, or while dead after fasting, freezing and defrosting. Depending on the group, the substrates and larvae were exposed to UVB for 15 min, 1 h, 2 h or 5 h. The exposure time of 15 min, which corresponded to a dose of approximately 2.4 J/cm^2, was the reference time. It was chosen to maximize VD_2 production in mushrooms. Indeed, although studies measuring VD_2 production in mushrooms under UVB exposure typically use a dose of approximately 1 J/cm^2 (Roberts et al., 2008; Simon et al., 2011), one study showed that higher VD_2 concentrations can be achieved by increasing this dose, with a plateau observed at 2.5 J/cm^2. This plateau was explained by the hypothesis that higher doses of UVB cause photodegradation of VD_2 (Kristensen et al., 2012).

The experimental protocol consisted of ten groups:

- **Group 1 (Control)**: BSFL were reared on mushrooms not exposed to UVB light.
- **Group 2**: BSFL were reared on mushrooms that had been exposed to UVB for 15 min.
- **Groups 3 and 4**: BSFL were reared on mushrooms and exposed to UVB on the first day of fasting for 15 min (Group 3) or 5 h (Group 4).
- **Groups 5 to 8**: BSFL were reared on mushrooms and exposed to UVB at the end of the fasting period for 15 min (Group 5), 1 h (Group 6), 2 h (Group 7) or 5 h (Group 8).
- **Groups 9 and 10**: BSFL were reared on mushrooms and, after undergoing fasting, freezing, and defrosting, the larvae were exposed to UVB for 15 min (Group 9) or 5 h (Group 10).

2.5. VD and ergosterol extraction

Firstly, 100 mg of BSFL and mushrooms samples finely ground with liquid nitrogen in a mortar grinder (Pulverisette 2, FRITSCH GmbH, Idar-Oberstein, Germany) were homogenized in 500 μL of distilled water. Then 500 μl of an internal standard solution, containing ethanol and retinyl acetate at a concentration of 0.4 mg/L, were added. A double extraction with hexane was performed on all samples. After centrifugation at 1257 x g during 10 min at 4 °C, the hexane phases were recovered and pooled and then evaporated under nitrogen until a dry lipid-rich film was obtained. This film was then solubilized in 1 mL of acetonitrile/methanol/water (60/38/2, v/v/v).

2.6. VD and ergosterol quantification

Volumes of 15 to 50 µL of the dry film resuspended in the solvent mixture mentioned above were injected in the HPLC apparatus. VD_2 and VD_3 were quantified at 265 nm and ergosterol at 282 nm. All the analyses were performed using a 250 × 4.6 nm ZORBAX Eclipse XDB-C18 5-µm column and the corresponding analytical guard column (Agilent Technologies, Montpellier, France). The column was maintained at a constant temperature (40 °C). Analysis was conducted with an acetonitrile/methanol/water (60/38/2, v/v/v) mobile phase and a flow rate of 1.5 mL/min. The HPLC system included an UltiMate 3000 system (Thermo Fisher Scientific, San Jose, CA, USA). Quantifications were performed using Chromeleon software comparing peak areas with standard reference curves.

2.7. Calculations and statistics

Results are expressed as the mean ± SEM of 4 independent samples and are reported on a fresh weight (FW) basis. The homogeneity of variances ($p > 0.05$) was assessed by the Levene's test. In the case of variance heterogeneity, the data were log-transformed. To test the normality of the data, Q-Q plots of the standardized residuals were used. Differences between groups were tested using several two-way ANOVA. When a significant effect was found by ANOVA, the comparison of means was carried out by pairwise comparisons using bilateral *t*-tests, with a Bonferroni adjustment. To compare the impact of different exposure times to UVB, one-way ANOVA was performed and Tukey-Kramer's test was used as a post hoc test for pairwise comparisons. To highlight the ergosterol and VD_2 bioaccumulation in the larvae and to compare the UVB exposed mushrooms and larvae, Student's *t*-tests were used with Welch's correction in case of inhomogeneous variances. Values of $p < 0.05$ were considered significant. All statistical analyses were performed using R version 4.1.1 for Windows. Finally, it should be noted that, as this is the first study on this topic, we did not have results in the literature that would allow us to perform the statistical power calculation necessary to determine the number of repetitions that would have been required for certain differences between the means to be statistically significant. However, the results of this study could be used to perform this power calculation in future studies on this topic.

3. Results

3.1. Ergosterol and VD_2 concentrations in mushrooms and BSFL

Fig. 1 shows the concentrations of ergosterol and VD_2 in mushrooms and BSFL. Concerning ergosterol (Fig. 1**A**), its concentration was 443.51 ± 55.55 mg/kg in mushrooms and 702.33 ± 95.15 mg/kg in BSFL. These two concentrations were not significantly different ($p = 0.09$), probably due to lack of statistical power. As expected, mushrooms and BSFL not exposed to UVB contained no detectable concentrations of VD_2 (data not shown). Concerning VD_2 (Fig. 1**B**), its concentration in mushrooms exposed to UVB was 7.51 ± 1.69 mg/kg and it was 8.81 ± 0.81 mg/kg in BSFL not exposed to UVB (but who were reared on the UVB-exposed mushrooms). Finally note that none of aforementioned samples contained detectable concentrations of VD_3 (data not shown).

3.2. VD_2 synthesis in mushrooms and in ergosterol-enriched BSFL exposed to the same dose of UVB

Fig. 2 shows VD_2 concentrations in mushrooms and in ergosterol-enriched BSFL exposed at 2.43 J/cm^2 UVB (exposure time of 15 min). The VD_2 concentration in BSFL was 14.07 ± 0.81 mg/kg which was about twice as high as that measured in the mushrooms ($p = 0.040$). Note that, as shown in Fig. 3, at this UVB dose, no VD_3 was detected in BSFL.

3.3. Effect of the time of UVB exposure on VD_2 and VD_3 concentrations in ergosterol-enriched BSFL

Fig. 3 illustrates the concentrations of VD_2 and VD_3 in BSFL treated at different UVB exposure times and thus at increasing UVB doses. The VD_2 concentration of BSFL exposed to UVB for 15 min was significantly ($p < 0.001$) lower than that of BSFL treated for 1 h (46.46 ± 2.01 mg/kg), 2 h (37.46 ± 1.67 mg/kg) and 5 h (42.90 ± 5.69 mg/kg). Concerning VD_3 (Fig. 3), none was detected after 15 min UVB. However, it was present in significant concentrations in BSFL exposed for 1 h (10.87 ± 0.53 mg/kg), 2 h (9.62 ± 0.32 mg/kg) and 5 h (11.49 ± 1.56 mg/kg). Nevertheless, the VD_3 concentrations obtained after 1, 2 and 5 h were not significantly different from each other ($p = 0.4$).

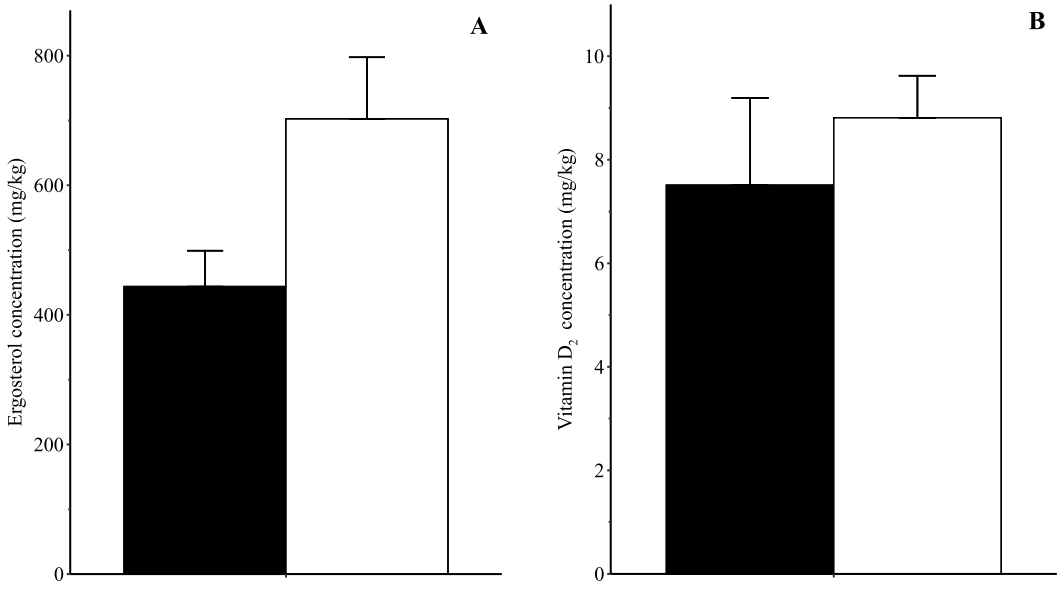


Fig. 1. Ergosterol and vitamin D_2 concentrations in mushrooms and in live BSFL reared on these mushrooms. A: Ergosterol concentrations in mushrooms (■) and in BSFL reared on these mushrooms (□). B: Vitamin D_2 concentrations in mushrooms exposed at a dose of 2.43 J/cm^2 UVB (see material and method for the justification of this dose) (■) and in BSFL reared on the exposed mushrooms (□). All concentrations are expressed on a fresh weight basis. Bars represent mean ± SEM ($n = 3$). Student-test showed no significant differences between ergosterol or Vitamin D_2 concentrations measured in mushrooms and BSFL.

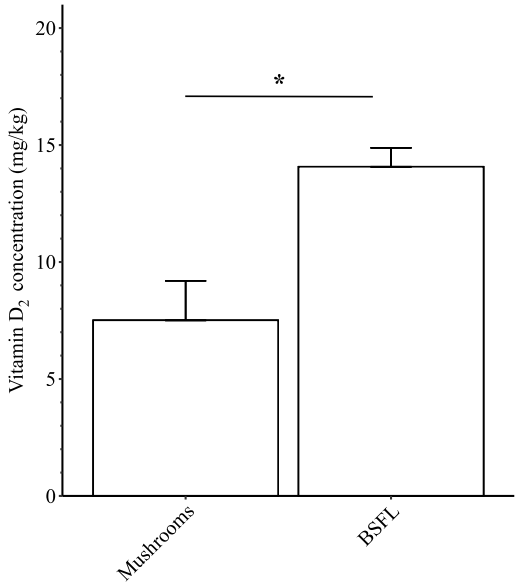


Fig. 2. Vitamin D_2 concentrations in mushrooms and live ergosterol-rich BSFL exposed to the same dose of UVB The UVB dose of 2.43 J/cm^2 was chosen in order to maximize the VD_2 concentration in the mushrooms (see material and method for more explanations). This experiment was carried out on larvae which had previously been enriched with ergosterol by being raised on mushrooms. Bars represent mean ± SEM ($n = 4$). Concentrations are expressed on a fresh weight basis. The asterisk means that the concentrations were significantly different ($p < 0.05$).

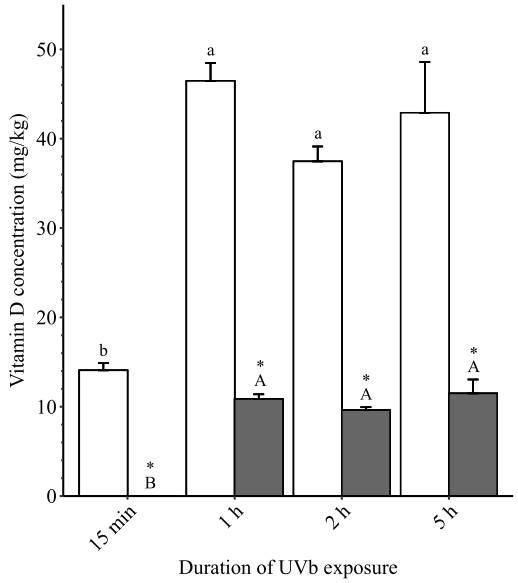


Fig. 3. Effect of UVB exposure time on vitamin D_2 and D_3 concentrations in live ergosterol-rich BSFL previously raised on mushrooms not exposed to UVB Vitamin D_2 (□) and vitamin D_3 (■) concentrations expressed on a fresh weight basis. Bars represent mean ± SEM ($n = 4$). For each vitamin, bars with different letters (capital letters for vitamin D_3 and lowercase letters for vitamin D_2) are significantly different ($p < 0.05$; two-way ANOVA, followed by pairwise comparisons using bilateral *t*-tests). For each time, an asterisk indicates that the concentration of vitamin D_3 is significantly different than that of vitamin D_2 ($p < 0.001$).

3.4. Effect of fasting and killing ergosterol-enriched BSFL prior to UVB exposure on their VD_2 and VD_3 concentrations

To evaluate the hypothesis that fasting enhances the efficiency of ergosterol conversion to VD_2 in larvae (see the discussion section for more explanations), we first compared the VD_2 concentrations of the larvae exposed alive to the same dose of UVB when they were fasted or not. As shown in Fig. 4**A**, the VD_2 concentration in larvae exposed alive 15 min to UVB after fasting was significantly higher ($p = 0.001$) than that of larvae exposed alive 15 min to UVB before fasting (14.07 ± 0.81 vs 7.19 ± 0.31 mg/kg). For the 5 h exposure time, the larvae exposed alive after fasting had also higher concentrations than the larvae exposed alive before fasting (42.90 ± 5.69 vs 36.35 ± 2.16 mg/kg) but this was not significant ($p = 1$). Concerning VD_3 (Fig. 4**B**), it was not detected in BSFL exposed to UVB for 15 min, whether they were fasting or not. However, after 5 h, VD_3 concentration of BSFL treated alive after fasting was significantly higher ($p < 0.01$) than that of BSFL exposed alive before fasting (11.49 ± 1.56 mg/kg vs 4.12 ± 0.36 mg/kg). In conclusion, fasting the larvae leads to an increase in their concentrations of VD_2 and VD_3.

Since our project was both fundamental and applied, we also studied the effect of exposing larvae to UVB after their death, rather than while they were alive, on their VD content. It is indeed likely that this would be easier to implement for manufacturers in this sector. Following the results on the effect of fasting larvae on their VD content (see previous paragraph), and with the aim of maximizing this content, we opted to fast the larvae prior to killing them by freezing. Fig. 4**A** shows that the VD_2 concentration in BSFL exposed to UVB for 15 min post-mortem (10.06 ± 1.37 mg/kg) was lower than that of BSFL treated with UVB alive (14.07 ± 0.81 mg/Kg), although this was not significant ($p = 0.055$). When exposure time was increased to 5 h, VD_2 concentration in BSFL treated after their death (29.28 ± 1.30 mg/kg) was also lower ($p = 0.09$) than that of BSFL treated alive (42.90 ± 5.69 mg/Kg), although this was also not significant ($p = 0.09$), likely because of insufficient statistical power. Concerning VD_3 (Fig. 4**B**), its concentration in dead BSFL after 5 h exposure (5.14 ± 0.41 mg/kg) was markedly lower ($p < 0.01$) than that measured in live BSFL (11.49 ± 1.56 mg/Kg). Overall, exposing larvae to UVB post-mortem, rather than while they are alive, results in a reduction in their VD_2 and VD_3 concentrations.

4. Discussion

The idea of this study came from a serendipity observation made in a previous study (Morand-Laffargue, Creton, et al., 2023). In that study on lutein bioaccumulation in BSFL, mushrooms were used as the control group because they do not contain lutein. As expected, no lutein was found in either mushrooms or the larvae that were fed with this substrate. However, an unknown compound was identified in these larvae, which was later confirmed to be ergosterol, the fungal equivalent of cholesterol. This compound was not found in larvae fed lutein-rich substrates, suggesting that BSFL cannot synthesize this compound and it bioaccumulates from the substrate consumed by the larvae. Since mushroom ergosterol can be converted into VD_2 under UVB exposure (Jiang et al., 2020; Lee and Aan, 2016), the aim of this project was to investigate the potential to produce VD_2-rich BSFL by first enriching them with ergosterol and then exposing them to UVB light.

The first objective was to assess ergosterol bioaccumulation in BSFL. We first observed that BSFL contained very low concentrations of this compound (between 1 and 6 % of the concentration found in those raised on mushrooms) when raised on substrates theoretically free of ergosterol, for example kale, parsley, endive and broccoli (data not shown). As it has never been observed that an animal can synthesize ergosterol, it is likely that these traces of ergosterol were due to yeasts or fungi present on the plant substrates or in larvae microbiota. When reared on a substrate rich in ergosterol, i.e. mushrooms, BSFL were able to bioaccumulate significant amounts of this compound (Fig. 1**A**). In

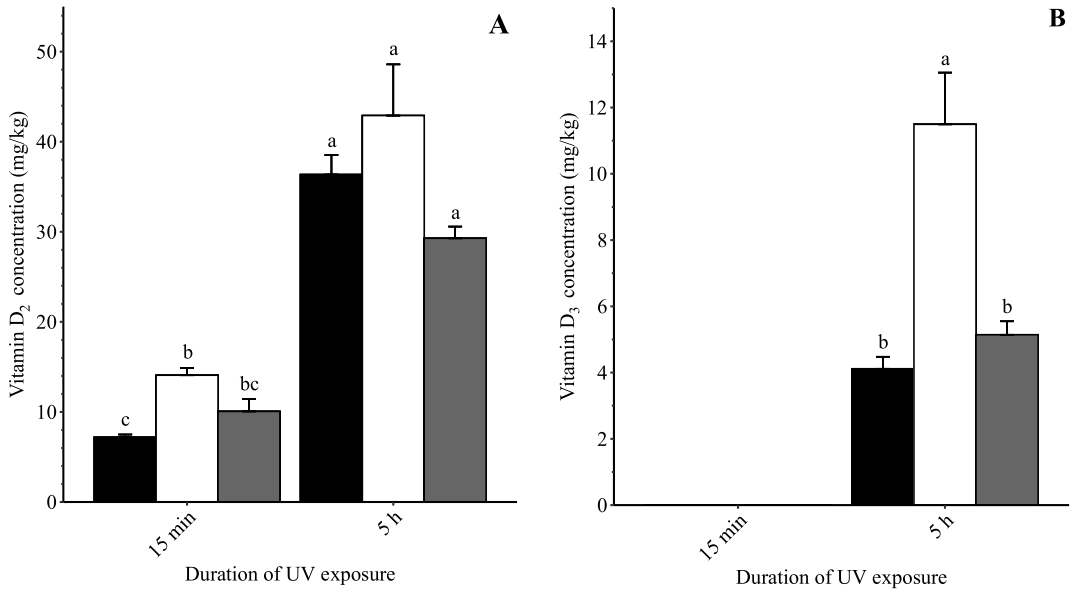


Fig. 4. Effect of fasting and killing ergosterol-rich BSFL prior to UVB exposure on their Vitamin D_2 and D_3 concentrations. Living larvae exposed to UVB before fasting (■); Living larvae exposed to UVB after fasting (□); Dead larvae exposed to UVB after fasting and freezing (■). This experiment was carried out on larvae which had previously been enriched with ergosterol by being raised on mushrooms. **A**: Vitamin D_2 concentrations. **B**: Vitamin D_3 concentrations. Both concentrations were expressed on a fresh weight basis. Bars represent mean ± SEM ($n = 4$). For each figure bars with different letters are significantly different ($p < 0.05$; two-way ANOVA, followed by pairwise comparisons using bilateral *t*-tests).

fact, the concentration of ergosterol was about 60 % higher in the larvae than in the substrate (although not significant, $p = 0.09$). We first hypothesize that this could be partially explained by the lower water content of the larvae as compared to the mushrooms (61 % vs 93 %, respectively, (Finke, 2013; Simon et al., 2011)). Nevertheless, the apparently lower bioaccumulation of VD_2 in larvae reared on VD_2 containing mushrooms (Fig. 1**B**), as compared to the bioaccumulation of ergosterol (Fig. 1**A**), suggests that this may not be the only explanation. Our data also show that BSFL can bioaccumulate VD_2 present in mushrooms exposed to UVB. This finding aligns with previous studies showing that BSFL can bioaccumulate lipid micronutrients with very different chemical structures (Borel et al., 2021; Morand-Laffargue, Creton, et al., 2023, 2023). Most importantly, our study showed that BSFL can bioaccumulate concentrations of ergosterol that are in the range of those found in mushrooms, which made them good candidates for obtaining interesting concentrations of VD_2 via the conversion of their ergosterol by UVB.

After confirming that BSFL can bioaccumulate relevant concentrations of ergosterol, the second objective was to assess whether it can be converted to VD_2 as efficiently as mushroom ergosterol under UVB exposure. Both mushrooms and ergosterol-enriched BSFL were exposed to the same dose of UVB, 2.5 J/cm^2. The result showed that, not only UVB exposed BSFL contained VD_2, but the concentration of VD_2 was about two times higher in the larvae as compared to the mushrooms exposed to the same dose of UVB (Fig. 2). We propose four hypotheses to explain this difference. The first, and simpler one is that, since the larvae contained about 60 % more ergosterol than the mushrooms, they naturally produced more VD_2. However, this alone cannot account for the doubling of the VD_2 concentration in the larvae. The second hypothesis is identical to the one proposed to explain the higher ergosterol concentration in the larvae than in the mushrooms. It assumes that part of this difference in concentration is due to the lower water content of larvae compared to mushrooms. A third hypothesis could be that the dose of UVB that have reached the ergosterol molecules in the two different substrates, i.e. the mushrooms and the BSFL, was not the same because the specific area (mm^2/g) of the substrates that received the light were not the same. A fourth hypothesis could be that UVB penetrates the skin and flesh of the larvae more effectively than the cuticle of the mushrooms and that consequently a greater amount of ergosterol is affected by UVB in the flesh of the larvae than in that of mushrooms.

Once it was established that ergosterol in larvae was converted to VD_2 as effectively as in mushrooms, we aimed to determine if higher UVB doses could further boost VD_2 production in larvae. Indeed, it has been shown in mushrooms that increasing the dose of UVB can further increase the concentration of VD_2 recovered in this substrate (Kristensen et al. 2012). Concerning the BSFL, the results obtained also show that increasing the UVB dose increases the concentration of VD_2 in the larvae, up to 45 ± 2 mg/kg (Fig. 3). However, as in mushrooms, VD_2 concentration plateaued with longer UVB exposure, indicating a possible saturation point. The plateau was reached at a dose greater than 2.5 J/cm2, and less than or equal to 9.9 J/cm2. By comparing ergosterol concentrations before and after 1 h of UVB exposure, we estimated that 6.6 % of ergosterol was converted to VD_2. Since this is a non-enzymatic process, it likely occurs only at the surface where ergosterol is directly exposed to UVB and converted, while deeper ergosterol remains unaffected. If the limiting factor for the conversion of ergosterol to VD_2 is indeed the amount of ergosterol that can be reached by UVB, the ergosterol in the digestive tract of non-fasted larvae, about 18 % of their total mass (Papin et al., 2024), is likely non-convertible, as it is likely not exposed to UVB. To test this hypothesis, larvae raised on mushrooms were fasted before being exposed to UVB. The aim was to allow digestion and absorption of gut contents, thereby increasing the amount of ergosterol bioaccumulated in the tissues of the larvae that are more accessible to UVB. Results showed that fasted larvae had higher VD_2 levels than non-fasted larvae exposed to the same dose of UVB, thus apparently supporting our hypothesis (Fig. 4**A**). However, we recognize that this increase could also be due to partial dehydration, or fat loss of the larvae during fasting, which would raise VD_2 concentration per unit mass. This second hypothesis is supported by the fact that fasting also significantly increased the concentration of VD_3 which is a priori synthesized in the epidermis of the larvae. Overall, fasting enhances both VD_2 and VD_3 concentrations in the larvae.

After attempting to maximize the VD_2 concentration in live larvae, we next aimed to determine the VD_2 concentration achieved by exposing the larvae to UVB post-mortem, considering its practicality for BSFL farmers. Results showed that exposing the larvae to UVB after death

reduced the concentrations of VD_2 (not significantly but the decrease was observed at the two UVB doses) and VD_3 obtained (Fig. 4). Since the conversion of ergosterol to VD_2 is a chemical mechanism, the decrease in this form of the vitamin cannot be attributed to a cessation of enzymatic reactions following death. Thus, we hypothesize that this decline resulted from ergosterol degradation after death and storage.

As reminded in the introduction, animals, including insects (Oonincx et al., 2018) can produce VD_3 from the UVB-mediated conversion of 7-DHC. Knowing that BSFL were exposed to UVB in our study, we hypothesized that they could also have produced VD_3 by this mechanism. We first observed that the VD_3 concentration was below our detection limit in larvae not exposed to UVB, contrasting with Oonincx et al., who measured approximately 5 μg/kg of VD_3 in UVB non-exposed BSFL (Oonincx et al., 2018). However, their larvae were fed diets containing at least 580 IU/kg of VD_3, suggesting that a fraction of food VD_3 had bioaccumulated in their larvae. In our case, the VD_3 concentration was also below our detection limit in the larvae exposed to 2.43 J/cm^2 UVB (15 min exposure time), in accordance with the results of Oonincx et al. (Oonincx et al., 2018). This limited synthesis of VD_3 in BSFL exposed to UVB is likely due to BSFL's low 7-DHC content (Grundmann et al., 2024) and/or, as suggested by Oonincx et al. (Oonincx et al., 2018), to the location of 7-DHC in the insect exoskeleton, which may filter UVB. Nevertheless, VD_3 synthesis occurred in the ergosterol-enriched BSFL exposed to UVB doses ranging from 2.4–9.7 J/cm2, though higher doses did not further increase VD_3 (Fig. 3). As previously proposed to explain the plateau in VD_2 concentration with increasing UVB doses, we hypothesize that only the existing fraction of 7-DHC in the larvae was converted to VD_3, and the synthesis rate of 7-DHC was too low to produce additional amounts for conversion into VD_3.

The synthesis of VD_3 in BSFL under UVB exposure was unexpected as OOnincx et al. found no significant increase in VD_3 concentration after exposure of BSFL to UVB (Oonincx et al., 2018). The main difference between our study and that study lies in the ergosterol enrichment of the larvae before UVB exposure. Our hypothesis is that the presence of ergosterol in the larvae boosted the synthesis of VD_3 when the BSFL were exposed to UVB. Since ergosterol, like 7-DHC, is a substrate of 7-DHC reductase (DHCR7) which converts 7-DHC to cholesterol, and that ergosterol also inhibits the synthesis of this enzyme (Kuwabara et al., 2022), our hypothesis is that the high ergosterol concentration in our larvae significantly decreased the 7-DHC conversion to cholesterol. Which leads to an increase in the amount of 7-DHC that is available to be converted to VD_3 by photolysis, as seen in studies of ergosterol supplementation in rodents (Baur et al., 2019; Kuwabara et al., 2023).

The results of this study went beyond our expectations since not only did our process allow us to obtain larvae rich in VD, but the maximum concentrations obtained were extremely high. Indeed, by combining the maximum VD_2 and VD_3 concentrations obtained after 1 h of exposure, we reach about 57 mg of VD/kg of larvae, approximately 260 times higher than what had been observed in a previous study on the same insect species (Oonincx et al., 2018). Compared to previous studies aiming to enhance VD levels in other insect species, our larvae contain about 10 times more VD than irradiated *Tenebrio molitor* flour (Nölle et al., 2024).

This is largely due to ergosterol enrichment, which serves as a VD_2 precursor and boosts VD_3 synthesis.

5. Main conclusions

This study showed that BSFL are capable of bioaccumulating ergosterol, and upon UVB exposure, ergosterol-enriched larvae produce not only VD_2 but also VD_3. Furthermore, the VD concentration of our larvae was approximately 23 times higher than that of the richest dietary source of VD—cod liver oil. Therefore, these VD-rich larvae could be a valuable source of this vitamin in animal food, or even human food if this species of insect were to be authorized in human. Indeed, the use of insects for human consumption remains limited in several countries due to several constraints, including their potential allergenicity, the lack of regulatory authorization for certain species such as BSFL, and the need for a more comprehensive assessment of their food safety.

Several questions remain unanswered following this study. First, it is essential to verify the bioavailability of VD in these larvae, or in derived products like flour and oil, as chitin could bind a significant part of VD and thus reduces its bioavailability. It is also necessary to assess whether processing treatments used to produce animal feed from larvae degrade significant amounts of VD. Finally, it would be valuable to determine whether we can enrich other insect species with VD by applying this process to them. The implementation of this method by manufacturers who would like to enrich BSFL with VD and put them on the market will face constraints that were not studied in this study. For example, we can assume that UV will not be able to reach all the larvae if they are exposed in the form of a more or less significant layer of superimposed dead larvae. It will therefore be necessary to set up a means of exposing them in the form of a monolayer of larvae. We can also anticipate that the installation of UV lamps and their maintenance will generate a cost, and additional adjustments, which will have to be included in the sale price. Finally, the marketing of these VD-enriched larvae will have to be authorized by EFSA.

This procedure was patented to obtain VD-rich BSFL. Given the general mechanism, this patent had been extended to all insect species that can bioaccumulate high concentrations of ergosterol from substrates rich in this compound (such as fungi, yeast, or certain algae).

Ethical statement

We declare that this manuscript does not involve any studies with human participants or animals performed by any of the authors.

CRediT authorship contribution statement

Lisa Morand-Laffargue: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Florence Charles:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Charlotte Sabran:** Investigation. **Patrick Borel:** Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Lisa Morand-Laffargue reports financial support and equipment, drugs, or supplies were provided by BioMiMetic (Boulbon, France). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors would like to thank A. Sefah and D. Sabatier (BioMiMetiC, F-13150 Boulbon, France) for making their larvae breeding premises available to us and for feeding the different groups of larvae by scrupulously following the experimental protocol that we had indicated to them.

Data availability

The data that has been used is confidential.

Bibliography

Baur, A.C., Kühn, J., Brandsch, C., Hirche, F., Stangl, G.I., 2019. Intake of ergosterol increases the vitamin D concentrations in serum and liver of mice. J. Steroid Biochem. Mol. Biol 194, 105435. https://doi.org/10.1016/j.jsbmb.2019.105435.

Borel, P., Hammaz, F., Morand-Laffargue, L., Creton, B., Halimi, C., Sabatier, D., Desmarchelier, C., 2021. Using black soldier fly larvae reared on fruits and vegetables waste as a sustainable dietary source of provitamin A carotenoids. Food Chem. 359, 129911. https://doi.org/10.1016/j.foodchem.2021.129911.

Brustad, M., Sandanger, T., Wilsgaard, T., Aksnes, L., Lund, E., 2003. Change in plasma levels of vitamin D after consumption of cod-liver and fresh cod-liver oil as part of the traditional north Norwegian fish dish "Molje. Int. J. Circumpolar Health 62 (1), 40–53. https://doi.org/10.3402/ijch.v62i1.17527.

Duffy, S.K., Rajauria, G., Clarke, L.C., Kelly, A.K., Cashman, K.D., O'Doherty, J.V., 2017. The potential of cholecalciferol and 25-hydroxyvitamin D3 enriched diets in laying hens, to improve egg vitamin D content and antioxidant availability. Innov. Food Sci. Emerg. Technol. 44, 109–116. https://doi.org/10.1016/j.ifset.2017.07.007.

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA Panel), Turck, D., Bohn, T., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K.I., Maciuk, A., Mangelsdorf, I., McArdle, H.J., Naska, A., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Aguilera-Gómez, M., Cubadda, F., Frenzel, T., Knutsen, H. K., 2023. Safety of UV-treated powder of whole yellow mealworm (Tenebrio molitor larva) as a novel food pursuant to regulation (EU) 2015/2283. EFSA J. 21 (6), e08009. https://doi.org/10.2903/j.efsa.2023.8009.

Finke, M.D., 2013. Complete nutrient content of four species of feeder insects: nutrient content of feeder insects. Zoo Biol. 32 (1). https://doi.org/10.1002/zoo.21012.

Grundmann, S., Jerathe, S., Pfeiffer, J., Most, E., Klüber, P., Rühl, M., Eder, K., 2024. Exploring insect meals as novel sources of vitamin D: evaluation of vitamin D precursors, biofortification by UV-B exposure, and in vivo efficacy. J. Insects Food Feed. 11 (3), 509–523. https://doi.org/10.1163/23524588-00001284.

Holick, M.F., Biancuzzo, R.M., Chen, T.C., Klein, E.K., Young, A., Bibuld, D., Reitz, R., Salameh, W., Ameri, A., Tannenbaum, A.D., 2008. Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. J. Clin. Endocrinol. Metab 93 (3), 677–681. https://doi.org/10.1210/jc.2007-2308.

Itkonen, S.T., Andersen, R., Björk, A.K., Brugård Konde, Å., Eneroth, H., Erkkola, M., Holvik, K., Madar, A.A., Meyer, H.E., Tetens, I., Torfadóttir, J.E., Thórisdóttir, B., Lamberg-Allardt, C.J.E., 2021. Vitamin D status and current policies to achieve adequate vitamin D intake in the Nordic countries. Scand. J. Public Health 49 (6), 616–627. https://doi.org/10.1177/1403494819896878.

Jiang, Q., Zhang, M., Mujumdar, A.S., 2020. UV induced conversion during drying of ergosterol to vitamin D in various mushrooms: effect of different drying conditions. Trends Food Sci. Technol. 105, 200–210. https://doi.org/10.1016/j.tifs.2020.09.011.

Kearns, M.D., Alvarez, J.A., Tangpricha, V., 2014. Large, single-dose, oral vitamin D supplementation in adult populations: a systematic review. Off. J. Am. Coll. Endocrinol. Am. Assoc. Clin. Endocrinol. 20 (4), 341–351. https://doi.org/10.4158/EP13265.RA.

Kristensen, HanneL., Rosenqvist, E., Jakobsen, J., 2012. Increase of vitamin D2 by UV-B exposure during the growth phase of white button mushroom (Agaricus bisporus). Food Nutr. Res. 56 (1), 7114. https://doi.org/10.3402/fnr.v56i0.7114.

Kuwabara, N., Ohta-Shimizu, M., Fuwa, F., Tomitsuka, E., Sato, S., Nakagawa, S., 2022. Ergosterol increases 7-dehydrocholesterol, a cholesterol precursor, and decreases cholesterol in human HepG2 cells. Lipids 57 (6), 303–311. https://doi.org/10.1002/lipd.12357.

Kuwabara, N., Sato, S., Nakagawa, S., 2023. Effects of long-term high-ergosterol intake on the cholesterol and vitamin D biosynthetic pathways of rats fed a high-fat and high-sucrose diet. Biol. Pharm. Bull 46 (12), 1683–1691. https://doi.org/10.1248/bpb.b23-00348.

Larouche, J., 2019. MSc-thesis [. Université de Laval.

Lee, N.K., Aan, B.-Y., 2016. Optimization of ergosterol to vitamin D2 synthesis in Agaricus bisporus powder using ultraviolet-B radiation. Food Sci. Biotechnol. 25 (6), 1627–1631. https://doi.org/10.1007/s10068-016-0250-0.

Mattila, P., Lampi, A.-M., Ronkainen, R., Toivo, J., Piironen, V., 2002. Sterol and vitamin D2 contents in some wild and cultivated mushrooms. Food Chem. 76 (3), 293–298. https://doi.org/10.1016/S0308-8146(01)00275-8.

Morand-Laffargue, L., Creton, B., Halimi, C., Sabatier, D., Desmarchelier, C., Borel, P., 2023a. Black soldier fly larvae (*Hermetia illucens*) as a sustainable and concentrated source of bioavailable lutein for feed. J. Insects Food Feed 10 (4), 559–570. https://doi.org/10.1163/23524588-20230107.

Morand-Laffargue, L., Vairo, D., Halimi, C., Chiarello, E., Creton, B., Sabatier, D., Borel, P., 2023b. Ability of black soldier fly larvae to bioaccumulate tocopherols from different substrates and measurement of larval tocopherol bioavailability in vitro. J. Insects Food Feed 10 (5), 795–807. https://doi.org/10.1163/23524588-20230183B.

Nölle, N., Hörnstein, A., Lambert, C., 2024. Vitamin D fortification of selected edible insect species through UVB-treatment. Food Chem. 444, 138679. https://doi.org/10.1016/j.foodchem.2024.138679.

Ogbonna, A.C., Chaudhry, A.S., Asher, L., 2023. Effect of dietary vitamin D3 and ultraviolet-B light on the behaviour and growth of broilers challenged with social isolation stress. Appl. Anim. Behav. Sci. 268, 106083. https://doi.org/10.1016/j.applanim.2023.106083.

Oonincx, D.G.A.B., van Keulen, P., Finke, M.D., Baines, F.M., Vermeulen, M., Bosch, G., 2018. Evidence of vitamin D synthesis in insects exposed to UVb light. Sci. Rep. 8 (1), 10807. https://doi.org/10.1038/s41598-018-29232-w.

Ovesen, L., Andersen, R., Jakobsen, J., 2003. Geographical differences in vitamin D status, with particular reference to European countries. Proc. Nutr. Soc. 62 (4), 813–821. https://doi.org/10.1079/PNS2003297.

Papin, M., Morand-Laffargue, L., Sabran, C., Sabatier, D., Sefah, A., Engel, E., Planche, C., & Borel, P., 2024. Black soldier fly larvae (*Hermetia illucens*) do not bioaccumulate ferulic and caffeic acids from wheat bran. *(published online ahead of print 2024)*. https://doi.org/10.1163/23524588-00001338.

Pludowski, P., Holick, M.F., Grant, W.B., Konstantynowicz, J., Mascarenhas, M.R., Haq, A., Povoroznyuk, V., Balatska, N., Barbosa, A.P., Karonova, T., Rudenka, E., Misiorowski, W., Zakharova, I., Rudenka, A., Łukaszkiewicz, J., Marcinowska-Suchowierska, E., Łaszcz, N., Abramowicz, P., Bhattoa, H.P., Wimalawansa, S.J., 2018. Vitamin D supplementation guidelines. J. Steroid Biochem. Mol. Biol 175, 125–135. https://doi.org/10.1016/j.jsbmb.2017.01.021.

Roberts, J., Teichert, A., Mchugh, T., 2008. Vitamin D 2 formation from post-harvest UV-B treatment of mushrooms (agaricus bisporus) and retention during storage. J. Agric. Food Chem. 56, 4541–4544. https://doi.org/10.1021/jf0732511.

Rondanelli, M., Moroni, A., Zese, M., Gasparri, C., Riva, A., Petrangolini, G., Perna, S., Mazzola, G., 2023. Vitamin D from UV-irradiated mushrooms as a way for vitamin D supplementation: a systematic review on classic and nonclassic effects in human and animal models. Antioxidants 12 (3), 736. https://doi.org/10.3390/antiox12030736.

Simon, R.R., Phillips, K.M., Horst, R.L., Munro, I.C., 2011. Vitamin D Mushrooms: comparison of the composition of button mushrooms (Agaricus bisporus) Treated Postharvest with UVB light or sunlight. J. Agric. Food Chem. 59 (16), 8724–8732. https://doi.org/10.1021/jf201255b.

Tangpricha, V., Pearce, E.N., Chen, T.C., Holick, M.F., 2002. Vitamin D insufficiency among free-living healthy young adults. Am. J. Med 112 (8), 659–662. https://doi.org/10.1016/S0002-9343(02)01091-4.

Thacher, T.D., Fischer, P.R., Obadofin, M.O., Levine, M.A., Singh, R.J., Pettifor, J.M., 2010. Comparison of metabolism of vitamins D2 and D3 in children with nutritional rickets. J. Bone Miner. Res. 25 (9), 1988–1995. https://doi.org/10.1002/jbmr.99.

Van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., Vantomme, P., 2013. Edible insects—future prospects for food and feed security. FAO For. 171.

Wicherts, I.S., Boeke, A.J.P., van der Meer, I.M., van Schoor, N.M., Knol, D.L., Lips, P., 2011. Sunlight exposure or vitamin D supplementation for vitamin D-deficient non-western immigrants: a randomized clinical trial. Osteoporos. Int. 22 (3), 873–882. https://doi.org/10.1007/s00198-010-1343-x.