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Review

Naturally enhanced eggs as a source of vitamin D: A review

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ABSTRACT

Background: It is estimated that on annual basis 40% of the European population is either vitamin D insufficient/deficient. A way to increase the vitamin D intake is to fortify a broader range of foods or by increasing the natural vitamin D content in food sources that already contain vitamin D. Eggs is once again considered part of a healthy varied diet and eggs contain a wide range of micro nutrients including vitamin D.
Scope and approach: Review of production methods to naturally enhance eggs with vitamin D, and discussion of the perspectives of vitamin D enhanced eggs as part of the strategy to increase the dietary intake of vitamin D.
Key findings and conclusions: There are three ways to naturally enhance the vitamin D content in eggs: feeding more vitamin D_3/25(OH)D_3 to the hens, exposing the hens to UVB and exposing liquid egg products to UVB. Naturally enhanced eggs can contribute to increased vitamin D intake. An inter-trial linear relationship between vitamin D_3 in feed and vitamin D_3 in eggs was found. Within the linear range a maximum of 20 μg/100 g yolk was obtained with feed contain 617.5 μg/kg feed. Feed can provide higher levels of vitamin D in eggs than UVB exposure of the hens. However, the European maximum for vitamin D in feed for layers at 80 μg/kg limits the beneficial effect. Vitamin D content in liquid egg products can be tailored by adjusting the UVB dose, however further research is needed.

1. Introduction

It is estimated that on annual basis 40% of the European population is either vitamin D insufficient (< 50 nmol/L total serum 25-hydroxyvitamin D (25(OH)D) or deficient (< 30 nmol/L) (Cashman et al., 2016). Although there is dispute about where to set the limit of insufficiency and deficiency, there is agreement that the vitamin D status (total serum 25(OH)D) of the general population has to be increased (Holick, 2017).

Vitamin D can be obtained either through food or sun exposure, where sun exposure is the major contributor to vitamin D in humans. 7-Dehydrocholesterol (7-DHC) in the skin is converted to previtamin D_3 when the skin is exposed to wavelengths between 290 and 315 nm (ultraviolet B, UVB) and previtamin D_3 is then converted to vitamin D_3 by thermal isomerization at body temperature (Holick et al., 1980; MacLaughlin, Anderson, & Holick, 1982); vitamin D_3 is transported to the liver where it is converted to 25-hydroxivitamin D_3 (25(OH)D_3) (Christakos, Dhawan, Verstuyf, Verlinden, & Carmeliet, 2016). However, from October through March at 52 °N no cutaneous vitamin D is produced as most of the solar photons below 315 nm is attenuated by the longer travel through the ozone layer during the so-called vitamin D winter (Webb, 2006; Webb, DeCosta, & Holick, 1989; Webb, Kline, & Holick, 1988). The duration of the vitamin D winter decreases with decreasing latitude and according to UVB data it is suggested that the duration is: 5 months in UK, Ireland and the Netherlands; 6 months in Denmark; 8 months in the north of Norway; while it is 2 to months in Greece and 0 in Crete (O'Neill et al., 2016).

Vitamin D exists in two forms: vitamin D_2 and vitamin D_3. Although there are multiple metabolites of vitamin D, only vitamin D and its major metabolite 25(OH)D is considered when determining the total vitamin D content in food (Ovesen, Brot, & Jakobsen, 2003). Vitamin D_3 and 25(OH)D_3 is found in food of animal origin, such as fish, meat, offal, eggs, milk and dairy products (Ovesen et al., 2003). Vitamin D_2 is found in yeast, mushrooms, and fortified milk and dairy products (Keegan, Lu, Bogusz, Williams, & Holick, 2013).

The recommended intake of vitamin D varies for the different age groups, and between the individual organisations setting the guidelines (Boiullion, 2017).In Europe and US, the guidelines for adults are between 10 and 20 μg/day (European Food Safety Authority, 2016; Institute of Medicine, 2011; Nordic Council of Ministers, 2012), however the estimated intake is 3–7 μg/day (Cashman & Kiely, 2016). In some countries with mandatory fortification programmes the intake of vitamin D is still suboptimal (Lehtonen-Veromaa et al., 2008; Whiting, Green, & Calvo, 2007). Since 2003 Finland have had a voluntary fortification programme which includes milk and fat spread with the effect that the adult

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population who have a diet based on nutritional recommendations have a sufficient vitamin D status, while the status in adolescent girls is still insufficient (Jääskeläinen et al., 2017; Lehtonen-Veromaa et al., 2008).

A way to increase the vitamin D intake is to fortify a broader range of foods or by increasing the natural vitamin D content in food sources that already contain vitamin D (Barnkob, Argyraki, Petersen, & Jakobsen, 2016; Black, Seamans, Cashman, & Kiely, 2012; Cashman & Kiely, 2016; Kiely & Black, 2012; O'Mahony, Stepien, Gibney, Nugent, & Brennan, 2011). Foods with natural increased vitamin D content could in some cases be more broadly accepted by consumers (Cashman, 2015).

For many years eggs have had a bad reputation due to a high content of cholesterol, however as reviewed by Gray (2018), eggs were wrongfully accused and is once again considered to be part of a healthy varied diet as they contribute with a range of micro nutrients, including vitamin D, and high quality, easily digestible proteins; and compared to other food sources eggs are easily prepared, they are relatively cheap and they are included in the diet of the majority of the population (Gray, 2018; Mejborn, Jacobsen, & Trolle, 2011; Pedersen et al., 2015).

The principle in the analytical methods used for quantification of vitamin D has since the introduction of an internal standard been the same (Indyk & Wollard, 1985), i.e. alkaline saponification, liquid-liquid extraction, clean-up step (preparative HPLC or/and solid-phase-extraction), and separation by HPLC combined with UV-/DAD-detector. Method for quantification of 25-hydroxyvitamin D was introduced at a later stage by applying the same principle (Mattila, Piironen, Uusi-Rauva, & Koivistoinen, 1995). A huge improvement in especially sensitivity and laboratory work came with the introduction of mass spectrometry detection (Dimartino, G, 2009). Due to the relatively high amount of vitamin D in eggs, the analytical improvement is essential for the amount of sample taken for analysis and the cost of an analysis, as a technician in 1990's could run 10 samples a week, a technician in 2020 may run 20 samples a day. Otherwise the improvement, did not affect the trueness of the methods, which essentially is due the use of internal standard. Thus, making it possible to combine old and new analytical data in this review.

The natural content of vitamin D in eggs can be increased by three methods, of which two have gained the most attention: adding more vitamin D to the feed of the hens; and exposing the hens to UVB light. The third method is to expose egg yolk directly to UVB. We will review the literature on these three methods and discuss their future perspectives.

2. Method

An extensive literature search was performed in January 2019. The search machine DTU Findit (DTU Library, n.d.) was used with the following search phrase: vitamin D OR eggs OR ultraviolet OR content OR yolk. Reference lists from relevant articles found in the search as well as the cited by function in Google were used to find additional references.

In order to make different reported results comparable all results have been recalculated to μg/100 g egg for vitamin D_3 and 25(OH)D_3; and μg/kg for vitamin D_3 and 25(OH)D_3 in feed. Some authors report the results per gram egg yolk, in these cases the data has been recalculated under the assumption that a whole egg consists of 33% yolk (Mattila, Lehikoinen, Kiiskinen, & Piironen, 1999a, Mattila, Ronkainen, Lehikoinen, & Piironen, 1999b; Mattila, Valkonen, & Valaja, 2011). Others have reported results per gram dry matter of egg yolk, it is assumed that an egg yolk contain 7 g of dry matter (DTU Food, 2018; Kühn et al., 2015; Schutkowski et al., 2013) based on an average egg yolk weight of 15 g. It is assumed that vitamin D is only present in the yolk.

To make UVB doses comparable all reported values have been recalculated to J/m^2.

2.1. Limitations

This review will primarily focus on the vitamin D content, if interested in how vitamin D and its metabolites affect performance and egg quality we refer to a recent review from Światkiewicz, Arczewska-Wlosek, Bederska-Lojewska, and Józefiak (2017).

Vitamin D_2 is not as effectively transferred to yolk as vitamin D_3 (Francis G. McDonald & Massengale, 1932), and only vitamin D_3 and 25(OH)D_3 is approved as vitamin D additives in feed for layers; therefore studies performed with vitamin D_2 are not included except for Kawazoe, Yuasa, Yamazaki, and Ando (1994) who studied the relation between feed intake and vitamin D content in eggs which have not been studied using vitamin D_3.

A lot of studies were published in the 1920'ties and 1930'ties regarding how to increase the vitamin D content in eggs. It was shown that the content of vitamin D in egg yolk could be increased by adding vitamin D to the feed of laying hens (Bethke, Kennard, & Sassaman, 1927; Branion, Drake, & Tisdall, 1935; DeVaney, Munsell, & Titus, 1933; Guerrant, Kohler, Hunter, & Murphy, 1935) or by irradiating non-supplemented hens from above (Farrell, 1924; Hart et al., 1925; Hendricks, 1931; Hughes & Payne, 1924; Hughes, Payne, Titus, & Moore, 1925; Maughan & Maughan, 1933). However, when Carson and Beall (1955) supplemented hens with approximately 29 μg vitamin D/kg feed and exposed them to UVB from above, no increase of vitamin D in the eggs were observed. The vitamin D content in these studies of older date was determined by hatchability of eggs, an endpoint, which is based on the fact that vitamin D in egg yolk is essential for embryonic survival (Sunde, Turk, & DeLuca, 1978), or by animal assays where the total vitamin D activity is determined. Only studies that measure the individual content of vitamin D_3 and/or 25(OH)D_3 have been included.

Two studies were excluded as they also included increased content of vitamin K in the feed (Park, Namkung, Ahn, & Paik, 2005; Zang et al., 2011). At high concentration vitamin K inhibits the uptake of vitamin D (Reboul, 2015) and as both vitamin D and vitamin K are involved in bone health (Torbergsen et al., 2015) it cannot be ruled out that they also interact at higher levels in vivo.

3. Bioavailability of vitamin D from eggs

As mentioned in the introduction eggs are an optimal vehicle for vitamin D in regard to its nutritional qualities and wide use. A recent review found no difference in the bioavailability of vitamin D_3 from fortified food compared to supplements (Borel, Caillaud, & Cano, 2015). Investigation of the bioavailability of vitamin D_3 and 25(OH)D_3 from natural food is limited, but no difference was shown for vitamin D_3 from cod liver oil and vitamin D_3 in multivitamin tables to increase vitamin D status (Holvik, Madar, Meyer, Lofthus, & Stene, 2007). Moreover, it has been shown than intake of vitamin D enhanced eggs is associated with increased vitamin D status (Hayes et al., 2016).

4. Cooking loss of vitamin D in eggs

Two studies regarding the loss of vitamin D during cooking of eggs have been published (Jakobsen & Knuthsen, 2014; Mattila, Ronkainen, et al., 1999b). Vitamin D and 25(OH)D in eggs will be lost, to the same extent, during household cooking: A hardboiled egg (10 min of cooking) will lose around 10% of both vitamin D and 25(OH)D, scrambling an egg for 3 min gives less than 20% loss of both, while during baking for 40 min around 60% of both is lost (Jakobsen & Knuthsen, 2014; Mattila, Ronkainen, et al., 1999b).

5. European legislation regarding vitamin D in feed for laying hens

The legal limit for adding vitamin D_3, 25(OH)D_3 or a combination of the two to feed is 80 μg/kg for laying hens in Europe (Commission Regulation (EU) 2017/1492 Commission Regulation (EC) No 887/2009); according to Commission Regulation (EU) 2017/1492 this level does not have adverse effects on animal health, human health or the environment based on scientific opinions from the European Food

Safety Authority (EFSA). The reasoning behind EFSAs conclusion regarding animal safety was that this level has been used for over a decade without any reported intolerances (EFSA, 2014); they were however unable to draw any final conclusion as their answer was solely based on data from the National Research Council (NRC) from 1987. In regard to human health, they conclude that as there has been no change in the level of vitamin D in feed during the last decade it is safe to continue with the same level (EFSA, 2014). The fact that the human intake of vitamin D is below the recommended was not taken into consideration.

6. Vitamin D content in commercial feed for laying hens

In 2014 one of the major producers of feed for laying hens in Denmark used 75 μg vitamin D_3 per kg feed (personal communication with Danish Agro). A survey from 1996 conducted in USA showed that the average content of vitamin D_3 in feed for laying hens was 61.5 μg/kg feed (BASF, 1998), while in China the average level is 60 μg/kg feed (Zang et al., 2011).

How common the use of 25(OH)D_3 in feed has become since it was legalized in 2009 in Europe is unknown but Mattila et al. (2011) concluded from their investigations of commercial eggs that egg producers in Finland so far continued to use vitamin D_3 as the only vitamin D supplement, however in 2011 one producer in Denmark launched an egg where 25(OH)D_3 was used in the feed (Hedegaard, n.d.) and Liu, Greenfield, and Fraser (2014) analysed eggs from an Australian producer known to use a combination of 25(OH)D_3 and vitamin D_3.

7. Vitamin D content in commercial eggs

The content of vitamin D_3 and 25(OH)D_3 in commercial eggs reported by various sources since 1982 is displayed in Table 1. On average the content of vitamin D_3 and 25(OH)D_3 in commercial eggs is 1.5 μg/100 g and 0.5 μg/100 g, respectively.

8. Effect of enhancing vitamin D content through feed

Vitamin D in feed can be given either as vitamin D_3, 25(OH)D_3 or a combination of the two. Hens have a binding protein for vitamin D_3 in the plasma that forms a complex that is actively transported into yolk; however this binding protein also has a small affinity for 25(OH)D_3 and when 25(OH)D_3 is present in high concentrations it can displace vitamin D_3 and thereby be actively transported into yolk (Fraser & Emtage, 1976). When feeding vitamin D_3 the content of both vitamin D_3 and 25(OH)D_3 in the egg will increase, however using 25(OH)D_3 alone will not increase the vitamin D_3 content of the egg (Browning & Cowieson, 2014); and when fed a combination the displacement of vitamin D_3 by 25(OH)D_3 mentioned above is assumed to be minimal. Therefore, studies that use a combination have been included in assessing the impact feeding vitamin D_3 has on the vitamin D_3 content in eggs.

When starting a new feeding regime it will take some time before the vitamin D_3 content in the eggs reach a stable plateau (equilibration). The equilibration time is reported to be between 8 days and 3 weeks and it is independent of the vitamin D_3 content of the feed; and when an equilibrium is reached the content stays relatively stable (Mattila et al., 2003; Park et al., 2005; Yao, Wang, Persia, Horst, & Higgins, 2013). The duration of the studies feeding vitamin D_3 was between 2 weeks and 48 weeks while for 25(OH)D_3 it was between 4 and 9 weeks.

The age of the hens varies between trials, but Mattila et al. (2003) found that age does not have an effect on the transfer of vitamin D_3 from feed to the egg and it is assumed that this holds true for 25(OH)D_3 as well. A variation of commercial breeds, both white and brown, has been used.

It should be noted that the reported values are averages and that the content in the individual eggs will vary as the feed intake per hen varies. Kawazoe et al. (1994) found an average feed intake of 117 g ± 7.6 g (standard deviation, SD) and they showed a linear relationship between vitamin D content in the egg and the feed intake; within a group the vitamin D content varied between 7 and 11 μg/100 g and the coefficient of variance (CV) of the mean was 15%.[1] Kawazoe et al. (1994) used irradiated shitake and thereby D_2, but it is assumed that relation will be the same for vitamin D_3 and 25(OH)D_3. The CV% of vitamin D_3 and 25(OH)D_3 determined in egg yolk, fed a combination of the two, were comparable with an average CV of 24% (Browning & Cowieson, 2014). Genetic differences between birds are most likely also a contributor to the observed variance (Browning & Cowieson, 2014).

8.1. Effect of using vitamin D_3 as feed additive

We found eight studies where hens have been fed vitamin D_3 at levels between 26.6 μg/kg and 2555 μg/kg (Browning & Cowieson, 2014; Hayes et al., 2016; Kawazoe, Yuasa, Noguchi, Yamazaki, & Ando, 1996; Mattila et al., 2003; Mattila, Valaja, Rossow, Venäläinen, & Tupasela, 2004; Mattila, Lehikoinen, et al., 1999; Plaimast, Kijparkorn, & Ittitanawong, 2015; Yao et al., 2013).

The transfer efficiency (= [vitamin D_3 in yolk • yolk weight • egg production per hen per day]/[vitamin D_3 in feed • feed intake per hen per day • 100]) of vitamin D_3 from diet to egg was determined by Kawazoe et al. (1996) and Yao et al. (2013). The data from the two studies have shown that the transfer efficiency increases when the content in feed is increased: it is 7–8% when using 55 μg/kg feed; 11–14% when using 242.5–617.5 μg/kg; and 41–47% when using 2555 μg/kg. Yao et al. (2013) also calculated transfer efficiencies using data from other studies where similar levels of supplementation was used and the results were within the ranges given above.

In long term trials (≥24 weeks) no negative health effects of feeding 300–2555 μg vitamin D_3/kg feed have been observed (Mattila et al., 2004, 2003; Persia, Higgins, Wang, Trample, & Bobeck, 2013). Reduced feed intake, egg weight, shell quality and fertility have been reported for laying hens fed with 5000 μg/kg (Ameenuddin, 1986).

The vitamin D_3 content in eggs as a function of the content vitamin D_3 in feed is displayed in Fig. 1. There is a linear relationship between the vitamin D_3 content in the feed and the content of vitamin D_3 in the egg with a Pearson correlation coefficient (Pearson's r) of 0.987 and a p-value of $6 \cdot 10^{-19}$ (F-test) in spite of the differences in the design of the studies. The equation for the linear regression is:

Vitamin D_3 in eggs (μg/100 g) = 0.033 • vitamin D_3 in feed (μg/kg) - 0.58.

The linear range covers up to 617.5 μg/kg feed and the highest vitamin D content in eggs is 40 times higher than the lowest. Yao et al. (2013) fed 2555 μg/kg feed to one of their treatment groups and the resulting vitamin D_3 content was 287 μg/100 g; this result is not included in Fig. 1 as it is outside the linear range; the expected content from the linear regression would be 84 μg/100 g. This fits with the observation that the transfer efficiency increases with increased content of vitamin D_3 in the feed (Kawazoe et al., 1996; Yao et al., 2013).

The 25(OH)D_3 content in eggs as a function of vitamin D_3 in feed is better described by a logarithmic equation (black line in Fig. 2) with a Pearson's r of 0.6 compared to 0.47 for the linear relationship (dotted line in Fig. 2). However, the data is limited and the p-value for the F-test of the regression of the log-transformed data is 0.08. The only conclusion that can be draw is that 25(OH)D_3 content in eggs will increase with increased supplementation of vitamin D_3 but to a lesser degree than what is observed for the vitamin D_3 content in eggs.

8.2. Effect of using 25(OH)D_3 as feed additive

We have found six studies that report on the vitamin D content of eggs after hens have been fed 25(OH)D_3 alone or in combination with

[1] Approximate values, as they are calculated from data points in Figure 6 in Kawazoe et al. (1994).

Table 1
Average content of vitamin D_3 and 25(OH)D_3 in commercial whole eggs.

Origin	Vitamin D_3 (μg/100 g)	25(OH)D_3 (μg/100 g)	Reference
England	1.6	–	Jackson, Shelton, & Frier (1982)[b]
England	1.1	–	Sivell, Wenlock, and Jackson (1982)
Japan	1.3	–	Takeuchi, Okano, Teraoka, Murakami, and Kobayashi (1984)
Finland	1.7	0.32	(Mattila et al., 1992; Mattila, Piironen, Uusi-rauva, & Koivistoinen, 1993)
Canada	0.92	0.36	(Bilodeau et al., 2011)[a]
Finland	1.4	0.38	Mattila et al. (2011)
USA	2.46	0.56	(USDA, 2019)[a]
Russia	2.2	–	Chirkin, Karpov, Selemenev, and Shumskiy (2013)
Australia	0.83	0.92	Liu et al. (2014)
UK	2.5	0.13	Public Health England (2019)[a]
Ireland	1.1	1.0	Hayes et al. (2016)
England	1.7	0.5	(Guo, Kliem, Lovegrove, & Givens, 2017)[b]
Australia	0.95	0.9	(Dunlop et al., 2017) [b]
Denmark	1.37	0.44	(DTU Food, 2018)[a,b]
Average (SD)	**1.5 (± 0.6)**	**0.6 (± 0.3)**	

SD = Standard deviation- = not analysed [a] Food composition table or data produced for a food composition table [b] calculated average of results from free range, indoor and organic hens.

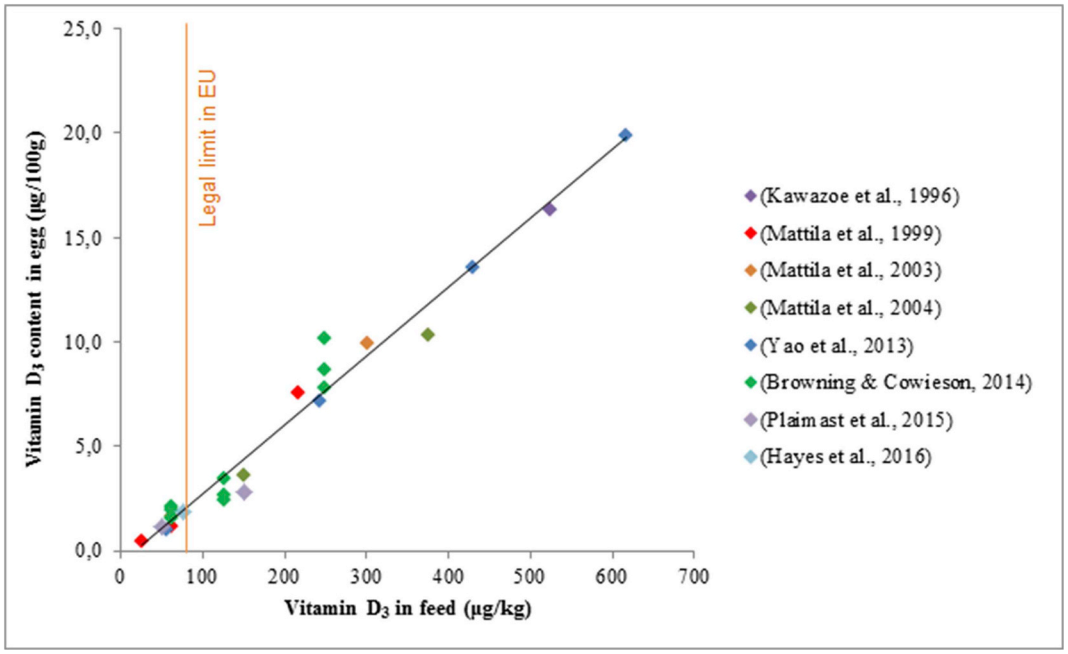

Fig. 1. Vitamin D_3 content in eggs as a function of vitamin D_3 content in feed.

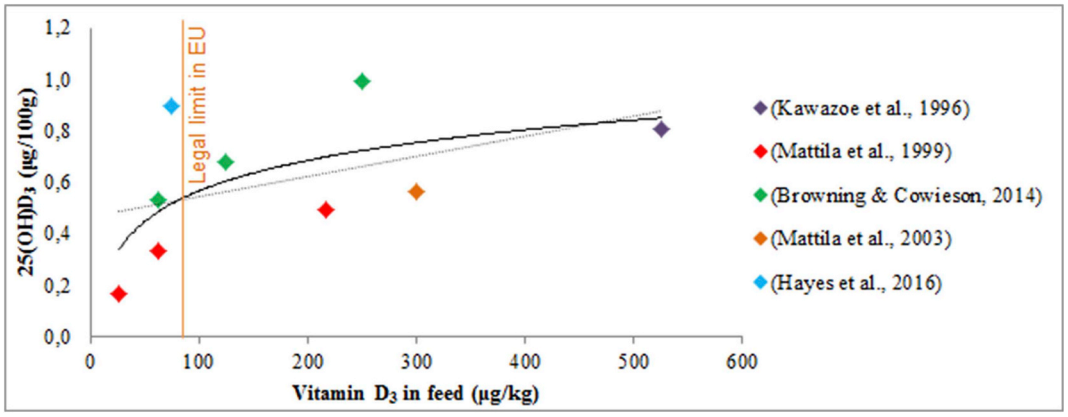

Fig. 2. 25(OH)D_3 content in eggs as a function of vitamin D_3 content in feed. Full black line is exponential regression while dotted line shows linear regression of the data.

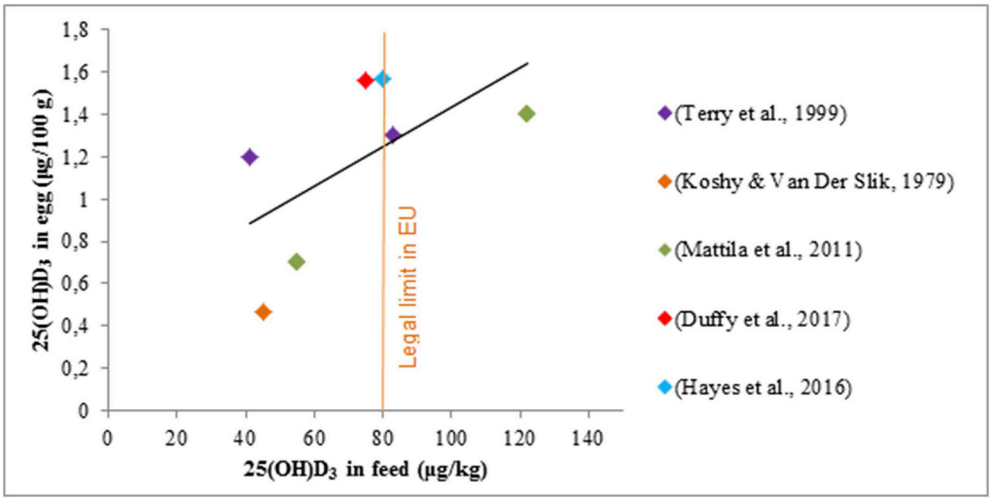

Fig. 3. 25(OH)D_3 content in eggs as a function of 25(OH)D_3 content in feed.

vitamin D_3 (Browning & Cowieson, 2014; Duffy et al., 2017; EFSA, 2005; Hayes et al., 2016; Koshy & Van Der Slik, 1979; Mattila et al., 2011).

Fig. 3 shows the 25(OH)D_3 content in eggs from hens that have been fed solely 25(OH)D_3. From Fig. 3 it is seen that only two studies have used at least two levels and that Mattila et al. (2011) is the only of the two that shows a significant increase; however, with a Pearson's r of 0.6 there is not the same linear inter-trial tendency as shown for vitamin D_3, but the limited amount of data should be taken into consideration. The dose-response is moderate compared to what is observed for vitamin D_3; the slope of the linear regression is 0.0093 which is approximately a factor 3 lower than the vitamin D_3 slope (see section 8.1).

Only two studies used doses above the legal limit of 80 µg/kg. Terry, Lanenga, McNaughton, and Stark (1999) supplemented laying hens with 41, 83, 413 and 825 µg 25(OH)D_3 per kg feed for 224 days, however eggs were analysed for vitamin D content after 112 days. No mortality was observed at any level. Based on decreased production parameters 825 µg were toxic while 413 µg 25(OH)D_3 per kg feed were just above the margin of the no effect level and for this reason the results of these two treatments are left out of Fig. 3. Eggs from hens fed 413 µg/kg had a 25(OH)D_3 content of 2.4 µg/100 g (Terry et al., 1999), therefore this could be considered to be the maximum obtainable level when using 25(OH)D_3 as the only vitamin source. Mattila et al. (2011) fed hens with 122 µg 25(OH)D_3/kg feed during 6 weeks without observing any negative effects on the egg shell quality.

Based on the results presented in Terry et al. (1999), p. 80 µg/kg was set as the safe level in the scientific opinion from EFSA regarding the use of 25(OH)D_3 in feed (EFSA, 2005).

Three studies used a combination of 25(OH)D_3 and vitamin D_3 (Browning & Cowieson, 2014; Duffy et al., 2017; Mattila et al., 2011); the results are displayed in Fig. 4. From the results of Browning and Cowieson (2014) ([2] in Fig. 4) it can be seen that increasing the 25(OH)D_3 supplementation while keeping the vitamin D_3 supplementation unchanged will give increased 25(OH)D_3 content in eggs. Feeding a combination of 250 µg vitamin D_3 and 69 µg 25(OH)D_3 results in a 25(OH)D_3 content of 2.7 µg/100 g in the eggs; this exceeds the maximum that can be obtained by feeding 25(OH)D_3 alone.

9. Enhancement using artificial UVB

9.1. Location and content of 7-DHC in hens

The legs and feet of hens have the highest content of 7-DHC when compared to the feathered part of the hens and the comb. Koch and Koch (1941) found that skin from the legs and feet of chicken had approximately 8 times as much 7-DHC compared to the body. Tian, Chen, Lu, Shau, and Holick (1994) measured the content of 7-DHC in various parts of the chicken skin. They found that the 7-DHC content in skin on the legs and feet were 29 and 23 times higher than the content in the back skin. After irradiation with 5000 J/m^2 no pre-vitamin D_3 was detected in the back skin (Tian et al., 1994). Schutkowski et al. (2013) found that the 7-DHC concentration in the un-feathered legs was 190 times higher than the concentration in the comb; the lowest concentration was found in the feathered parts of the hen. Edwards (2003) irradiated young chickens with UVB and looked at growth and bone development; he found that exposure from below was more than twice as effective as from above.

Uva, Mandich, and Vallarino (1983) found that vitamin D_3 concentration was 3 times higher in the uropygial gland (an oil gland located just above the base of the tail feathers) than in the unfeathered skin from the legs and concluded that this is the major site for production of vitamin D_3 (from 7-DHC). They also found vitamin D_3 in the extracts of feathers, the origin was hypothesized to be from the gland and that the oil was spread in the act of preening. Tian et al. (1994) found 7-DHC in lipid extracts from feathers; this could originate from the uropygial gland as Uva et al. (1983) hypothesized as the major production site of 7-DHC.

9.2. Effect of exposing hens to UVB from above

It has been shown possible to increase vitamin D in eggs when irradiating hens from above, if the hens do not receive vitamin D through the feed (Chiang, Hwang, & Holick, 1996, 1997). However, Lietzow et al. (2012) irradiated layers, with UVB light in the range of 280–310 nm for 4 weeks from above, with a dose of 540 J/m^2/day. Both non-supplemented hens and hens supplemented with 75 µg vitamin D_3/kg feed were exposed. They found no effect of UVB exposure on the content of vitamin D in eggs. However, as no increase was observed in the non-supplemented group, as would have been expected from the trials of Chiang et al. (1996, 1997) and older findings, the dose might have been too low. We have repeated the trial of Lietzow et al. (2012) with a dose of 547 J/m^2/day using UVB light emitting diodes (LED) with a central wavelength of 307 ± 2 nm (standard deviation) which should be more optimal for vitamin D_3 production (Barnkob et al., 2016); the outcome was however the same (Argyraki, 2017). We also tried with a dose of 4000 J/m^2/day, however, the treatment was stopped after 7 days as changes of the comb was observed and interpreted as erythema; in retrospect the changes could be harmless as free-range hens have more red and stiff comb than hens secluded from sunlight. Eggs collected before UVB exposure had a content of 1.1 µg/

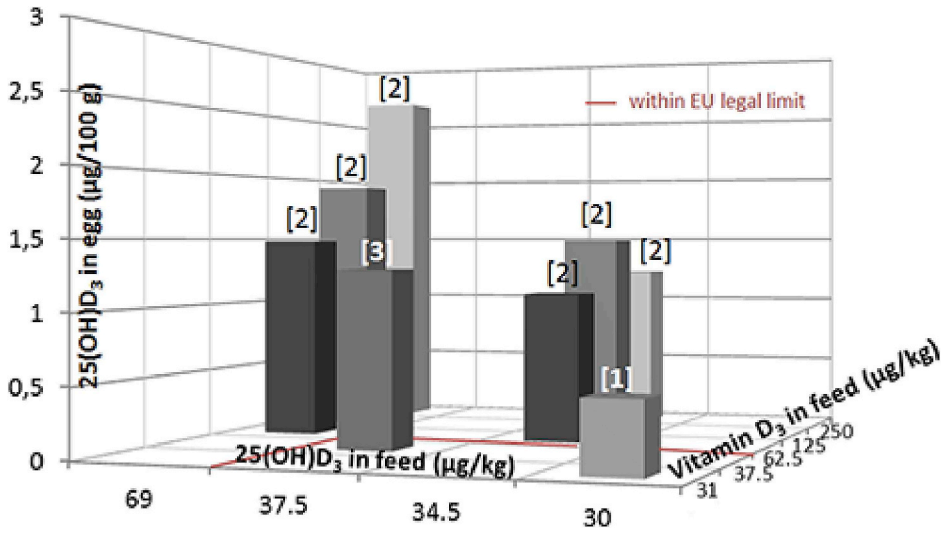

Fig. 4. 25(OH)D_3 content in eggs from hens fed 25(OH)D_3 in combination with vitamin D_3. [1] Mattila et al. (2011); [2] Browning and Cowieson (2014); [3] Duffy et al. (2017).

100 g while eggs collected after 7 days of exposure, where the treatment was stopped, had a vitamin D_3 content of 1.6 µg/100 g; equilibrium was probably not reached as the content 5 days after ceased exposure was 1.7 µg/100 g (Argyraki, 2017).

9.3. Effect of exposing the legs of hens to UVB

Schutkowski et al. (2013) exposed the legs of hens, supplemented with 75 µg vitamin D_3/kg feed, to a dose of 8208 J/m^2/day for 4 weeks; the reported content of vitamin D_3 and 25(OH)D_3 is 4,9 µg/100 g and 0,57 µg/100 g respectively. Kühn et al. (2015) irradiated the legs of hens to 7 different doses of UVB between 0 and 13680 J/m^2/day during 4 weeks and showed that vitamin D_3 in the eggs increased non-linearly with dose and at the highest dose the content was 4.5 µg/100 g and it was almost at equilibrium. 25(OH)D_3 content of eggs also increased non-linearly but did not increase beyond a dose of 5472 J/m^2/day where equilibrium was reached with a content of 0.6 µg/100 g.

10. Vitamin D enhancement of liquid egg products by direct exposure to UVB

Egg yolk contain around 140 µg 7-DHC per 100 g (Kühn, Schutkowski, Kluge, Hirche, & Stangl, 2014); therefore there is a potential that egg yolk can be enhanced with vitamin D by exposing it directly to UVB. In a small pilot study, described in Argyraki (2017), we exposed egg yolk and whole egg mix to UVB light using the UVB-LED with a central wavelength of 296 nm described in Barnkob et al. (2016). Layers of 3–4 mm liquid egg were placed in small weigh boats and then exposed to a dose of 3000 J/m^2; the exposure time was approximately 3.5 min. In whole egg the vitamin D_3 content increased from 1.1 to 4.3 µg/100 g and in egg yolk the content increased from 5.2 to 18.9 µg/100 g; close to a factor of 4 in increased content. By adjusting the UVB dose it would be possible to tailor the vitamin D content in liquid egg products. The penetration depth of UVB at 296 nm is 0.01–0.08 mm (Argyraki, 2017) therefore any practical application would have to be on a very thin film or in a dynamic treatment (e.g. stirring).

Dynamic treatment of liquid egg products with an UVC dose of 42,000 J/m^2 obtained in 30 min has the same disinfectant properties as heat pasteurization but with only minor changes to the rheological properties (de Souza & Fernández, 2013). It has been shown that UVB is more effective than UVC in inactivating the bacteria *Pseudomonas aeruginosa* in a trial where the exact same UV-LED as used for UVB exposure of hens in (Argyraki, 2017) was used; a dose of 10,000 J/m^2 left no viable colonies, and the exposure time was less than 12 min (Argyraki, Markvart, Bjørndal, Bjarnsholt, & Petersen, 2017). Thereby, there is potential that a dynamic treatment with UVB, alone or in combination with UVC could produce both safe and vitamin D enriched liquid egg products (yolk and whole egg mix).

11. Discussion on future trends

In Denmark the average intake of eggs per person per day is 25 g (Pedersen et al., 2015), in Ireland it is 16 g (O'Mahony et al., 2011). 10% of males in Denmark ingest more than 55 g per day (Pedersen et al., 2015).

How effective 25(OH)D_3 is compared to vitamin D_3 in increasing serum 25(OH)D_3 depends on the dose of 25(OH)D_3. The factor between the efficiencies is 1.04 at 5 µg/day, 1.5–5.5 at doses below 25 µg/day and 8 at doses above 50 µg/day (reviewed by Quesada-Gomez and Bouillon (2018) (Jakobsen et al., 2018),). The daily intake of 25(OH)D_3 from food is assumed to be less than 5 µg/day, for this reason we have used factor 1 when calculating the total vitamin D content. Cooking loss and bioavailability have not been included in the calculation of the average intake of vitamin D from eggs.

According to the findings in Section 7 the average total vitamin D content in commercial eggs is 2 µg/100 g (using factor 1 for 25(OH)D_3, see above). The maximum obtainable total vitamin D content in eggs from hens exposed to UVB, directed at the legs (in cages), is 5.1 µg/100 g (see Section 9.3). If eggs in Denmark and Ireland were increased to have a vitamin D content of 5.1 µg/100 g the daily average intake per person would increase with 0.8 µg and 0.5 µg, respectively (the actual intake will be lower as cooking loss was not included in the calculations). However, implementing UVB-lamps directed at the feet of the hens in a barn will be a challenging task. If placed near the floor the lamps will get dirty and a vast amount of cleaning will be required. In addition, only the hens nearby the lamp will be exposed and the dose each hen receives will be hard to control. If UVB-treatment is to be implemented in barn egg production facilities it will be necessary to develop solutions where the hens are irradiated from above to ensure even exposure and minimisation of costs for both maintenance and cleaning.

The gap between the recommended and the actual vitamin D intake is 3–17 µg/day/person and it therefore might be desirable to increase the vitamin D content of eggs further than what is possible with UVB exposure. There is an inter-trial linear relationship between the vitamin D_3 content in the feed and the content of vitamin D_3 in the egg (see section 8.1) and within the linear range the highest content is 20 µg/

100 g which is achieved with a vitamin D_3 content of 617.5 μg/kg feed. This level is well within the reported safe level for laying hens (Mattila et al., 2004, 2003; Persia et al., 2013). Feeding vitamin D_3 will also increase the content of 25(OH)D_3 in the egg, however the relationship between vitamin D_3 in feed and the 25(OH)D_3 is most likely logarithmic with a maximum around 1 μg/100 g (see Fig. 2). The total vitamin D content in eggs from hens supplemented with 617.5 μg/kg feed would be 21 μg/100 g; such a level would increase of the daily average intake from eggs per person with 4.8 μg in Denmark and 3.0 μg in Ireland. Although this content might be too high, for a single source of vitamin D, it illustrates that it is possible to design the vitamin D_3 content to any desired level through feed.

The use of 25(OH)D_3 in feed was legalized in 2009 in Europe, and trials have shown that 25(OH)D_3 content of eggs will increase when 25(OH)D_3 is added to the feed; however, there is not the same linear inter-trial tendency as observed for vitamin D_3, and the dose-response is a factor 3 less compared to vitamin D_3. Also, 25(OH)D_3 is more toxic to hens than vitamin D_3 as 413 μg/kg feed is on the margin of being toxic (Terry et al., 1999) whereas no negative effects of vitamin D_3 has been observed with a dose of 2555 μg/kg feed (Yao et al., 2013).

Feeding a combination of 250 μg/kg feed of vitamin D_3 and 69 μg/kg feed of 25(OH)D_3 gives a total vitamin D content of 12.9 μg/100 g (Browning & Cowieson, 2014). To obtain the same level using vitamin D_3 alone would require approximately 378 μg/kg feed.

In order to increase the content of vitamin D in eggs through feed the European legislation has to be changed, as the current limit is 80 μg vitamin D/kg feed. Fortunately, these limits are not set in stone; according to the procedure described in Regulation (EC) No 1831/2003 one can send an application with new information to the European Commission (using the application form found in Commission Regulation (EC) No 492/2008) the Commission shall send it to EFSA who shall provide a new opinion. If the opinion is positive it will most likely result in a draft Regulation made by the Standing Committee on Plant, Animals, Food and Feed (PAFF committee - section animal nutrition), on behalf of the European Commission, who will also decide if the draft Regulation should eventually be put into force. The Norwegian Food Safety Authority (NFSA) applied for increasing the maximum limit 20 times, from 75 μg/kg feed to 1.5 mg/kg feed for salmonids and received a positive opinion from EFSA (EFSA, 2017, 2019). The opinion was discussed at a PAFF committee meeting in April 2019 and was voted for with a favourable opinion (Standing Committee on Plant, Animals, 2019); and in May 2019 a feeding level of 60,000 IU/kg (~1.5 mg/kg feed) for salmonids was implemented into the existing regulation (Regulation (EU) 2019/849).

12. Conclusion

If choosing between feed and UVB exposure for natural enhancement of eggs with vitamin D, increasing the vitamin D_3 in the feed seems to give the most predictable and cost-effective results as only the feed has to be changed. In order to use this option in the EU an application must to be submitted to, and approved by, the European Commission; for salmonids the maximum limit was in 2019 increased 20 times upon such application.

A third option is to expose liquid egg products directly to UVB. With this method the vitamin D content can be tailored by adjusting the dose, and in addition the bacterial load is reduced due to the dual effect of UVB; however further research and development of equipment is needed for this to be implemented and approved as a novel food.

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