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Effect of production system, supermarket and purchase date on the vitamin D content of eggs at retail

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ABSTRACT

The vitamin D content of eggs from three retail outlets was measured over five months to examine the effects of production system (organic vs. free range vs. indoor), supermarket and purchase date on the concentration of vitamin D_3 and 25-hydroxyvitamin D_3. Results demonstrated a higher vitamin D_3 concentration in free range (57.2 ± 3.1 μg/kg) and organic (57.2 ± 3.2 μg/kg) compared with indoor (40.2 ± 3.1 μg/kg) ($P < 0.001$), which was perhaps related to increased vitamin D synthesis by birds having more access to sunlight, while 25-hydroxyvitamin D_3 concentration was higher ($P < 0.05$) only in organic eggs. The interaction ($P < 0.05$) between system and supermarket for both forms of vitamin D may relate to some incorrect labelling. Concentration of 25-hydroxyvitamin D_3 was higher ($P < 0.05$) in July and September than in August. The results indicate variations in vitamin D concentrations in eggs from different sources, thus highlighting the importance of accurate labelling.

1. Introduction

The two major sources of vitamin D for humans are *in vivo* synthesis by exposure to sunlight and dietary intake. Holick and Chen (2008) reported the links of vitamin D deficiency with increased risk of many common and serious diseases, such as cardiovascular disease, osteoporosis, common cancers and diabetes, in addition to its association with calcium homeostasis. Maintaining a serum 25-hydroxyvitamin D (25(OH) D) concentration of at least 75 nmol/L is regarded as being necessary for prevention of most vitamin D-related diseases (Vieth, 2011). There are many factors which limit *in vivo* synthesis of vitamin D via ultraviolet radiation, such as a more indoor lifestyle, latitude, skin pigmentation, ageing and sunscreen use (Holick, 1995). Thus, the prevalence of vitamin D deficiency in Europe has become a very concerning issue (Cashman et al., 2016). In the UK, a study showed that 87% of 7437 white British participants (92% Scotland residents) had plasma concentrations of 25(OH) D of below 75 nmol/L during winter and spring (Hypponen & Power, 2007). Therefore, the vitamin D intake from dietary sources has become more important in maintaining adequate vitamin D status. However, only certain foods (e.g. fish, meat, offal, eggs) are naturally rich in vitamin D (Schmid & Walther, 2013), and many of these are not consumed widely.

Eggs contain, not only vitamin D_3, but also significant quantities of 25-hydroxyvitamin D_3 (25(OH) D_3) (Mattila, Piironen, Uusi-Rauva, & Koivistoinen, 1993; Schmid & Walther, 2013), with the accumulation of vitamin D in the egg yolk rather than egg white (Fraser & Emtage, 1976). Studies have shown that the 25(OH) D_3 metabolite is five times more effective at raising plasma 25(OH) D_3 concentration in humans and has been reported to be absorbed at a faster rate when compared with an equivalent dose of vitamin D_3 (Cashman et al., 2012; Jetter et al., 2013).

Recently, the vitamin D concentration of whole eggs was given as 3.2 μg/100 g in the UK official food database (McCance & Widdowson, 2015). Eggs are available from different husbandry production systems, including indoor, free-range and organic in the UK retail outlets (Department for Environment & Rural Affairs, 2010). Evidence from a previous enhancement study demonstrated that vitamin D in eggs was increased from birds exposed to ultraviolet radiation (Kühn, Schutkowski, Kluge, Hirche, & Stangl, 2014). Thus, vitamin D concentrations of eggs may vary due to different production systems which give the birds varying lengths of sunlight exposure. However, there are limited data on the vitamin D content of retail eggs from the different UK production systems. As customers will expect more expensive eggs to be of better quality, it is important to inform the consumer about the effect of different production systems on the nutritional

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composition of eggs. One previous UK study suggested that the vitamin D_3 concentration of hens' eggs was significantly affected by housing system, with the vitamin D_3 content of egg yolk produced outdoors being significantly higher (44.1–69.2 nmol/L) than that of egg yolk produced indoors (17.3–18.7 nmol/L) (Hobbs-Chell, Stickland, & Wathes, 2010). However, egg yolk 25 (OH) D_3 concentration was not reported, and the study was not concerned with retail eggs.

The main objective of the current study was to explore the effects of production system (as labelled), supermarket and time of the year on the concentrations of vitamin D_3 and 25(OH) D_3 in the egg yolk from UK hens' eggs at retail. Although variation in vitamin D_3 content of eggs collected from UK farms due to production system has been reported (Hobbs-Chell et al., 2010), the data are unlikely to reflect eggs currently in the UK market. Accordingly, the current study focussed on the effect, not only of labelled production system, but also on supermarket and seasonal variation of two forms of vitamin D, vitamin D_3 and 25(OH) D_3 in UK retail eggs. This study also updates information on the vitamin D_3 and 25(OH) D_3 contents of eggs sold in the UK, which may improve the estimation of the contribution of eggs to vitamin D intakes of the general population.

2. Materials and methods

2.1. Sample collection

Eggs were purchased from three supermarkets (Supermarket 1, Supermarket 2, and Supermarket 3) in the Reading, Berkshire area, once per month, from July to November in 2012. On each occasion, packs of six eggs per box from three production systems (indoor, organic and free range, as identified on the label) were purchased from each supermarket, so a total of 270 eggs was collected. Following collection, eggs were transported directly to the laboratory, the yolks and whites of each egg were separated manually. The yolk was homogenised and decanted into 10 ml tubes before storage at −80 °C prior to vitamin D analysis. In total, 259 egg yolks (129 egg yolks for vitamin D_3 analysis; 130 egg yolks for 25(OH) D_3 analysis) were stored frozen, prior to analysis, as the egg whites and egg yolks of 11 eggs failed to separate during the processing. Nutritional information on the label of the purchased egg boxes was recorded for each sample.

2.2. Vitamin D_3 and 25(OH) D_3 analyses

The vitamin D_3 and 25(OH) D_3 concentrations of egg yolk samples were analysed by DSM Nutritional Products Ltd., (Basel, Switzerland). Vitamin D_3 analysis was carried out according to the method of Schadt, Gössl, Seibel, and Aebischer (2012).

The concentration of 25(OH) D_3 in the egg yolk samples was quantified by the standard method of the DSM Nutritional Products Ltd. using a LC-MS system (Agilent 1946). In brief, the sample was combined with d_6-25(OH) D_3 as an internal standard and the mixture dispersed in water. The suspension was extracted with tert-butyl methyl ether (TMBE). An aliquot of the TMBE phase was purified by semi-preparative normal-phase HPLC with a YMC-Pack-Sil column. An appropriate fraction was collected and analysed after solvent exchange by reversed-phase HPLC equipped with Aquasil C18 column and a mass selective detector.

2.3. Data analysis

A General Linear Model ANOVA (Minitab version 16; Minitab Inc., State College, PA, USA) was used to investigate the effect of (a) month of purchase (July to November 2012), (b) production system (indoor, organic or free-range) and (c) supermarket (S1, S2 or S3) on vitamin D_3 and 25(OH) D_3 concentrations. Tukey's pairwise multiple comparison test was used for *post hoc* analysis. Effects were considered significant when $P < 0.05$.

Total vitamin D concentration was calculated by using concentrations of vitamin D_3 + (5 × 25(OH) D_3) (McCance & Widdowson, 2015).

3. Results

3.1. Effect of production system

Concentrations of both vitamin D_3 and 25(OH) D_3 in egg yolk differed ($P < 0.001$), depending on production system (Table 1). Egg yolk from free range and organic systems contained a 42% greater concentration of vitamin D_3 than did those from the indoor system (Table 1). In addition, organic egg yolks had a higher ($P = 0.001$) concentration of 25(OH) D_3 than had egg yolks from free range and indoor systems, although no differences were observed between caged and free range systems.

3.2. Effect of purchase month

There was no effect of month purchased on the concentration of vitamin D_3 in egg yolks (Table 1; Fig. 1a). However, there was a significant effect of the system by month interaction ($P = 0.001$; Table 1), meaning that the vitamin D_3 concentration changes across different months varied by production system (Fig. 1a). The greatest ($P < 0.05$) concentration of vitamin D_3 in egg yolks tended to be found during summer months for indoor and organic eggs but, for free range eggs, the highest ($P < 0.05$) concentration was observed during the autumn months (Fig. 1a).

Month of collection had an effect ($P < 0.001$) on egg yolk 25(OH) D_3 concentration; however, as with vitamin D_3, no clear trend over time was observed (Fig. 1b). Again, a production system by month interaction was observed ($P = 0.001$; Fig. 1b). The lowest ($P < 0.05$) concentration of 25(OH) D_3 across all production systems was measured during August (Fig. 1b), but highest ($P < 0.05$) concentrations were observed during different months for each production system. In addition, no interaction ($P > 0.05$) was observed between supermarket and month on both vitamin D concentrations of the eggs.

3.3. Effect of supermarket

An effect of supermarket ($P = 0.009$) was observed for vitamin D_3 (Table 1; Fig. 2a) but not for 25(OH) D_3 (Table 1; Fig. 2b). The interaction effects of production system with supermarket were significant for both vitamin D_3 ($P < 0.001$) and 25(OH) D_3 ($P = 0.033$) (Table 1). For Supermarket 1, free range eggs were higher ($P < 0.05$) in vitamin D_3 concentration than were both caged and organic eggs. In addition, there was no interaction ($P > 0.05$) between supermarket and month for vitamin D_3 and 25(OH) D_3 (Table 1).

4. Discussion

4.1. General

The main objective of this study was to identify any differences in egg yolk vitamin D_3 or 25(OH) D_3 concentrations between three different production systems (indoor, free range and organic). To our knowledge, this is the first comparison study of both vitamin D forms between indoor and outdoor eggs from different UK retail supermarkets among varied months of the year.

Table 1
Concentrations of vitamin D_3 and 25(OH) D_3 (µg/100 g) of egg yolk as influenced by production system, month and supermarket (least square means ± pooled SE).

	Production system				*P* –Value for					
Vitamin D	Indoor	Free range	Organic	SEM	System	Month	Supermarket	System × month	System × supermarket	Supermarket × month
Vitamin D_3 (n = 130)	4.0^b	5.7^a	5.7^a	0.3	<0.001	NS[1]	0.009	0.001	<0.001	NS
25(OH)D_3 (n = 129)	1.3^b	1.4^b	1.6^a	0.06	0.001	<0.001	NS	0.001	0.033	NS
Total vitamin D[2]	10.4	12.6	13.8							

[a,b,c]Mean values with different superscripts within a row are significantly different (P < 0.05).
[1] NS, not significant (P > 0.05).
[2] Calculated as the sum of vitamin D_3 and (5 × 25(OH) D_3) concentrations (McCance & Widdowson, 2015).

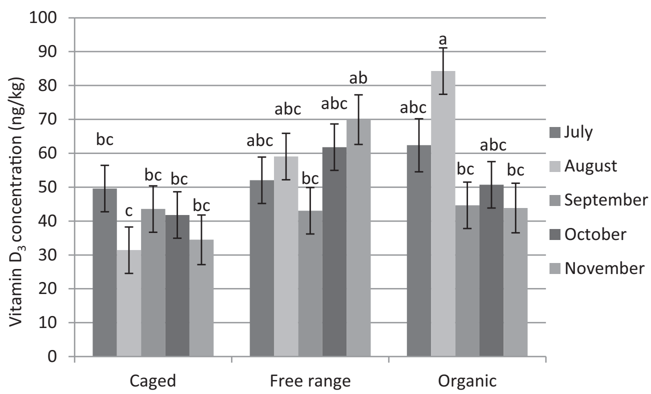

Fig. 1a. Effect of month on concentration (ng/kg) of vitamin D_3 in egg yolk from three production systems (least square means ± pooled SE). [a–d]Mean values with different letters are significantly different ($P < 0.05$) according to Tukey's pairwise multiple comparison test across all systems and months.

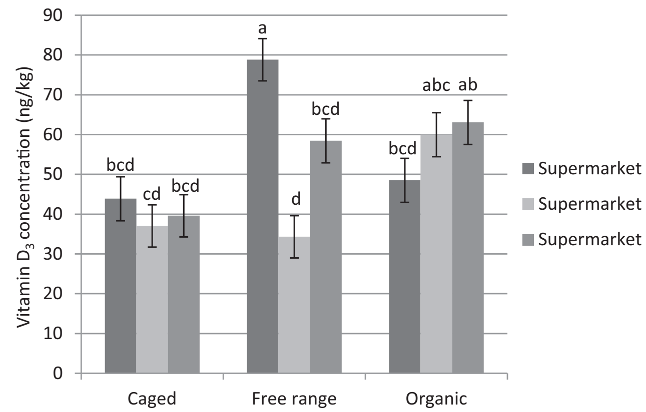

Fig. 2a. Effect of supermarket on concentration (ng/kg) of vitamin D_3 in egg yolk from three production systems (least square means ± pooled SE). [a–d]Mean values with different letters are significantly different ($P < 0.05$) according to Tukey's pairwise multiple comparison test across all systems and supermarkets.

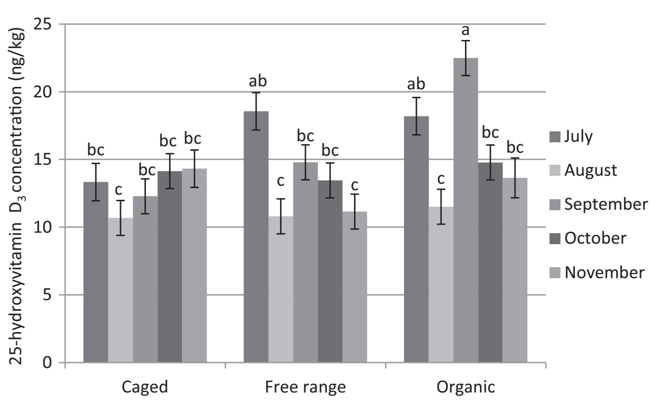

Fig. 1b. Effect of month on concentration (ng/kg) of 25-hydroxyvitamin D_3 in egg yolk from three production systems (least square means ± pooled SE). [a–d]Mean values with different letters are significantly different ($P < 0.05$) according to Tukey's pairwise multiple comparison test across all systems and months.

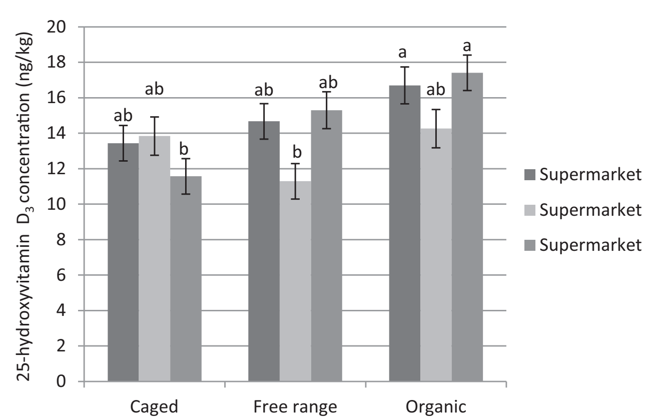

Fig. 2b. Effect of supermarket on concentration (ng/kg) of 25-hydroxyvitamin D_3 in egg yolk from three production systems (least square means ± pooled SE). [a–d]Mean values with different letters are significantly different ($P < 0.05$) according to Tukey's pairwise multiple comparison test across all systems and supermarkets.

4.2. Effect of production system

The vitamin D nutrition of birds is similar to that of humans (Bar, Sharvit, Noff, Edelstein, & Hurwitz, 1980); vitamin D is either synthesised *in vivo* by ultraviolet radiation from sunlight or consumed in the diet. In the UK, eggs produced by free range and indoor systems account for the majority of production systems (Department for Environment & Rural Affairs, 2013). Unlike the conventional indoor egg production system, free range and organic birds have more opportunity to be exposed to sunlight, as they can access pasture continuously during the day time with at least 4 square metres of range for one bird (RSPCA, 2014). As expected, the key finding of the current study is that both vitamin D_3 and 25(OH) D_3 were significantly different, according to production system. It is probable that the main reason for greater concentrations of vitamin D_3 and 25(OH) D_3 in eggs from free range and/or organic systems is higher sun exposure of the laying birds. Two previous studies also reported the effects of production systems on vitamin D concentration of eggs (Hobbs-Chell et al., 2010; Matt, Veromann, & Luik, 2009). Our results support previous UK data from Hobbs-Chell et al. (2010), who reported that eggs from free range and organic systems had higher vitamin D_3 concentrations than had those from a conventional indoor husbandry system. However, an Estonian study (Matt et al., 2009) demonstrated that eggs from organic systems have lower vitamin D_3 content than have indoor eggs. The inconsistency in results of these earlier studies can probably be explained by the variation in production system management between different countries, such as the difference in the diet or pasture usage for the birds.

The variation of vitamin D_3 concentrations between production systems was of greater magnitude than that observed for 25(OH) D_3 concentrations in the current study. An enhancement study (Kühn et al., 2014) also reported that the concentration of 25 (OH) D_3 can be increased in response to sunshine exposure (free range vs indoor system) but the increase was less pronounced than that of vitamin D_3. In our study, no difference in the 25(OH) D_3 concentration of eggs was seen between the indoor and free-range eggs, but there was a significantly higher amount of the 25 (OH) D_3 in the organic eggs. The reason for this is unclear, as the vitamin D content of the diets from different production systems in the present study is not known.

If levels of vitamin D_3 and/or 25(OH) D_3 in eggs from free range and organic systems were consistently higher than those from a conventional production system; this would provide the consumer of free range and organic eggs with an advantage in terms of vitamin D intake and potentially status. However, the significant interaction between the production system and supermarket for both vitamin D_3 and 25(OH) D_3 reflects inconsistencies in the ranking of both vitamin D forms by production systems between different supermarkets. This may indicate that the diets fed to birds at the farms supplying each supermarket were different or maybe some incorrect labelling exist, which would result in egg choice according to production system being less valuable.

The interaction between production system and collection month may suggest that the vitamin D_3 and 25(OH) D_3 concentrations in eggs produced indoors were more consistent than were the concentrations in eggs from free range and organic systems, possibly due to less variability in vitamin D synthesis from sunlight, since indoor birds only obtain vitamin D from their diet. For free range and organic birds, the potentially beneficial effect of exposure to sunshine may introduce unpredictable and changeable influences on vitamin D concentrations in eggs. There are several studies that have shown that vitamin D_3 and 25(OH) D_3 in eggs can be enhanced effectively by supplementing indoor birds with vitamin D_3- and 25(OH) D_3-enriched diets (Browning & Cowieson, 2014; Mattila, Lehikoinen, Kiiskinen, & Piironen, 1999; Yao, Wang, Persia, Horst, & Higgins, 2013). Therefore, for greater enrichment of total vitamin D in eggs, the combination of enhanced vitamin D in the hen's diet, together with exposure to sunlight, may present opportunities in the future. It may be noted, however, that, within the EU, there are upper limits imposed on the concentrations of vitamin D_3 (75 μg/kg diet; European Commission, 2004) and 25(OH) D_3 (80 μg/kg diet; European Commission, 2009) that may be added to the diet of laying hens. Moreover, the total dietary concentrations of vitamin D_3 and 25 (OH) D_3 in poultry must not exceed 80 μg/kg diet (European Commission, 2009). These regulations may reduce the opportunity for dietary enrichment.

4.3. Effect of purchase month and supermarket

In terms of the seasonal effect on vitamin D content of the eggs, an earlier study (Mattila, Vakonen, & Valaja, 2011) reported that egg yolk vitamin D_3 and 25(OH) D_3 contents were not significantly different between the spring and autumn. Our results for vitamin D_3 agree with Mattila et al. (2011) in that vitamin D_3 did not vary with month, but 25(OH) D_3 was affected by purchase month. Surprisingly, the lowest concentration of 25(OH) D_3 was observed during August for all production systems. With the limitations of a retail study (such as not knowing farm locations, the diet and vitamin D status of the producing birds and weather conditions at these locations during egg production), the reason for effect of purchase month on 25(OH) D_3 is unclear. It might be that there was less sunshine, or the ambient temperature was too high for the birds to be outside in the year the eggs were produced, or changing of the vitamin D content of the feed. Other factors, such as fearfulness or stress, can also influence the length of outdoor time of the birds (Mahboub, Müller, & Von Borell, 2004).

Variations observed between supermarkets for vitamin D_3 in the current study may be related to different conditions employed by egg producers supplying the supermarkets. A similar finding was reported in an US-based retail study (Exler, Phillips, Patterson, & Holden, 2013) where eggs were collected from twelve supermarkets, which found a wider range of vitamin D_3 (0.71–12.1 μg/100 g) or 25(OH) D_3 (0.43–1.32 μg/100 g) content of the hen's eggs. Due to the nature of retail studies, it is difficult to assess the reasons why supermarket affected vitamin D_3 but not 25(OH) D_3 concentrations in the egg yolks. One possible reason may be variation of vitamin D_3 concentration of the birds' diet. Previous egg enrichment studies (Browning & Cowieson, 2014; Mattila et al., 1999) have shown that supplementing the birds' diet with vitamin D_3 can result in higher vitamin D_3 and 25(OH) D_3 concentrations of the egg yolk, but the increased 25(OH) D_3 content is much less than that of vitamin D_3. This may be because 25(OH) D_3 is a metabolite of vitamin D_3. In addition, Mattila et al. (2011) showed that supplementing birds with a high dose of 25(OH) D_3 only increased 25(OH) D_3 in the egg yolk but not vitamin D_3.

4.4. Vitamin D intake from eggs

The results from the current study indicate that the mean concentrations of vitamin D_3 and 25(OH) D_3 for egg yolk are 5.14 μg/100 g and 1.42 μg/100 g, respectively. Assuming that the bioactivity of 25(OH) D_3 is five times that of the same dose of vitamin D_3 (McCance & Widdowson, 2015), the mean total effective vitamin D concentration ($D_3 + (5 \times 25(OH)\ D_3)$) of the egg yolk in this study would be 12.25 μg/100 g, which agrees well with those of the most recently published data (Benelam et al., 2012) which reported a mean egg yolk vitamin D concentration ($D_3 + (5 \times 25(OH)\ D_3)$) of 12.8 μg/100 g. Furthermore, if the average egg yolk weight 16.31 g is taken into account, one egg yolk in the current study contains a total of 2 μg vitamin D.

For UK adults aged up to 65 years, a daily 10 μg of vitamin D has been recommended by SACN (SACN, 2015). Thus, one egg per day, from the current study, would contribute about 20% of the RNI of vitamin D. It must be noted that cooking may lead to loss of vitamin D in eggs (Jakobsen & Knuthsen, 2014; Mattila, Ronkainen, Lehikoinen, & Piironen, 1999); thus, the cooking temperature and method need to be considered to avoid reduction of active vitamin D intake, and this suggests an area for further research.

4.5. Strengths and limitations of the study

Whilst the current study has limitations in terms of the relatively small sample of eggs, the new data on vitamin D in retail eggs from differing production systems provide new information of value to the UK public. This study only collected samples between July and November, which does not represent all seasonal changes throughout the whole year. Also, since all of the eggs were purchased from retail outlets with no indication of producer location, these data may not be totally representative of the UK. Furthermore, observed variations in yolk concentration due to purchase months and/or supermarkets were difficult to explain, given that the producer details, bird diets, farming practices and weather conditions at time of production were not known.

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Subsequent investigations should study variations in vitamin D content of eggs from different producers throughout the UK, taking account the effects of the birds' diet and sun exposure.

5. Conclusions

Results from the current study confirm that vitamin D_3 and 25 (OH) D_3 concentrations in egg yolk vary over time and between production systems. Eggs from outdoor production systems are likely to contain higher amounts of both vitamin D forms, but this may not be a consistent effect. Future work is needed on eggs collected from different areas of the UK throughout the whole year to provide more information on vitamin D content of retail eggs. In addition, further studies should focus on identifying the reasons behind these variations to enable a greater understanding of how variation in vitamin D content can be minimised, for the benefit of the consumer.

The current study indicates that the average effective vitamin D content of each egg is about 2 μg (excluding any effect of factors such as cooking), which would mean that one egg per day would contribute 20% of the UK RNI for vitamin D. However, in the absence of up to date information on the vitamin D content of other relevant foods, such as fish and meat, it is difficult to reliably estimate vitamin D intake from the diet of the general population in the UK. So future retail studies should investigate the vitamin D content of other vitamin D- containing foods to improve estimates of dietary vitamin D intake of the UK population.

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References

Bar, A., Sharvit, M., Noff, D., Edelstein, S., & Hurwitz, S. (1980). Absorption and excretion of cholecalciferol and of 25-hydroxycholecalciferol and metabolites in birds. *The Journal of Nutrition, 110*, 1930–1934.

Benelam, B., Roe, M., Pinchen, H., Church, S., Buttriss, J., Gray, J., & Finglas, P. (2012). New data on the nutritional composition of UK hens' eggs. *Nutrition Bulletin, 37*, 344–349.

Browning, L. C., & Cowieson, A. J. (2014). Vitamin D fortification of eggs for human health. *Journal of the Science of Food and Agriculture, 94*, 1389–1396.

Cashman, K. D., Dowling, K. G., Skrabakova, Z., Gonzalez-Gross, M., Valtuena, J., De Henauw, S., et al. (2016). Vitamin D deficiency in Europe: Pandemic? *The American Journal of Clinical Nutrition, 103*, 1033–1044.

Cashman, K. D., Seamans, K. M., Lucey, A. J., Stocklin, E., Weber, P., Kiely, M., & Hill, T. R. (2012). Relative effectiveness of oral 25-hydroxyvitamin D3 and vitamin D3 in raising wintertime serum 25-hydroxyvitamin D in older adults. *The American Journal of Clinical Nutrition, 95*, 1350–1356.

Department for Environment, Food and Rural Affairs (2010). Eggs Marketing Standards. URL <http://archive.defra.gov.uk/foodfarm/food/industry/sectors/eggspoultry/faq/marketing.htm#eps>. Accessed 25.02.2015.

Department for Environment, Food and Rural Affairs (2013). UK egg packing station throughput and prices dataset. URL <https://www.gov.uk/government/statistics/egg-statistics>. Accessed 09.03. 2015.

European Commission (2004). Council Directive 70/524/EEC concerning additives in feedingstuffs. URL <http://ec.europa.eu/food/food/animalnutrition/feedadditives/docs/c_50_en.pdf>. Assessed 18.04.16.

European Commission (2009). Commission Regulation (EC) No 887/2009 concerning the authorisation of a stabilised form of 25-hydroxycholecalciferol as a feed additive for chickens for fattening, turkeys for fattening, other poultry and pigs. URL <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32009R0887>. Assessed 18.04.16.

Exler, J., Phillips, K. M., Patterson, K. Y., & Holden, J. M. (2013). Cholesterol and vitamin D content of eggs in the U.S. retail market. *Journal of Food Composition and Analysis, 29*, 110–116.

Fraser, D. R., & Emtage, J. S. (1976). Vitamin D in the avian egg. Its molecular identity and mechanism of incorporation into yolk. *Biochem Journal, 160*, 671–682.

Hobbs-Chell, H., Stickland, N., & Wathes, C. (2010). Vitamin D and calcium concentrations in eggs from commercial laying hen husbandry systems. In *Proceedings of 2010 Annual Meeting of World's Poultry Science Association (UK Branch). Belfast* (pp. 16).

Holick, M. F. (1995). Environmental factors that influence the cutaneous production of vitamin D. *Americal Journal of Clinical Nutrition, 61*, 638S–645S.

Holick, M. F., & Chen, T. C. (2008). Vitamin D deficiency: A worldwide problem with health consequences. *American Journal of Clinical Nutrition, 87*, 1080s–1086s.

Hypponen, E., & Power, C. (2007). Hypovitaminosis D in British adults at age 45 y: Nationwide cohort study of dietary and lifestyle predictors. *American Journal of Clinical Nutrition, 85*, 860–868.

Jakobsen, J., & Knuthsen, P. (2014). Stability of vitamin D in foodstuffs during cooking. *Food Chemistry, 148*, 170–175.

Jetter, A., Egli, A., Dawson-Hughes, B., Staehelin, H. B., Stocklin, E., Gossl, R., Henschkowski, J., et al. (2013). Pharmacokinetics of oral vitamin D3 and calcifediol. *Bone, 59*, 14–19.

Kühn, J., Schutkowski, A., Kluge, H., Hirche, F., & Stangl, G. I. (2014). Free-range farming: A natural alternative to produce vitamin D-enriched eggs. *Nutrition, 30*, 481–484.

Mahboub, H. D. H., Müller, J., & Von Borell, E. (2004). Outdoor use, tonic immobility, heterophil/lymphocyte ratio and feather condition in free-range laying hens of different genotype. *British Poultry Science, 45*, 738–744.

Matt, D., Veromann, E., & Luik, A. (2009). Effect of housing systems on biochemical composition of chicken eggs. *Agronomy Research, 7*, 662–667.

Mattila, P., Lehikoinen, K., Kiiskinen, T., & Piironen, V. (1999). Cholecalciferol and 25-hydroxycholecalciferol content of chicken egg yolk as affected by the cholecalciferol content of feed. *Journal of Agricultural and Food Chemistry, 47*, 4089–4092.

Mattila, P., Piironen, V., Uusi-Rauva, E., & Koivistoinen, P. (1993). Determination of 25-hydroxycholecalciferol content in egg yolk by HPLC. *Journal of Food Composition and Analysis, 6*, 250–255.

Mattila, P., Ronkainen, R., Lehikoinen, K., & Piironen, V. (1999). Effect of household cooking on the vitamin D content in fish, eggs, and wild mushrooms. *Journal of Food Composition and Analysis, 12*, 153–160.

Mattila, P. H., Vakonen, E., & Valaja, J. (2011). Effect of different vitamin D supplementations in poultry feed on vitamin D content of eggs and chicken meat. *Journal of Agricultural and Food Chemistry, 59*, 8298–8303.

McCance, R. A., & Widdowson, E. M. (2015). *Composition of foods* (7th ed.). Cambridge: The Royal Society of Chemistry.

RSPCA (2014). The Welfare of Laying Hens. URL <http://www.rspca.org.uk/adviceandwelfare/farm/layinghens>. Accessed 28.06.2016.

SACN (2015). Draft Vitamin D and Health Report. URL <https://www.gov.uk/government/consultations/consultation-on-draft-sacn-vitamin-d-and-health-report>. Assessed 18.04.16.

Schadt, H. S., Gössl, R., Seibel, N., & Aebischer, C. P. (2012). Quantification of vitamin D3 in feed, food, and pharmaceuticals using high-performance liquid chromatography/tandem mass spectrometry. *Journal of AOAC International, 95*, 1487–1494.

Schmid, A., & Walther, B. (2013). Natural vitamin D content in animal products. *Advances in Nutrition, 4*, 453–462.

Vieth, R. (2011). Why the minimum desirable serum 25-hydroxyvitamin D level should be 75 nmol/L (30 ng/ml). *Best Practice & Research Clinical Endocrinology & Metabolism, 25*, 681–691.

Yao, L. X., Wang, T., Persia, M., Horst, R. L., & Higgins, M. (2013). Effects of vitamin D3-enriched diet on egg yolk vitamin D3 content and yolk quality. *Journal of Food Science, 78*, C178–C183.

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