

# *Food fortification and biofortification as potential strategies for prevention of vitamin D deficiency*

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**Food fortification and biofortification as potential strategies for prevention of vitamin D deficiency**

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Running title: 25-hydroxyvitamin D<sub>3</sub> fortified foods

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1 **Abstract**

2 Hypovitaminosis D is widespread throughout the world. The cutaneous production of vitamin  
3 D through sunlight can be limited by several factors (*e.g.* skin pigmentation, sunscreen usage  
4 and, increasingly, indoor lifestyle). Thus, diet has become an important strategy to increase  
5 vitamin D intake and status. However, there are a limited number of foods (*e.g.* eggs, oily fish  
6 and wild mushroom) naturally enriched with vitamin D, and concentrations can vary  
7 significantly between and within species. Therefore, the need for vitamin D fortified foods  
8 (including via direct fortification and biofortification) to support adequacy of vitamin D status  
9 [blood 25-hydroxivitamin D (25(OH) D)] is a corollary of several limitations to synthesise  
10 vitamin D from sunlight. Ergocalciferol (vitamin D<sub>2</sub>) and cholecalciferol (vitamin D<sub>3</sub>) can be  
11 found in some mushrooms and animal-derived foods, respectively. Evidence has shown  
12 vitamin D<sub>3</sub> is more effective than vitamin D<sub>2</sub> at raising 25(OH) D blood concentrations. The  
13 vitamin D metabolite, 25(OH) D<sub>3</sub>, is present in animal-derived foods (*e.g.* meat, eggs and fish),  
14 and several intervention trials have shown 25(OH) D<sub>3</sub> to be more effective at raising blood  
15 25(OH) D concentrations than vitamin D<sub>3</sub>. In addition, 25(OH) D<sub>3</sub> supplements may prove to  
16 be preferable to vitamin D<sub>3</sub> for patients with certain clinical conditions. However, there is  
17 limited evidence on the effect of 25(OH) D<sub>3</sub> fortified foods on human vitamin D status and  
18 health. Therefore, long-term randomised controlled trials to evaluate the effect of 25(OH) D<sub>3</sub>  
19 fortified foods on vitamin D status are needed for both the general population and patients with  
20 certain conditions.

21

22 **Key words**

23 Vitamin D, 25(OH) D, fortification, biofortification, randomised controlled trial, dairy

## 24 **Introduction**

25 Vitamin D is a lipid soluble vitamin that acts as a hormone (Nair & Maseeh 2012), which  
26 generally refers to ergocalciferol (vitamin D<sub>2</sub>) and cholecalciferol (vitamin D<sub>3</sub>) (Tripkovic *et*  
27 *al.* 2012). Vitamin D<sub>2</sub> and vitamin D<sub>3</sub> are produced by fungi and the skin of vertebrates,  
28 respectively (Wacker & Holick 2013). The role of vitamin D in musculoskeletal health is well  
29 established (Wolff *et al.* 2008). Recently, vitamin D deficiency has been suggested to be  
30 associated with several non-musculoskeletal health outcomes, such as cardiovascular disease,  
31 certain cancers and type 2 diabetes, although mechanisms are not clear (Wang *et al.* 2017).  
32 Vitamin D status is assessed by measuring the blood concentration of circulating 25-  
33 hydroxyvitamin D (25(OH) D) (Holick 2009). Widespread hypovitaminosis D is now  
34 acknowledged (Hilger *et al.* 2014), although there is some dispute about the thresholds for  
35 vitamin D deficiency and insufficiency (Spiro & Buttriss 2014). In the UK, vitamin D  
36 deficiency is defined as 25(OH) D <25 nmol/L (SACN 2016). The UK *National Diet and*  
37 *Nutritional Survey (NDNS)* reported that in 2008-2012 24% men and 21.7% of women (aged  
38 19-64 years) had vitamin D deficiency (Bates *et al.* 2014). With seasonal variation, the  
39 prevalence of hypovitaminosis D in the UK was alarmingly high during winter and spring. A  
40 cross-sectional study conducted in the UK by Hypponen and Power (2007) reported that during  
41 the winter and spring months 25(OH) D concentrations were <25 nmol/L, <40 nmol/L and <75  
42 nmol/L in 15.5%, 46.6% and 87.1% of participants, respectively. There are several additional  
43 contributors to hypovitaminosis D, such as skin pigmentation, sunscreen usage, and an  
44 increasingly indoor lifestyle, all of which reduce the cutaneous production of vitamin D (Holick  
45 2004). Furthermore, vitamin D supplement can also contribute to vitamin D intake, however,  
46 uptake of supplements tends to be low (Hennessy *et al.* 2017; Datta *et al.* 2016 ). As a result,  
47 dietary intake of vitamin D has become more important than before (O'Mahony *et al.* 2011)  
48 and in recognition of this, in 2016, the UK Scientific Advisory Committee on Nutrition

49 (SACN) recommended the national population dietary of 10 µg vitamin D daily for everyone  
50 aged 4 years and older (SACN 2016). As there are a limited number of foods naturally enriched  
51 with vitamin D (such as egg yolk, oily fish and wild mushroom) (Schmid & Walther 2013),  
52 other strategies to improve vitamin D dietary intake are essential.

53

#### 54 **Vitamin D forms, metabolites and absorption**

55 The two forms of vitamin D, D<sub>2</sub> and D<sub>3</sub>, have similar chemical structures apart from vitamin  
56 D<sub>2</sub> having an additional methyl group and double bond (Hollis 1984). Humans and animals  
57 usually synthesise vitamin D<sub>3</sub> in the skin by converting 7-dehydrocholesterol in the epidermis  
58 to pre-vitamin D<sub>3</sub> in response to exposure to ultraviolet B radiation (UVB). Pre-vitamin D<sub>3</sub> then  
59 undergoes a temperature-dependent isomerisation to produce vitamin D<sub>3</sub> over approximately 3  
60 days (Holick & Chen 2008). Vitamin D<sub>2</sub> and D<sub>3</sub>, obtained from the diet, are absorbed with  
61 long-chain triglycerides in the small intestine and then incorporated into chylomicrons and  
62 transported via lymph to the circulation (Guo *et al.* 2018b).

63 After entering the blood circulation, vitamin D<sub>2</sub> and D<sub>3</sub> follow the same pathways to  
64 synthesise the biologically active form of 1, 25(OH)<sub>2</sub>D. There are two hydroxylation reactions:  
65 the first reaction occurs in the liver where vitamin D<sub>2</sub> and vitamin D<sub>3</sub> are hydroxylated to  
66 25(OH) D<sub>2</sub> and 25(OH) D<sub>3</sub> by the vitamin D-25-hydroxylase; the second occurs in the kidney  
67 where 25(OH) D<sub>2</sub> and 25(OH) D<sub>3</sub> are converted to 1α,25(OH)<sub>2</sub> D<sub>2</sub> and 1α,25(OH)<sub>2</sub> D<sub>3</sub>,  
68 respectively, by the 25-hydroxyvitamin D-1α-hydroxylase (DeLuca 1974).

69

#### 70 **Food sources and content of vitamin D**

71 Vitamin D<sub>2</sub> and D<sub>3</sub> can be found in fungi (*e.g.* mushrooms) and animal-derived foods (*e.g.* eggs,  
72 oily fish), respectively (McCance & Widdowson 2015). In addition, there are significant  
73 quantities of the 25(OH) D metabolite in animal-derived foods (Ovesen *et al.* 2003). Previous

74 studies (Guo *et al.* 2017b; Lu *et al.* 2007; Phillips *et al.* 2011) have showed that the vitamin D  
75 concentrations of these foods can vary significantly between and within species (O'Mahony *et*  
76 *al.* 2011). For example, Phillips *et al.* (2011) collected and analysed the vitamin D<sub>2</sub>  
77 concentrations in 10 types of mushrooms from retail suppliers in the US, and reported that they  
78 were low (0.1-0.3 µg/100 g) in *Agaricus bisporus* (White Button, Crimini, Portabella) and  
79 Enoki, moderate in Shiitake and Oyster (0.4-0.7 µg/100 g), and high in Morel, Chanterelle,  
80 Maitake (5.2-28.1 µg/100 g). Furthermore, the vitamin D content of foods may relate to  
81 different production systems and the time of the year. For example, our study (Guo *et al.* 2017b)  
82 investigated eggs from three different production systems (organic, free range and indoor) over  
83 5 months and showed a higher vitamin D<sub>3</sub> content in free range eggs (57.2 ± 3.1 µg/ kg) and  
84 organic eggs (57.2 ± 3.2 µg/ kg) compared with indoor eggs (40.2 ± 3.1 µg/ kg) (*P* <0.001). A  
85 seasonal effect on the vitamin D content of eggs has also been reported by others (Mattila *et*  
86 *al.* 2011a). The study of Lu *et al.* (2007) evaluated the vitamin D content of salmon, and found  
87 that farmed salmon had only ~ 25% of the vitamin D content of wild salmon and cooking may  
88 also cause detrimental loss of vitamin D. The study of Jakobsen & Knuthsen *et al.* (2014)  
89 investigated the loss/ retention of vitamin D during different cooking methods (frying, baking  
90 and boiling) in eggs and margarine. The results showed there was 39-45% retention of vitamin  
91 D content in eggs and margarine during baking in an oven for 40 minutes, while frying resulted  
92 in vitamin D retention of 82-84%. The author concluded that the loss/ retention of vitamin D  
93 during typical household cooking should be taken into account when calculating the dietary  
94 intake of vitamin D.

95 In general, there are two approaches to fortify foods with vitamin D: 1) 'direct fortification'  
96 by adding vitamin D into foods and 2) 'biofortification' of food by fortifying animal's diet with  
97 vitamin D (Cashman & Kiely 2016). For countries such as the UK where vitamin D  
98 fortification of foods is not mandatory (Kiely & Black 2012), populations have to rely on

99 dietary sources (including supplements) to maintain an adequate vitamin D status when there  
100 are limited sunlight. In the UK, the mean daily vitamin D dietary intake (excluding  
101 supplements) was 2.9 and 2.5 µg/day for men and women, respectively (NDNS 2008/2009-  
102 2011/2012; Bates *et al.* 2014)), which is far less than the current UK dietary reference nutrient  
103 intake (RNI) for vitamin D of 10 µg/day (SACN 2016). Therefore, approaches to increase  
104 vitamin D dietary intake have become necessary and urgent.

105

## 106 **Comparative effectiveness of different forms of vitamin D at raising blood 25(OH) D** 107 **concentrations**

### 108 *Vitamin D<sub>2</sub> and vitamin D<sub>3</sub>*

109 Blood 25(OH) D [the summation of 25(OH) D<sub>2</sub> and 25(OH) D<sub>3</sub>] concentration is widely used  
110 as a biomarker of vitamin D status (SACN 2016). Early studies reported conflicting results on  
111 the relative effectiveness of dietary vitamin D<sub>3</sub> compared with vitamin D<sub>2</sub> for increasing  
112 serum/plasma 25(OH) D concentrations (Tripkovic *et al.* 2017). Tripkovic *et al.* (2012)  
113 conducted a systematic review and meta-analysis comparing the effects of dietary vitamin D<sub>2</sub>  
114 and vitamin D<sub>3</sub> on serum 25(OH) D concentrations in humans. Data were included from seven  
115 randomised controlled trials (RCTs) and the results showed that vitamin D<sub>3</sub> intake led to a  
116 greater absolute change in serum/plasma 25(OH) D levels from baseline than vitamin D<sub>2</sub>, with  
117 a weighted mean difference of 15.23 (95% CI: 6.12, 24.34; Z=3.28; I<sup>2</sup>=81%; P=0.001).  
118 Recently, a review by Wilson *et al.* (2017) summarised the evidence to date on the relative  
119 effectiveness of vitamin D<sub>3</sub> and vitamin D<sub>2</sub> at raising 25(OH) D concentrations and concluded  
120 that most RCTs showed that vitamin D<sub>3</sub> is more effective.

121

### 122 *Vitamin D<sub>3</sub> and 25(OH) D<sub>3</sub>*



123 Of the few studies performed, most have found that the vitamin D metabolite 25(OH) D<sub>3</sub> given  
124 orally increases vitamin D status more efficiently than oral vitamin D<sub>3</sub>, although no consensus  
125 has been established for the relative potency of 25(OH) D<sub>3</sub> and vitamin D<sub>3</sub> (Jakobsen 2007).  
126 Our recent review (Guo *et al.* 2018b) summarised the available evidence (Cashman *et al.* 2012;  
127 Catalano *et al.* 2015; Jetter *et al.* 2014; Navarro-Valverde *et al.* 2016) comparing 25(OH) D<sub>3</sub>  
128 with vitamin D<sub>3</sub> on serum or plasma 25(OH) D<sub>3</sub> concentrations, and concluded that the relative  
129 effectiveness of 25(OH) D<sub>3</sub> to vitamin D<sub>3</sub> ranged from 3.13 to 7.14. These variable results  
130 probably reflect differences in study designs and/or characteristics of the investigated subjects.  
131 In addition, evidence from available RCTs (Guo *et al.* 2018b) indicates that 25(OH) D<sub>3</sub> fortified  
132 dairy drink resulted in plasma 25(OH) D reach its peak significantly earlier than with vitamin  
133 D<sub>3</sub> fortified dairy drink. Thus, supplementation with 25(OH) D<sub>3</sub> might increase vitamin D status  
134 more efficiently and effectively than vitamin D<sub>2</sub> and vitamin D<sub>3</sub>. Moreover, since the use of  
135 25(OH) D<sub>3</sub> avoids the need for the liver to convert vitamin D<sub>3</sub> to 25(OH) D<sub>3</sub> it may be of  
136 particular value to patients with impaired liver function.

137

## 138 **Food fortification with vitamin D**

### 139 *Direct fortification*

140 In the US and Canada, several common foods, such as milk, orange juices, breakfast cereals,  
141 yogurts and cheeses are fortified with vitamin D (Holick *et al.* 2011). In Europe, vitamin D  
142 mandatory and voluntary fortification policies and practice vary from country to country (Spiro  
143 & Buttriss 2014). A meta-analysis was performed by Black *et al.* (2012), which included  
144 sixteen RCTs to evaluate the efficacy of vitamin D food fortification for improving vitamin D  
145 status. The results showed a mean intake of vitamin D of 11 µg/day from fortified foods (range  
146 3-25 µg/day) increased serum/plasma 25(OH) D by 19.4 nmol/L (95% CI: 13.9-24.9), which  
147 corresponded to a 1.2 nmol/L (95% CI: 0.72, 1.68) increase in serum/plasma 25(OH) D for

148 each 1 µg ingested. Thus, vitamin D direct fortification could be an effective strategy to  
149 increase vitamin D status in the general UK population.

150 In the US and Canada, much of the vitamin D intake is from fortified foods (Fulgoni *et al.*  
151 2011; Langlois *et al.* 2010). The major fortified foods contributing to vitamin D intake in these  
152 countries are fluid milk, ready-to-eat cereals and margarine (Calvo *et al.* 2004; Feldman *et al.*  
153 2011). The study by Langlois *et al.* (2010) estimated vitamin D status among 5306 individuals  
154 aged 6- 79 years in the 2007- 2009 *Canadian Health Measures Survey* and showed that the  
155 mean 25(OH) D concentration was 67.7 nmol/L, and that 4% and 10% of the population had  
156 vitamin D deficiency (<27.5 nmol/L) and inadequacy (<37.5 nmol/L), respectively. In addition,  
157 subjects who consumed vitamin D fortified milk had higher 25(OH) D concentrations than  
158 non-consumers. In addition, voluntary fortification of foods with vitamin D has occurred in  
159 Finland since 2003 (Pilz *et al.* 2018), and the data from the *Finnish Health 2011 Survey* showed  
160 that mean serum 25(OH) D increased from 47.6 nmol/L in year 2000 to 65.4 nmol/L in 2011  
161 (Jaaskelainen *et al.* 2017). However, a recent review (Calvo & Whiting 2013) questioned the  
162 adequacy of vitamin D fortified foods in the US and Canada to meet the needs of all race,  
163 gender and age groups. Furthermore, a review by Kiely *et al.* (2012) pointed out well-designed  
164 sustainable fortification strategies are needed to take account for diversity in food consumption  
165 patterns. In the UK, the food fortification policy was effective in preventing rickets in the  
166 1950s; however, the mandatory vitamin D fortification policy was banned when over-  
167 fortification of some milk products led to cases of hypercalcaemia in young children (British  
168 Pediatric Association 1956). More research is needed to explore the safety of vitamin D  
169 fortification, including the range of products and doses of vitamin D added in each.

170

171 *Biofortification*

172 Biofortification of vitamin D is an alternative strategy to increase vitamin D intakes in countries  
173 and regions where policies and practices limit use of ‘direct fortification’.

174 Our previous review provides an overview of recent vitamin D biofortification studies (Guo  
175 *et al.* 2018b), and found that the amount of vitamin D<sub>3</sub> and 25(OH) D<sub>3</sub> in eggs, fish and milk  
176 increases in response to vitamin D<sub>3</sub> supplementation of the diets of hens, fish and cows.  
177 However, evidence relating to 25(OH) D<sub>3</sub> supplementation of animals’ diets is very limited,  
178 with the only available data for hens (Guo *et al.* 2018b). Interestingly, egg enrichment studies  
179 (Duffy *et al.* 2017; Mattila *et al.* 2011b) showed that supplementing hens’ diets with 25(OH)  
180 D<sub>3</sub> results in an increase in the 25(OH) D<sub>3</sub> concentration, but not vitamin D<sub>3</sub>, of the egg yolk.  
181 Thus, foods biofortified or fortified with either vitamin D<sub>3</sub> or 25(OH) D<sub>3</sub> are likely to have a  
182 variable effect on human vitamin D status (Mattila *et al.* 2011b).

183 Our recent milk biofortification study (Guo *et al.* 2018a) used a total of 60 dairy cows  
184 randomised to vitamin D<sub>3</sub> or 25(OH) D<sub>3</sub> dietary supplementing treatments, within the maximum  
185 permitted European Union (EU) vitamin D<sub>3</sub> concentration (2 mg/day vitamin D<sub>3</sub>) for feed. The  
186 results showed that supplementing dairy cows’ feed with 25(OH) D<sub>3</sub> significantly increased  
187 circulating plasma concentrations of 25(OH) D<sub>3</sub> in the cows. However, there was also no  
188 significant effect of the treatment on milk 25(OH) D<sub>3</sub> concentrations ( $P=0.193$ ), the mean  
189 25(OH) D<sub>3</sub> concentrations for non-fortified and 25(OH) D<sub>3</sub> dietary treatments were 869 and  
190 1001 ng/kg, respectively. In addition, the vitamin D concentration (100-3,300 ng/kg) of the  
191 biofortified milk was negligible and far less than the current UK vitamin D recommended  
192 intake of 10 µg/day (SACN 2016). In the future, more studies are needed to explore which  
193 forms and doses of vitamin D added to animal diets, within the bounds of EU regulation (EC  
194 2017; EFSA 2012), including those of fish, may have the greatest impact on human dietary  
195 quality.

196

197 *Evidence from human intervention studies with 25(OH) D<sub>3</sub> fortified foods*

198 Evidence of the effect of 25(OH) D<sub>3</sub> fortified food on increasing vitamin D status is limited.  
199 We were the first to compare the effects of dairy drinks fortified with either 20 µg 25(OH) D<sub>3</sub>  
200 or 20 µg vitamin D<sub>3</sub> on changes in human 24-hour vitamin D status (Guo *et al.* 2017a). The  
201 results showed plasma 25(OH) D<sub>3</sub> was significantly higher after the 25(OH) D<sub>3</sub> fortified dairy  
202 drink compared with the vitamin D<sub>3</sub> fortified dairy drink and control (non-fortified dairy drink),  
203 which was reflected in the 1.5-fold and 1.8-fold greater incremental area under the curve of  
204 plasma 25(OH) D<sub>3</sub> for the 0-8 hour response, respectively. However, we did not investigate the  
205 long-term effects of consuming the 25(OH) D<sub>3</sub> and vitamin D<sub>3</sub> fortified dairy drinks.

206 Hayes *et al.* (2016) conducted an 8-week RCT to compare the effects of consuming vitamin  
207 D<sub>3</sub> or 25(OH) D<sub>3</sub> biofortified eggs (7 per week for 8 weeks), obtained from feeding hens with  
208 the maximum concentration of vitamin D<sub>3</sub> or 25(OH) D<sub>3</sub> lawfully allowed in their diets, with a  
209 control treatment ( $\leq 2$  commercial eggs/week), on winter serum 25(OH) D concentrations in  
210 healthy adults. At the 8 week follow-up in winter the vitamin D status of the subjects who  
211 consumed the vitamin D<sub>3</sub> or 25(OH) D<sub>3</sub> biofortified eggs was maintained [50.4 nmol/L  
212 (SD=21.4) and 49.2 nmol/L (SD=16.5) for vitamin D<sub>3</sub> and 25(OH) D<sub>3</sub> group, respectively],  
213 while the control group's vitamin D status significantly decreased over winter ( $-6.4 \pm 6.7$   
214 nmol/L). In contrast with our study (Guo *et al.* 2017a), there was no significant difference  
215 between vitamin D<sub>3</sub> and 25(OH) D<sub>3</sub> biofortified egg consumption on the participants' serum  
216 25(OH) D concentrations. The reason is unknown, but maybe because baseline vitamin D status  
217 (mean 46.2 nmol/L) was much higher than our study (mean 31.7 nmol/L), and vitamin D dose  
218 (3.5-4.5 µg/egg) for fortified eggs (Hayes *et al.* 2016) was only 20% of ours (20 µg/day) (Guo  
219 *et al.* 2017a)..

220

221 **25(OH) D<sub>3</sub> supplementation and human health**

222 As an alternative strategy to increase vitamin D status, it is possible that supplementation with  
223 25(OH) D<sub>3</sub> may benefit human health more than with vitamin D<sub>3</sub>, although the evidence is  
224 limited. A study of Bischoff-Ferrari *et al.* (2012) provided 20 µg/day of 25(OH) D<sub>3</sub> or vitamin  
225 D<sub>3</sub> to 20 healthy postmenopausal women over 4 months [mean baseline serum 25(OH) D  
226 concentration was 42 nmol/L]. The results showed 25(OH) D<sub>3</sub> supplementation resulted in a  
227 more immediate and sustained increase of serum 25(OH) D concentrations than vitamin D<sub>3</sub>  
228 supplementation. The mean 25(OH) D concentration increased to 221 nmol/L and 99 nmol/L  
229 for 25(OH) D<sub>3</sub> and vitamin D<sub>3</sub> supplementation, respectively. In addition, 25(OH) D<sub>3</sub>  
230 supplementation was found, on average, to result in a 2.8-fold increased odds of maintained or  
231 improved lower extremity function (OR=2.79, 95% CI: 1.18-6.58), and a 5.7 mmHg decrease  
232 in systolic blood pressure compared with vitamin D<sub>3</sub> ( $P=0.0002$ ). In another study, Jean *et al.*  
233 (2008) provided 10-30 µg/day 25(OH) D<sub>3</sub> to haemodialysis patients for 6 months, and the  
234 results showed vitamin D status increased from 30 nmol/L to 126 nmol/L, and 25(OH) D<sub>3</sub>  
235 supplementation corrected their excess bone turnover.

236 A review by Brandi & Minisola (2013) summarised the available evidence in this area and  
237 concluded that for populations that have specific conditions (such as long-lasting vitamin D  
238 osteomalacia, liver failure, iatrogenic inhibition of liver 25-hydroxylases, inactivating  
239 mutations of genes encoding liver 25-hydroxylase, kidney failure with elevated PTH,  
240 nephrosis, transplanted patients, male hypogonadism), supplementation with 25(OH) D<sub>3</sub> may  
241 prove to be preferable to vitamin D<sub>3</sub>. The reasons might be because 25(OH) D<sub>3</sub> avoids the need  
242 for hepatic metabolism of vitamin D<sub>3</sub> to 25(OH) D<sub>3</sub>, which results in 25(OH) D<sub>3</sub> more quickly  
243 entering the blood circulation (Holick 1995; Ross *et al.* 2011).

244 Currently, vitamin D<sub>2</sub> and vitamin D<sub>3</sub> are legally permitted to be added to foods, but addition  
245 of 25(OH) D<sub>3</sub> is not (EC No 1925/2006). Future studies should focus on better defining the

246 long-term effects of 25(OH) D<sub>3</sub> fortified foods on vitamin D status and human health, compared  
247 to vitamin D<sub>3</sub> and vitamin D<sub>2</sub>.

248

### 249 **Conclusions and future directions**

250 Vitamin D deficiency and insufficiency have become global problems, especially where  
251 sunlight is limited by latitude, cultural reasons or lifestyle (Hilger *et al.* 2014). The UK  
252 government advisory committee, SACN, recommends an intake of 10 µg/day of vitamin D for  
253 the UK general population (SACN 2016). However, it is a great challenge to meet this  
254 recommendation from solely natural dietary sources and uptake of supplements tends to be  
255 low. Two potential strategies to increase vitamin D content of food are direct fortification and  
256 biofortification via animal diet supplementation. However, evidence from RCTs is limited on  
257 the effect of vitamin D fortified foods on human vitamin D status and human health. The  
258 available evidence suggests that the vitamin D metabolite, 25(OH) D<sub>3</sub>, might be more efficient  
259 than vitamin D<sub>2</sub> and D<sub>3</sub> at raising serum or plasma 25(OH) D<sub>3</sub> concentrations in both general  
260 healthy subjects and clinical patients. In addition, 25(OH) D<sub>3</sub> may have an advantage of  
261 improving the health of certain clinical patients, although the evidence for this is limited.  
262 Therefore, 25(OH) D<sub>3</sub> fortified foods (including direct fortification and biofortification) should  
263 be further explored in the future, and additional RCTs should be conducted to investigate the  
264 effect of 25(OH) D<sub>3</sub> fortified foods on vitamin D status and human health in both healthy  
265 subjects and clinical patients.

266

### 267 **Conflict of interest**

268 The authors have no conflict of interest to disclose.

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