## Natural Vitamin D Content in Animal Products<sup>1</sup>

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## ABSTRACT

Humans derive most vitamin D from the action of sunlight in their skin. However, in view of the current Western lifestyle with most daily activities taking place indoors, sun exposure is often not sufficient for adequate vitamin D production. For this reason, dietary intake is also of great importance. Animal foodstuffs (e.g., fish, meat, offal, egg, dairy) are the main sources for naturally occurring cholecalciferol (vitamin D-3). This paper therefore aims to provide an up-to-date overview of vitamin D-3 content in various animal foods. The focus lies on the natural vitamin D-3 content because there are many countries in which foods are not regularly fortified with vitamin D. The published data show that the highest values of vitamin D are found in fish and especially in fish liver, but offal also provides considerable amounts of vitamin D. The content in muscle meat is generally much lower. Vitamin D concentrations in egg yolks range between the values for meat and offal. If milk and dairy products are not fortified, they are normally low in vitamin D, with the exception of butter because of its high fat content. However, as recommendations for vitamin D intake have recently been increased considerably, it is difficult to cover the requirements solely by foodstuffs. *Adv. Nutr. 4: 453–462, 2013.* 

## Introduction

The term vitamin D, as used here, refers to the secosteroids ergocalciferol (vitamin D-2)<sup>2</sup> and cholecalciferol (vitamin D-3). Their structural difference lies only in the C-17 side chain, the former having an additional C-22 to C-23 double bond and a C-24 methyl group (**Fig.** 1). Vitamin D-2 is formed by UV radiation (in particular UVB radiation) of ergosterol, which is found in plants, fungi, and invertebrates. For vitamin D-3, the genesis is the same, but the provitamin is 7-dehydrocholesterol, which is found in vertebrates. In humans as well, vitamin D-3 is synthesized in the skin with the help of sunlight (1).

After the production of vitamin D-3 in the skin or after its absorption from the gut, the vitamin is transported to the liver, bound in blood to vitamin D–binding protein. In the liver, it is hydroxylated to 25-hydroxycholecalciferol [25(OH)-D-3] (calcidiol), the rate being related to substrate supply (2). 25(OH) D<sub>3</sub> is the major circulating metabolite of vitamin D, and its concentration in blood is used as a measure of vitamin D status (1,3,4). In the kidney, 25(OH)D is activated with a second hydroxylation to 1,25-dihydroxycholecalciferol [1,25(OH)<sub>2</sub>-D-3] (calcitriol), the production being tightly regulated by calcium (via parathyroid hormone), by phosphate (via fibroblast growth factor 23), and during growth and pregnancy (1,5,6). The main target tissues of  $1,25(OH)_2$ -D-3 are intestine, bone, and kidney where it carries out hormonal functions. 25(OH)-D-3 as well as  $1,25(OH)_2$ -D-3 are catabolized and the final products are excreted in the bile (2).

Humans derive most vitamin D from the action of sunlight in their skin. However, the current Western lifestyle with most activities taking place indoors often prevents sufficient sun expose. In addition, season and latitude may diminish the intensity of the sun, and also clothing, sunscreen, and skin pigmentation interfere with vitamin D synthesis (6). In this case, dietary intake becomes increasingly important. However, only a few foodstuffs, mainly of animal origin (e.g., fish, meat, offal, egg, dairy), are recommended in literature to be a valuable source of naturally occurring vitamin D-3 (2). This report compiles the available information on vitamin D content in various animal foods in relation to the prevention of vitamin D deficiency. The focus is on the natural vitamin D-3 content because there are many countries (e.g., most of the European countries) in which foods are not regularly fortified with vitamin D. 25-Hydroxycholecalciferol is included because this metabolite contributes to the total biological activity (7).

## **Functions of vitamin D**

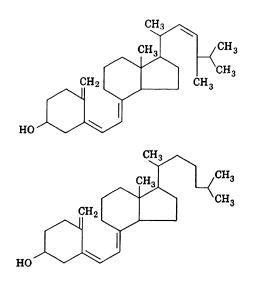
The vitamin D metabolite  $1,25(OH)_2$ -D-3 acts as a hormone in the regulation of calcium and phosphorus metabolism

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<sup>&</sup>lt;sup>2</sup> Abbreviations used: 25(OH)-D, 25-hydroxyvitamin D [25(OH)-D-2 and 25(OH)-D-3]; 25(OH)-D2, 25-hydroxyergocalciferol; 1,25(OH)<sub>2</sub>-D-3, 1,25-dihydroxycholecalciferol; 25(OH)-D-3, 25-hydroxycholecalciferol; UHT, ultra heat treated; vitamin D-2, ergocalciferol; vitamin D-3, cholecalciferol

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**Figure 1** Structure of ergocalciferol (top) and cholecalciferol (bottom).

with the aim of maintaining normal calcium and phosphorus concentrations in serum ensuring a normal mineralization of bone (1,2). Vitamin D may also play a role in muscle development because vitamin D receptor is located in muscle tissue and vitamin D deficiency leads to muscle weakness. Several further tissues exhibit a vitamin D receptor, among other brain, prostate, breast, and colon tissues as well as immune cells. Epidemiologic findings suggest benefits of vitamin D on cardiovascular mortality, hypertension, colorectal cancer, multiple sclerosis, type 1 diabetes, immune function, and inflammation (1,8). However, apart from the positive influence of vitamin D on fracture risk and falls, data are not yet compelling but need further evaluation in clinical studies.

# Requirements of vitamin D and vitamin D deficiency

25(OH)-D-3 concentrations in blood are used to determine the vitamin D status (9). Serum levels of <25 nmol/L come along with the risk of rickets (deformation of bones due to inadequate development) in children and of osteomalacia (brittle bones) in adults. Levels of 25 to 49 nmol/L are seen as insufficient (10). The question about the optimal serum concentration cannot yet be answered clearly. In adults, data suggest that serum levels of >75 nmol/L are optimal for bone health as well as for nonskeletal health benefits of vitamin D. However, concentrations between 50 and 60 nmol/L are often seen as (more conservative) target because above that there is no further increase in bone density, muscle function, and parathyroid hormone suppression (6,11).

With sufficient UVB exposure, dietary intake of vitamin D is not necessary. However, due to environmental influences (see above) synthesis of vitamin D is not always adequate; therefore, dietary intake is advisable. The RDA of vitamin D for females and males between 9 and 70 y of age in the United States is set at 15  $\mu$ g/d (600 IU/d) and also the Endocrine Society suggests this amount for adults

aged 19–50 y but indicates that to increase the blood level of 25(OH)-D-3 consistently above 30  $\mu$ g/L, at least 37.5–50.0  $\mu$ g/d (1500–2000 IU/d) of vitamin D may be required. The German-speaking nutrition societies (D-A-CH recommendations) recently increased their recommendation for children and adults from 5  $\mu$ g/d (200 IU/d) to 20  $\mu$ g/d (800 IU/d), whereas the European RDA for adults remains at a daily intake of 5  $\mu$ g/d (200 IU/d), but the upper limit from 50  $\mu$ g/d (200 IU/d) was increased to 100  $\mu$ g/d (4000 IU/d) (11–15).

## Vitamin D in animal food

Only a few foodstuffs naturally contain vitamin D, and these foodstuffs are mainly of animal origin. Above all, vitamin D-3 is found in these products together with its metabolite 25(OH)-D-3. As the latter is also biologically active and such contributes to dietary intake, it is included in the following compilation (7). There is still no consensus, if compared with vitamin D-3, that the bioactivity of 25(OH)-D-3 is higher. A bioactivity up to 5 times higher is proposed, but could not be demonstrated in all investigations (16). However, the outcome of a recent study supports a factor of 4-5 (17). Other metabolites are only contained in trace amounts and do not contribute much to biological vitamin D activity (7). In dairy, varying amounts of vitamin D-2 and 25-hydroxyergocalciferol [25(OH)-D-2] are documented and therefore are also mentioned in the overview. However, supplementation studies suggest that vitamin D-2 is not as potent as vitamin D-3 in increasing serum 25-hydroxyvitamin D [25(OH)-D] concentrations (18). Described below are results of studies determining the vitamin D content by HPLC or liquid chromatography-linear mass spectrometry (LC-MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods. Investigations based on classic bioassays measuring antirachitic activity are not included in this overview.

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#### Meat, offal, and meat products

A search of the national food composition databases of Denmark, France, Germany, Switzerland, Canada, and the United States for vitamin D content in various raw meat cuts yielded the following values:  $0.0-9.0 \ \mu g/kg$  for beef,  $1.0-23.0 \ \mu g/kg$  for pork,  $1.0-61.0 \ \mu g/kg$  for lamb,  $0.0-50.0 \ \mu g/kg$  for veal,  $0.0-14.0 \ \mu g/kg$  for poultry, and  $0.0-23.0 \ \mu g/kg$  for various meat products (19–24). However, the vitamin D content is not available for all meat cuts, and the databases do not give any information about how the data were obtained. A few values are inexplicably high and may be based on a single analysis and/or that the analyzed meat originated from animals supplemented with vitamin D.

**Table 1** presents vitamin D-3 and 25(OH)-D-3 content of meat and offal found in literature. Data on animals whose diets were supplemented beyond the normal with vitamin D-3 or 25(OH)-D-3 were excluded. Koshy and VanDerSlik (25) examined 25(OH)-D-3 contents in raw liver, muscle, and kidney from cows in Michigan. The highest concentrations

Reference	Foodstuff	D-3	25(OH)-D-3
		μg/kg	µg∕kg
Koshy and VanDerSlik (25)	Cow, liver		2.7–5.3
	Cow, muscle		1.5–3.4
	Cow, kidney		5.1–9.8
Mattila et al. (26)	Beef, steak	<0.5	0.8
	Beef, chuck	<0.5	0.5
	Beef, liver	< 0.5	3.4
Kobayashi et al. (27)	Beef (separable lean)	0 <sup>2</sup>	
	Beef, liver	0 <sup>2</sup>	
Montgomery et al. (30)	Beef, top round steak	2.8	1.3
<b>JJJJJJJJJJJJJ</b>	Beef, strip loin steak	4.1	1.4
	Beef, liver	8.6	7.7
	Beef, kidney	7.4	23.3
Montgomery et al. (29)	Beef, strip loin steaks	9.5	4.1
	Beef, liver	140.8	1.9
Wertz et al. (28)	Beef, longissimus muscle	110.0	0.9
Foote et al. (33)	Beef, longissimus dorsi muscle	1.1	1.7
0010 01 01. (55)	Beef, semimembranosus muscle	0.8	1.7
	Beef, infraspinatus muscle	1.1	
			0.9
	Beef, liver	1.9	2.6
	Beef, kidney	1.3	3.0
Montgomery et al. (32)	Beef, longissimus muscle	5.3	0.3
	Beef, liver	13.3	0.8
	Beef, kidney	12.2	1.6
Montgomery et al. (31)	Beef, strip loin	2.6-10.0	0.2–0.4
	Beef, liver	12.3-14.2	0.7–0.9
	Beef, kidney	4.2-27.1	0.9–2.0
Purchas et al. (34)	Beef, shoulder roast	1.0	4.8
	Beef, rump mince	1.3	5.8
	Beef, strip loin	1.1	2.7
	Beer, topside roast	0.9	4.8
Mattila et al. (26)	Pork, fillet	1.1	<0.6
	Pork, Boston butt	3.4	0.7
	Pork, liver	4.0	4.4
Kobayashi et al. (27)	Pork (separable lean)	7.0 <sup>2</sup>	
, , , , , ,	Pork (total edible)	13.8 <sup>2</sup>	
	Pork, liver	12.5 <sup>2</sup>	
Clausen et al. (35)	Pork, loin	1.5	0.9
	Pork, leg inside	0.5	0.7
	Pork, thin belly	2.1	1.4
	Pork, neck	1.6	1.3
Wilborn et al. (36)	Pork, longissimus muscle	13.9	69.3
Bilodeau et al. (37)	Pork, lean ground	2.1	0.9
biloueau et al. (37)	Pork, medium ground		
		2.4	0.9
	Pork, center chops boneless (whole)	1.7	0.8
	Pork, tenderloin (whole)	1.8	1.4
	Pork, back ribs (whole)	3.9	1.3
	Pork, shoulder blade roast boneless (whole)	3.7	1.2
	Pork, loin rib roast (whole)	3.1	1.0
Kobayashi et al. (27)	Sheep (mutton and lamb)	0 <sup>2</sup>	
Purchas et al. (34)	Lamb, leg roast	0.6	12
	Lamb, leg steak	0.4	10.4
	Lamb, rack roast	0.3	5.7
	Lamb, shoulder chop	0.9	8.4
Kobayashi et al. (27)	Chicken, breast	0 <sup>2</sup>	
	Chicken, liver	2.0 <sup>2</sup>	
	Turkey	1.0 <sup>2</sup>	
	Domestic duck	23.0 <sup>2</sup>	
Mattila et al. (26)	Chicken	2.9	2.5
Mattila et al. (38)	Chicken, leg and thigh	3.0	≤2.0
	Chicken, fillet	2.0	≦2.0 ≤2.0

Table 1.	Published	natural	vitamin	D-3	and	25(OH)-D-3	content	in	raw	meat	and	offal	1
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<sup>1</sup> Mean values. 25(OH)-D-3, 25-hydroxycholecalciferol. <sup>2</sup> Sum of vitamins D-3 and D-2.

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were found in kidney and the lowest in muscle meat. Mattila et al. (26) investigated raw beef meat and liver samples that were purchased in retail stores in the Helsinki area in autumn and spring. Vitamin D-3 concentrations were <0.5  $\mu$ g/kg in all beef samples; 25(OH)-D-3 concentrations ranged from <0.5 to 3.5  $\mu$ g/kg, with the highest values in liver. The results did not vary substantially between the 2 seasons. Kobayashi et al. (27) did not find any vitamin D (sum of vitamins D-3 and D-2) in bovine meat and offal when analyzing 69 different Japanese foods purchased from markets. Between 2000 and 2004, several experiments of the Department of Animal Science at the Iowa State University regarding the effect of vitamin D-3 supplementation on beef tenderness were published (28-33). The vitamin D-3 concentrations in the control groups of the steers (receiving 90% concentrate diet with a commercial nutrient supplement) ranged between 0.8 and 10.0  $\mu$ g/kg in raw meat, between 1.9 and 140.8  $\mu$ g/kg in raw liver, and between 1.3 and 27.1 µg/kg in raw kidney. For 25(OH)-D-3, the concentrations in meat were 0.2–4.1  $\mu$ g/kg, in liver 0.7–7.7  $\mu$ g/kg, and in kidney 0.9–23.3  $\mu$ g/kg. Muscle concentrations of vitamin D-3 and 25(OH)-D-3 vary significantly according to biological type of cattle, liver concentrations, however, do not (31). Additional vitamin D-3 supplementations up to 7.5 million IU/steer for 8 or 9 days before slaughter increased vitamin D-3 and 25(OH)-D-3 values in meat and offal (29,30,32,33). Purchas et al. (34) found vitamin D-3 concentrations between 0.9 and 1.3  $\mu$ g/kg and 25(OH)-D-3 concentrations between 2.7 and 5.8  $\mu$ g/kg in raw beef meat (various cuts) of animals raised on pasture without any supplements.

Analyses by Mattila et al. (26) in raw pork meat purchased in several retail stores and pork liver bought from 1 meat wholesaler in the Helsinki area in spring and autumn showed only minor variations in the results of the 2 seasons. In the case of the muscle samples, a positive correlation between the fat and vitamin D-3 content was found. Compared with Mattila et al. (26), Kobayashi et al. (27) found rather high vitamin D (sum of vitamins D-3 and D-2) amounts in meat and liver of pork, respectively. In raw pork cuts of varying fat content, Clausen et al. (35) measured vitamin D-3 concentrations from 0.5 to 2.1 µg/kg and 25(OH)-D-3 concentrations from 0.7 to 1.4 µg/kg. Vitamin D-3 and 25(OH)-D-3 were significantly associated with the fat content of whole cuts, and in the cuts 8 to 10 times more vitamin D-3 and 2 to 3 times more 25(OH)-D-3 was found in lard and intramuscular fat than in the lean parts. Wilborn et al. (36) investigated the effect of supplemental vitamin D on pork quality. In the control group, vitamin D-3 and 25(OH)-D-3 concentrations in longissimus muscle were 13.9 and 69.3  $\mu$ g/kg, respectively, which is substantially higher than the values given in the other publications. Last, but not least, Bilodeau et al. (37) determined vitamin D-3 and 25(OH)-D-3 concentrations in various raw pork cuts collected from major retail centers in Canada. Vitamin D-3 concentrations ranged from 0.8 to 4.2  $\mu$ g/kg and 25(OH)-D-3 concentrations from 0.8 to 1.4  $\mu$ g/kg.

In raw samples from 4 lamb cuts (lambs raised on pasture), Purchas et al. (34) determined vitamin D-3 concentrations of 0.3–0.9  $\mu$ g/kg and 25(OH)-D-3 concentrations of 5.7–12.0  $\mu$ g/kg. By contrast, Kobayashi et al. (27) found no vitamin D in lamb and mutton.

Chicken samples purchased in spring and autumn from retail stores in Finland were analyzed for their vitamin D-3 and 25(OH)-D-3 content by Mattila et al. (26). Mean vitamin D-3 content was 2.9  $\mu$ g/kg and 25(OH)-D-3 2.5  $\mu$ g/kg with no significant seasonal difference. In a more recent repetition of this investigation similar vitamin D-3 concentrations were detected, but 25(OH)-D-3 concentration was below detection limit (38). Kobayashi et al. (27) found no vitamin D in chicken breast but slight amounts in chicken liver and turkey and a high amount (325  $\mu$ g/kg) in domestic duck. The latter might be due to supplements in feedstuff.

In meat products, the vitamin D content is dependent on the vitamin D concentration of the processed fresh meat and the fat content. In various Swiss meat products, vitamin D-3 content from below detection limit (<2.5  $\mu$ g/kg) to 23  $\mu$ g/kg was found (39–42). Values below detection limit were, for instance, found in different types of cooked ham and in dried beef with little fat. The largest amounts provided salami type meat products, Vienna sausages, and lard.

#### Dairy

A search in different national food composition databases of Denmark, France, Germany, Switzerland, Canada, and the United States for vitamin D content in dairy products yielded the following values: whole milk, 0.3–1.0  $\mu$ g/kg (US and Canada present only values for fortified whole milk: 7.05  $\mu$ g/kg and 9.9  $\mu$ g/kg, respectively); cream, 3.7–10.8  $\mu$ g/kg; butter, 5.9–14.1  $\mu$ g/kg; yogurt, 0.4–6.0  $\mu$ g/kg; curd cheese, 2.0–7.05  $\mu$ g/kg; soft cheese, 2.8–5.8  $\mu$ g/kg; semihard and hard cheese, 2.0–18.1  $\mu$ g/kg. Only few data are available in these databases for dairy products from non-cow origin: goat's milk, 0.6–2.8  $\mu$ g/kg; ewe's milk, 1.8  $\mu$ g/kg; feta, 3.0–4.0  $\mu$ g/kg. The databases do not differentiate between vitamins D-2 and D-3, nor give any information about inclusion or exclusion of the vitamin D metabolites and how the data were obtained (19–24).

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The influence of different factors like fodder, supplementation, sunlight, seasons, and races on the vitamin D content in (cow's) milk and dairy products has been a topic for >80 y (43). Modern analytical methods like HPLC, LC-MS, and LC-MS/MS confirmed the results from earlier studies obtained by indirect detection by biological assays (44). On the other hand, they also opened the possibility to differentiate between the several metabolites and to give more quantified results (37). In both periods, the findings agree that vitamin D content in milk and dairy products is originally rather low. **Table 2** presents vitamin D-3 and 25(OH)-D-3 content of milk and dairy products found in literature. However, the detected values partly vary considerably among the different studies (7,44). Animal supplementation influences significantly the concentration of vitamin D in

Reference	Foodstuff	D-3	25(OH)-D3	D-2	25(OH)-D2
Mouillet et al. (45)	Raw milk	0.60-1.38			
Kunz et al. (46)	Cow's milk	0.05 <sup>2</sup>	0.50		
Takeuchi et al. (47)	Cow's milk	0.42	0.27		
Kneifel (48)	Raw milk	0.1-2.0 <sup>2</sup>			
	Whole milk PAST	0.8			
	UHT milk	0.8			
Mattila et al. (26)	Milk	<0.2	< 0.2		
Trenerry et al. (49)	Milk (0.1% fat)	< 0.02			
	Milk (1.0% fat)	< 0.02			
	Whole milk (3.8% fat)	0.02			
	Raw milk (4.5% fat)	0.06			
Hollis et al. (57)	Raw milk	0.04 <sup>3</sup>	0.37 <sup>3</sup>		
McDermot et al. (58)	Raw milk	0.075	0.250		
Jakobsen and Saxholt (50)	Whole milk	0.092	0.075	0.034	0.031
	Milk semiskimmed	0.046	0.042		
	Whole milk (organic)	0.076	0.057		
Takeuchi et al. (63)	Butter <sup>4</sup>	7.25			
Kneifel (48)	Butter <sup>4</sup>	10			
Mattila et al. (26)	Butter <sup>4</sup>	2.0	0.5	0.5	
Jakobsen and Saxholt (50)	Butter <sup>4</sup>	1.96	0.96	0.61	0.58
Kneifel (48)	Cheese (curd, Camembert, Edam, Gouda, Emmental) <sup>4</sup>	0-10			
Mattila et al. (26)	Edam cheese <sup>4</sup>	1.1	0.5		
Mattila et al. (26)	Cream	0.7	0.9	0.1	
Jakobsen and Saxholt (50)	Cream	0.94	0.59		
Jakobsen and Saxholt (50)	Coffee cream	0.44	0.27		

Table 2.	Published	natural	vitamin D-3	, vitamin D-	2, 25(OH)-D3	, and 25(OH)-D	2 content in milk and d	airy
products <sup>1</sup>								

<sup>1</sup> All values are means in μg/L unless indicated otherwise. 25(OH)-D-3, 25-hydroxycholecalciferol; 25(OH)-D2, 25-hydroxyergocalciferol; UHT, ultra heat-treated.

<sup>2</sup> Unclear whether vitamins D-3 + D-2 or D-3 or D-2.

<sup>3</sup> Cows supplemented with 4000 IU/d.

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 $^4$  All values for butter and cheese are means in  $\mu$ /kg.

milk, which explains the relatively high values found in the milk of these animals. Data of animals whose diets were supplemented with vitamin D-3 or 25(OH)-D-3 were therefore excluded. On the other hand, in milk and dairy products vitamin D-2 and 25(OH)-D<sub>2</sub> are partially found in relevant concentrations, so we decided to include these values in Table 2 where available. Mouillet et al. (45) collected raw milk samples from different regions in France. The concentration of vitamin D-3 varied between and within the regions. Kunz et al. (46) distinguish between the various metabolites and found 10 times more 25(OH)-D than vitamin D, but they give no information on whether the values are the sum of vitamins D-2 and D-3 or only 1 of them. In commercially available milk samples in Japan, 0.42  $\mu$ g/L vitamin D-3 and 0.27 µg/L 25(OH)D<sub>3</sub>, but neither vitamin D-2 nor its metabolites were detected (47). In his review, Kneifel (48) listed a range of 0.1–2.0  $\mu$ g/L for raw milk and 0.8  $\mu$ g/L for pasteurized whole milk and ultra heattreated (UHT) milk, respectively, but it is not clear which method was used to analyze these samples. In all samples of the study carried out by Mattila et al., the values for vitamins D-2 and D-3 as well as for the metabolites were below the detection limit of 0.2  $\mu$ g/L (26). Analysis with the LC-MS and LC-MS/MS method of whole milk (3.8% fat) and fresh cow's milk (~4.5% fat) detected 0.2  $\mu$ g/L and 0.5-0.6  $\mu$ g/L of vitamin D-3, respectively. The vitamin D-3 content in fat-reduced milk (0.1% and 1.0%) was below the

detection limits (49). The apparent correlation of the fatsoluble vitamin with the fat content of the milk was later clearly demonstrated in a Danish study (50).

In some countries like the United States, Canada, and the United Emirates, milk and dairy products for sale are fortified with vitamin D either by law (Canada) or by choice (United States and United Emirates) (51,52). Another possibility to improve the vitamin D content in milk and dairy products is to supplement the cows. But neither the supplementation over the fodder nor the intravenous or intramuscular application of vitamin D significantly improves the vitamin D content in the milk (53-56). However, a direct oral supplementation of cows with doses from 4000 IU/d to 40,000 IU/d of vitamin D resulted in an increase of vitamin D (57), and another experiment also showed a positive correlation between increasing oral supplementation and the concentration of vitamin D in milk (58). In several countries, the oral supplementation of dairy cows is recommended with varying doses (50,59). Although this is done to secure the health of the cows and not with the aim to increase the vitamin D content in milk, it could nevertheless have an impact on it.

For some time, a water-soluble vitamin D metabolite found in whey was held responsible for an additional vitamin D activity in milk (60). But this hypothesis was not confirmed, and all vitamin D activity was explained by the sum of vitamin D-3,  $24,25(OH)_2$ -D<sub>3</sub>,  $1,25(OH)_2$ -D<sub>3</sub>, and 25(OH)-D-3 (61,62). Hollis et al. (57) realized that vitamin D and its metabolites initially present in whey fraction migrate with time into the fat fraction of the milk.

Few data are available for the vitamin D content in dairy products. Because of the high fat content, the values are significantly higher in butter than in milk; they vary from 1.96  $\mu$ g/kg to 10  $\mu$ g/kg (26,48,50,63). Some but not all of the authors also reported mentionable concentrations for 25(OH)-D-3, vitamin D-2, and 25(OH)-D<sub>2</sub> (26,50). Insufficient data are available for cheese. In the list of Kneifel (48), a range of 0–10  $\mu$ g/kg is given for a summary of different cheese types (curd, Camembert, Edam, Gouda, and Emmental). More detailed are the results of the analysis of an Edam type cheese in which vitamin D-3 and 25(OH)-D-3 but not vitamin D-2 and 25(OH)-D<sub>2</sub> were detected (26).

Cream samples collected in Finland and Denmark contained 0.7  $\mu$ g/kg and 0.94  $\mu$ g/kg vitamin D-3, respectively, and 0.9  $\mu$ g/kg and 0.59  $\mu$ g/kg 25(OH)-D-3, respectively. The Finnish research group additionally reports vitamin D-2. The amounts of vitamin D-3 and 25(OH)-D-3 in coffee cream are only half of the values for whipping cream because of the reduced fat content (26,50).

The Danish study shows significant seasonal variation of the vitamin D content in milk and dairy products, which confirms earlier observations (26,50,64).

#### Eggs

In the above-mentioned national food composition databases, vitamin D values for the whole egg and the egg yolk in particular can be found. The vitamin D content in eggs is practically all in the yolk. Based on the whole egg, the content varies between 14.4 and 29.3  $\mu$ g/kg and between 32.5 and 55.8  $\mu$ g/kg for egg yolks (19–24). **Table 3** shows the values found in literature. Mattila et al. (38) analyzed pools of egg yolk from commercial chicken eggs collected in the spring and autumn. Vitamin D-3 and 25(OH)-D-3 content in the egg yolk was slightly higher in spring. The values correspond to previous analyses in 1992 and 1993 (65,66); slightly lower content of vitamin D-3 and 25(OH)-D-3 was found in egg yolks by the same authors in 1999,however (67). Overall, the vitamin D-3 values found in literature vary

**Table 3.** Published natural vitamin D-3 and 25(OH)-D-3 content in chicken  $\mbox{eggs}^1$ 

Reference	Foodstuff	D-3	25(OH)-D-3
		$\mu$ g/kg	$\mu$ g/kg
Koshy and VanDerSlik (70)	Egg, yolk		5.0-8.0
Jackson et al. (68)	Egg, whole	16	
Sivell et al. (69)	Egg, whole	8-14	
Takeuchi et al. (63)	Egg, yolk	39	
Mattila et al. (66)	Egg, yolk (autumn)	40	
	Egg, yolk (spring)	56	
Mattila et al. (65)	Egg, yolk		9.8
Kobayashi et al. (27)	Egg, whole	30 <sup>2</sup>	
	Egg, yolk	58 <sup>2</sup>	
Mattila et al. (67)	Egg, yolk	34	9.3
Mattila et al. (38)	Egg, whole	14	3.8
	Egg, yolk (autumn)	40	10.0
	Egg, yolk (spring)	49	13.0

<sup>1</sup> Mean values. 25(OH)-D-3, 25-hydroxycholecalciferol.

<sup>2</sup> Sum of vitamins D-3 and D-2.

between 8.0 and 30.0  $\mu$ g/kg for whole chicken egg and between 34.0 and 58.0  $\mu$ g/kg for egg yolks (27,38,66–69). The 25(OH)-D-3 is documented to range from 5.0 to 13.0  $\mu$ g/ kg in egg yolks (38,65,67,70). The concentrations of vitamin D in eggs can be increased in a linear dose-dependent manner by supplementing chicken feed with vitamin D-3 (67,71).

## Fish

Fish and fish products are regarded as the major dietary source of vitamin D. National food composition databases show values in the range of 0 to 300  $\mu$ g/kg (19–24). Amounts found in literature are presented in Table 4. In 1984, Takeuchi et al. (63) reported the vitamin D-3 content of 8 different fish products. The values ranged between 5 and 356  $\mu$ g/kg. The highest amounts were found in fresh eel and shiokara, a Japanese fish product. Kobayashi et al. (27) analyzed 18 different kinds of fish and reported vitamin D (total of vitamins D-2 and D-3) concentrations from 18 to 350  $\mu$ g/kg. Fish liver was as high as 1200  $\mu$ g/kg of vitamin D. Various fresh fish from the Baltic sea and 2 lakes in Finland as well as frozen fish and fish products were analyzed by Mattila et al. (72). Large variations were not only found between different fish species but also in the same species caught in different locations. The observed variations in vitamin D content between fish of the same species seem not to be related to the weight, sex, or age of the fish but may depend on the diet (i.e., the vitamin D-3 content of zooplankton). Contrary to general belief, no significant correlation between fat and vitamin D content was detected (72,73). The vitamin D-3 content of the frozen fish and fish products ranged between <2 (shrimp) and 196  $\mu$ g/kg (roe of vendace) (72). Lu et al. (74) and Bilodeau et al. (37) found vitamin D-3 concentrations between 6 and 453  $\mu$ g/kg in various fish. Where 25(OH)-D-3 was analyzed in fish and fish products, the results were consistently very low and often there was no detectable content (37,72).

#### Bioavailability

Like other fat-soluble vitamins, vitamin D is absorbed incorporated in mixed micelles from the intestine into the enterocytes by nonsaturable passive diffusion. From there, vitamin D is transported along with chylomicrons via lymph to the circulation (2,75). The more polar metabolite 25(OH)D is absorbed better and faster than vitamin D because it is also taken up directly from the proximal jejunum into the portal vein (7,75).

Almost all studies on vitamin D bioavailability were done with different kinds of vitamin supplements. Vitamin D absorption from supplements varies depending on the used vehicle substance (oils, powders, and ethanol) (76). There are not a lot of data on its availability from natural sources. Based on an investigation with special pig meat (pigs receiving only vitamin D-2 as the sole vitamin D source), van den Berg (75) estimated the average relative bioavailability of vitamin D-2 from meat sources to be  $\sim 60\%$  compared with a vitamin D-2 supplement. It has also been shown that the bioavailability of vitamin D from fortified hard cheese

Reference	Foodstuff	D-3	25(OH)-D-3
		$\mu$ g/kg	$\mu$ g/kg
Egaas and Lambertsen (89)	Tuna, liver	32,500	
	Mackerel, liver	2400	
	Mackerel, fillet	155	
	Mackerel, red muscle	155	
	Coalfish, liver	165	
Takeuchi et al. (63)	Japanese pilchard	1361	
	Skipjack	187	
	Tuna, fatty meat	37	
	Fel	268	
Kobayashi et al. (27)	Anglerfish, liver	1100 <sup>2</sup>	
	Indo-Pacific blue marlin	350 <sup>2</sup>	
	Chum salmon	325 <sup>2</sup>	
	Herring	275 <sup>2</sup>	
	Flat fish	230 <sup>2</sup>	
	Bastard halibut (cultured)	180 <sup>2</sup>	
	Bluefin tuna, fatty meat	180 <sup>2</sup>	
	Sand eel	150 <sup>2</sup>	
	Grunt	150 <sup>2</sup>	
	Rainbow trout	150 <sup>2</sup>	
	Fel	140 <sup>2</sup>	
		140 130 <sup>2</sup>	
	Red sea bream (cultured)	130 $110^{2}$	
	Mackerel	110 110 <sup>2</sup>	
	Pacific saury		
	Skipjack	100 <sup>2</sup> 98 <sup>2</sup>	
	Japanese pilchard		
	Yellowtail	85 <sup>2</sup>	
	Cod	18 <sup>2</sup>	
Mattila et al. (72)	Baltic herring	171.0	ND
	Bream	138.0	ND
	Cod	69.0	ND
	Perch	2.9-244.0	ND
	Pike	12.0-47.0	ND
	Pikeperch	245.0	ND
	Rainbow trout (cultured)	76.0	1.4
	Vendace	23.3-245.0	<1.0
	Whitefish	122.0-444.0	<1.0-2.5
Lu et al. (74)	Wild salmon	249.0	
	Farmed salmon	60.5	
	Blue fish	70.6	
	Farmed trout	97.8	
	Tuna ahi	101.8	
	Mackerel	6.0	
Bilodeau et al. (37)	Mahi mahi	11.1	<0.2
	Canned pink salmon	223.0	1.1
	Tilapia	453.0	0.6

#### Table 4. Published natural vitamin D-3 and 25(OH)-D-3 content in fish<sup>1</sup>

 $^{\rm 1}$  Mean values. 25(OH)-D-3, 25-hydroxycholecalciferol; ND, not detected.  $^{\rm 2}$  Sum of vitamins D-3 and D-2.

Sum of vitamins D-3 and D-2.

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(cheddar and low-fat cheese) is equivalent to supplements (77) and that vitamin D bioavailability is not influenced by the fat content of the fortified milk (78). Apparently vitamin D-3 strongly binds to  $\beta$ -lactoglobulin A as well as  $\beta$ -casein, protecting it from a polar environment in fatfree dairy products. This stabilization of vitamin D-3 may ensure the availability for absorption within the human body (79). These strong binding affinities may not have a negative impact on the bioavailability because vitamin D-2 was more efficiently absorbed from fortified cheese than from water (80). Vitamin D-3 encapsulated in reformed casein micelles was highly bioavailable in a clinical study, comparable to a commercial aqueous supplement (81). Other investigations report no influence of the vehicle on the

bioavailability of vitamin D supplements but a higher response of vitamin D-3 compared with vitamin D-2 (16). An influence of calcium, phosphorus, vitamin A, and cholesterol on vitamin D bioavailability is assumed, but further investigation is needed to determine the mechanism (82). Additionally, growing evidence suggests an interaction of a genetic polymorphism in key genes with bioavailability, transport, distribution in body pools, metabolism, and action of vitamin D (82,83).

## Influence of processing and cooking

Cooking does not much influence the vitamin D content of animal foods. Mattila et al. (84) found that, in eggs boiled for 10 min, the vitamin D-3 concentration was 1–6% lower and 25(OH)-D-3 content was 6-11% lower compared with raw eggs. Also in fish, the cooking effect was moderate: baking various kinds of fish (e.g., perch, rainbow trout, Baltic herring) in the oven at 172°C or 200°C for 20 min induced a vitamin D-3 loss of <10%, calculated on a dry matter basis. Only 1 lot of Baltic herring showed an exceptionally large decrease of 23%, which was attributed to the loss of fat during baking (84). Another investigation reported a loss of ~50% when salmon was fried in vegetable oil but not when it was baked (74). Cooking pork loin in the oven at 250°C for 20 min followed by 150°C until meat core temperature reached 80°C significantly increased vitamin D-3 and 25(OH)-D-3 content in all parts, presumably due to water loss and hence increased dry matter content (35). However, calculating the true retention for vitamin D-3 and 25(OH)-D-3 in the whole cut shows losses of 23% and 5%, respectively. Bennink and Ono (85) reported that for beef, 35-42% of vitamin D was lost with cooking. Purchas et al. (34) investigated the impact of several cooking procedures on vitamin D-3 and 25(OH)-D-3 content in various beef and lamb cuts. Vitamin D-3 retention in beef ranged from 79% to 101% and in lamb from 75% to 126%; retention of 25(OH)-D-3 in beef was 77%-130% and in lamb 55%-79%. In beef cuts, longer, slower cooking induced higher losses than rapid cooking. Again, water loss caused higher vitamin D-3 and 25(OH)-D-3 levels in cooked beef samples, and also in lamb, the concentrations tended to increase with cooking (34). Montgomery et al. (31,32) as well found increased vitamin D-3 and 25(OH)-D-3 concentrations in cooked (71°C internal temperature) longissimus steak, which was attributed to moisture losses.

Storing eggs at room temperature for 2 and 3 wk only leads to slight vitamin D-3 and 25(OH)-D-3 decreases (<6% on a dry matter basis) (84). Renken and Warthesen (86) investigated vitamin D stability in fortified skim milk and found some losses through exposure to light but not to air. Thermal stress like pasteurization, ultra heat treatment, sterilization, or even spray drying does not provoke a significant loss of added vitamin D in milk (87,88).

## Conclusion

Studies focusing on the determination of the natural vitamin D content in different animal foods are limited. Most of the data derive from the development of new methods of analysis or from experiments studying the influence of feed supplementation. Although we tried to concentrate on products from animals without excessive vitamin D supplementation, often the information about the upbringing of the animals is not available (especially in retail products). This makes it difficult to compare and judge the given results, and it may also be an explanation for the variations found. Besides this, varying fat content of the products as well as other season of production may also result in different concentrations of vitamin D. The highest values of vitamin D are found in fish and especially in fish liver, ranging from  $<2 \mu g/kg$  to 477  $\mu$ g/kg and up to 1200  $\mu$ g/kg, respectively, depending on fish species and locations. Also offal provides considerable

amounts of vitamin D up to 140  $\mu$ g/kg, whereas the content in muscle meat is generally much lower (up to 10  $\mu$ g/kg). Variations between species and meat cuts are seen. With vitamin D concentrations of up to 57  $\mu$ g/kg egg yolk features values between the vitamin D values of meat and offal. Milk and dairy products are normally low in vitamin D if they are not fortified with it. The highest natural values are reported in butter and cheese (up to 10  $\mu$ g/kg) due to its high fat content.

Processing does not influence the concentration of vitamin D in meat and dairy products very much because the vitamin is rather heat and oxygen tolerant. However, exposure to light can significantly reduce vitamin D content.

Because recommendations for vitamin D intake have recently been increased considerably, the possibility to cover the requirements with foodstuff is even more difficult. Nutrition societies often recommend an intake of 3 portions of dairy and 1 portion of meat, fish, or eggs per day. We estimate that by complying with these recommendations, the maximal intake of vitamin D through animal food would be 3  $\mu$ g (dairy plus meat), 7  $\mu$ g (dairy plus eggs), and 49  $\mu$ g (dairy plus fish) per day.

Further research is needed to improve and optimize the natural vitamin D content in dairy products and meat because fortification of food is not well accepted in several countries.

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