



## Original article

### Vitamin D in Commonly Consumed Freshwater and Marine fish

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#### ABSTRACT

Insufficiency/deficiency of vitamin D has been a worldwide health problem. Fish has been used as a protein source among Thai population, but data on vitamin D content of fish in Thailand is not available. This study aimed to generate a database of vitamin D in freshwater and marine fish and determine the good sources of vitamin D for consumption. Ten species of freshwater (5 species) and marine (5 species) fish which were commonly consumed in Thailand were studied. Three wholesale markets were selected and the samples came from three retail shops in each market. The fish were prepared by common household practices. The edible portion of raw fish was homogenized, freeze-dried, re-homogenized, and packed in aluminum foil bags. The samples were kept at -20°C until analysis of moisture and vitamin D. Vitamin D<sub>2</sub> and D<sub>3</sub> were determined by HPLC method (AOAC, 2016, method no. 995.05). The results indicated that vitamin D<sub>3</sub> was the major form (82-100%) of vitamin D in the studied fish. Common silver barb, red Nile tilapia, and Nile tilapia contained high levels of vitamin D, with 48.5, 31.0, and 19.8 µg per 100g fresh weight (FW), respectively. Two species of freshwater fish - walking catfish and striped snake-head fish contained low levels of vitamin D, 2.4 and 5.7 µg per 100g FW, respectively. All raw marine fish contained low levels of vitamin D, ranging from 2.9 to 4.7 µg per 100g FW. Fish that lived in the limnetic zone exhibited high levels of vitamin D. Consuming one serving (55 g or 3-4 tbsp.) of common silver barb, red Nile tilapia, and Nile tilapia provides 11-30 µg of vitamin D, contributing 218-534% of Thai Recommended Daily Intake. Consuming fish high in vitamin D and exposing oneself to sunlight regularly could reduce or prevent the incidence of vitamin D deficiency.

**Key words:** vitamin D, freshwater fish, marine fish

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## นิพนธ์ต้นฉบับ

### วิตามินดีในปลาน้ำจืดและปลาน้ำเค็มที่นิยมบริโภค

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#### บทคัดย่อ

การขาดวิตามินดีเป็นปัญหาสุขภาพสำคัญของคนทั่วโลก ปลาน้ำจืดเป็นแหล่งสำคัญของโปรตีนที่คนไทยนิยมบริโภค แต่ยังไม่มียางานข้อมูลของวิตามินดี การศึกษานี้จึงมีวัตถุประสงค์เพื่อจัดทำฐานข้อมูลวิตามินดีในปลาน้ำจืดและปลาน้ำเค็มที่นิยมบริโภค และเพื่อค้นหาชนิดของปลาที่เป็นแหล่งของวิตามินดีที่ควรนำมาส่งเสริมการบริโภค ดำเนินการศึกษาปลา 10 ชนิด ด้วยการคัดเลือกปลาน้ำจืดและปลาทะเลที่นิยมบริโภคอย่างละ 5 ชนิด โดยซื้อจากตลาด 3 แหล่งใหญ่ ตลาดละ 3 ร้านค้า เตรียมตัวอย่างด้วยวิธีที่นิยมทำในครัวเรือน นำส่วนที่กินได้มาปั่นให้เป็นเนื้อเดียวกัน นำไปทำให้แห้งด้วยระบบแช่เยือกแข็ง และปั่นให้ละเอียดอีกครั้งหนึ่ง เก็บในช่องอุณหภูมิต่ำ -20°C จนกว่าจะวิเคราะห์วิตามินดีและความชื้น วิเคราะห์ปริมาณวิตามิน D<sub>2</sub> และ D<sub>3</sub> ด้วยเทคนิค HPLC ตามวิธีมาตรฐานสากล (AOAC 2016, method no. 995.05) ผลการศึกษาพบว่าในปลาที่ศึกษาทั้งหมดมีวิตามิน D<sub>3</sub> เป็นหลัก คิดเป็นร้อยละ 82-100 ปลาตะเพียน ปลาทับทิม และปลานิล พบว่ามีวิตามินดีสูงมาก (48.5, 31.0, 19.8 ไมโครกรัมต่อ 100 กรัมน้ำหนักสด ตามลำดับ) ปลาดุกและปลาช่อนพบค่าวิตามินดีต่ำ 2.4 และ 5.7 ไมโครกรัมต่อ 100 กรัม สำหรับปลาทะเลพบวิตามินดีต่ำตั้งแต่ 2.9 - 4.7 ไมโครกรัมต่อ 100 กรัม ปลาน้ำจืดที่อาศัยอยู่บริเวณผิวน้ำ พบว่ามีวิตามินดีสูง การบริโภคปลาตะเพียน ปลาทับทิม และปลานิล เพียงหนึ่งหน่วยบริโภค (55 กรัม หรือ 3-4 ช้อนโต๊ะ) ได้รับวิตามินดีสูงมาก คิดเป็นร้อยละ 218-534 ของความต้องการวิตามินดีต่อวัน ดังนั้นการบริโภคปลาเหล่านี้เป็นประจำร่วมกับการได้รับแสงแดดสม่ำเสมอจะช่วยลดความเสี่ยงหรือป้องกันการขาดวิตามินดีได้

**คำสำคัญ:** วิตามินดี ปลาน้ำจืด ปลาน้ำเค็ม



## Introduction

Vitamin D is a fat-soluble vitamin which has two major forms; vitamin D<sub>2</sub> (ergocalciferol) and vitamin D<sub>3</sub> (cholecalciferol). Vitamin D is primarily synthesized in the skin and partly derived from dietary sources<sup>1</sup>. During exposure to sunlight, ultraviolet B (UVB, wavelength 290–305 nm) penetrates the skin and converts 7-dehydrocholesterol to pro-vitamin D<sub>3</sub>. Pro-vitamin D<sub>3</sub> is then rapidly converted by a heat dependent process to vitamin D<sub>3</sub><sup>2,3</sup>. Vitamin D<sub>3</sub> is metabolized in the liver to 25-hydroxyvitamin D (25(OH)D), which is the major circulating form of vitamin D and used to determine an individual's vitamin D status<sup>4</sup>. Vitamin D<sub>2</sub> is derived from dietary sources and has potentially lower bioavailability than vitamin D<sub>3</sub>. Most of the vitamin D (usually between 50% and 90%) is produced via exposure of the skin to UVB through sunlight exposure<sup>5</sup>. Many factors can alter cutaneous vitamin D production such as aging, skin pigmentation, sun-screen usage, time of the day, season, and latitude setting<sup>3</sup>.

Vitamin D is important for growth and development and is associated with bone mineral density. Thai Dietary Reference Intake (DRIs) suggests 'adequate intakes' (AI) of vitamin D for those without sun-mediated synthesis in the skin. For both sexes, the AI required for ages 0–50 years (including pregnancy and lactation), is 5 µg/day (200 IU), for 51–70 years is 10 µg/day (400 IU), and for those over 70 years of age is 15 µg/day (600 IU)<sup>6</sup>. Vitamin D insufficiency may be a risk factor of many chronic illnesses, including common cancer<sup>7</sup>, autoimmune disease<sup>8</sup>, infectious disease<sup>9</sup>, and cardiovascular

disease<sup>10</sup>. Vitamin D insufficiency (serum Vitamin D <50 nmol/L) has worldwide prevalence<sup>11</sup>. The tropical countries, Malaysia<sup>12</sup> and Thailand, reported high prevalence in several populations including children, adolescents, adults and elders. Vitamin D status in healthy elderly Thai women revealed vitamin D insufficiency in two-third of the studied group and vitamin D deficiency in one-third of the group<sup>13</sup>. The prevalence of hypovitaminosis D in healthy Thai children is very high, despite their exposure to sunlight, and that prevalence increases in children with a high BMI percentile<sup>14</sup>. Evaluation of vitamin D status in healthy young Thai men and women (age 25–54 years) found that the prevalence of vitamin D deficiency in females was three-fold higher than males (43.1% in females compared to 13.9% in males). Thai women are at risk for vitamin D insufficiency likely due to sun-screen usage and sun avoidant behavior due to the desire to maintain a fair complexion. Living in urban areas such as in Bangkok increases the risk of vitamin D insufficiency due to increased pollution, which decreases the amount of UVB available for cutaneous vitamin D synthesis. Young Thai people living in urban areas of Thailand, who have less leisure time and spend less time in the sunlight are also at an increased risk for vitamin D insufficiency<sup>15</sup>.

Dietary sources of vitamin D are limited only in fish and mushrooms. USDA databases reported foods of animal origin (e.g., fish, egg, dairy products) as the main naturally-occurring sources of vitamin D<sub>3</sub> and mushrooms as the main source of vitamin D<sub>2</sub><sup>16</sup>. Vitamin D-fortified food products are mandatory in several countries but not in Thailand. Fish are the most significant natural source of vitamin D. Previous

studies found that fatty fish (such as salmon, bluefish, mackerel and tuna) is the best source of vitamin D<sup>17</sup>. Vitamin D<sub>3</sub> content of different fish species showed high variation due to several factors such as environment, season, climate, age, food supply, and type of species<sup>18</sup>. Vitamin D in fish probably comes from diverse sources in the food chain starting from plankton. The amount and quality of plankton could vary in different lakes and seas<sup>19</sup>. Fish is the primary source of animal protein for most of Thailand's population, particularly in the coastal and near coastal areas. Data on the vitamin D content of consumed fish in Thailand is not available. Additionally, environmental and dietary factors can affect the vitamin D content of particular samples of a given species from different sources. Estimation of vitamin D intake in populations needs reliable vitamin D values of food composition databases. For these

reasons, information on natural sources of vitamin D is very important for identifying the best sources for consumption. This study aims to set up a method of vitamin D analysis and to develop a database of vitamin D in commonly consumed fish. Hopefully, this information could be useful for promoting the consumption of fish to prevent vitamin D deficiency in the country and to access fish with high vitamin D content in Thailand.

## Materials and methods

### Sample selection and collection

The most commonly consumed freshwater and marine fish species, five each, were selected based on the data reported by the Agricultural Statistics of Thailand (2019)<sup>20</sup> and Food Consumption Data of Thailand (2016)<sup>21</sup>, as shown in **Table 1**.

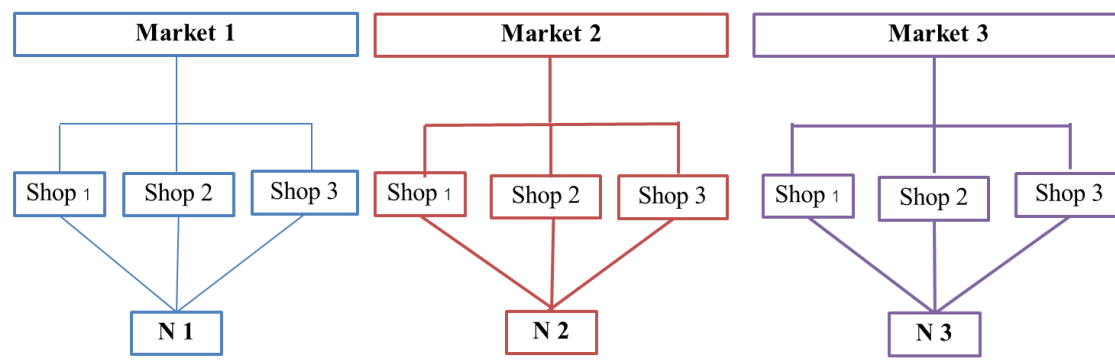
**Table 1** List of selected freshwater and marine fish used for the study

English name	Thai Name	Scientific name
<b>Freshwater fishes</b>		
Striped snake-head	ปลาช่อน	<i>Channa striatus</i>
Walking catfish	ปลาดุก	<i>Clarias macrocephalus</i>
Nile tilapia	ปลานิล	<i>Oreochromis niloticus</i>
Nile tilapia (red)	ปลาพื้ทิม	<i>Oreochromis niloticus-mossambicus</i>
Common silver barb	ปลาตะเพียน	<i>Puntius gonionotus</i>
<b>Marine fishes</b>		
Giant sea perch	ปลากะพงขาว	<i>Lates calcarifer</i>
Grey mullet	ปลากะบอก	<i>Mugil cephalus</i>
Black-banded trevally	ปลาสำลี	<i>Seriolina nigrofasciata</i>
Short-bodied mackerel	ปลาทุสด	<i>Rastrelliger brachysoma</i>
Indo-Pacific king mackerels	ปลาอินทรี	<i>Scomberomorus</i>

Simple random sampling (SRS) and standard guidelines for food composition database<sup>22</sup> were used as guidelines for sample collection. Ten species of selected fish (**Table 1**), 1-2 kg each, were purchased during July to November 2018. Each species of studied fish

was brought from three main markets (Market 1, 2 and 3). From each market, samples were purchased from 3 retail shops (Shop 1, 2 and 3). Fish from each market were made into a single composite sample to obtain 3 independent samples (**Figure 1**).

**Figure 1** Diagram of sample collection for each studied fish



Freshwater fish were brought from Klong-toey market (representative of Bangkok area), Ying-cha-roen or Sa-pan-mai market (representative of northern and/or north-eastern parts of Thailand), and Nakhon Pathom market (representative of southern and/or western part of Thailand). Marine fish, were brought from Fish Marketing Organization (FMO) market located in Bangkok, Samutsakorn, and Chonburi provinces (samples came from the gulf of Thailand).

#### **Sample handling, transportation, and preparation**

Fish bought from each market were kept in an ice-box and transported directly to the laboratory at the Institute of Nutrition, Mahidol University (INMU). On arrival at the laboratory, the size (length and body width), and weight of the fish were measured and recorded and photographs were taken. They

were washed with tap water several times to remove adhering blood and slime. The fish was prepared using common household practices, i.e., eviscerating, descaling (without removing skin), and then washed again twice with deionized water. Each species of fish from each market were prepared separately. Edible portions were collected and inedible portions are discarded. The edible parts were cut into pieces, homogenized in a food blender (Wongdec, WTI-1684A), put in a screw-cap plastic bottle, and kept at -20°C until analyze moisture content.

For freeze-drying, the homogenized samples were spread on trays and kept in the freezer at -80°C overnight before being transferred to the freeze dryer system. Frozen fish samples were freeze dried (Heto powerdry PL 9000 Freeze Dryer) for 3 days or until it was



completely dried. The weight of the sample was recorded before and after freeze-drying process. The fish samples were re-homogenized with a food blender (Wongdec, WTI-1684A), packed in aluminum foil bags, and kept at  $-20^{\circ}\text{C}$  until analyze vitamin D content.

#### **Analysis of moisture and vitamin D content in fish**

##### **Determination of moisture content by hot air oven method**

Moisture content of all samples were determined by drying the samples with sand in a water bath and then in a hot air oven (Memmert ULE 400) at  $100\pm 2^{\circ}\text{C}$  for 2 hours, cooled in a desiccator and weighed with a 4-digit analytical balance (Mettler AT201). The drying step was repeated for 1 hour until a constant weight was obtained (AOAC method no. 990.19 and 925.23, 2016)<sup>23</sup>.

##### **Determination of vitamin D content by HPLC method**

AOAC Official Method no. 995.05 (AOAC, 2016)<sup>23</sup> was used for the determination of vitamin D<sub>3</sub>. In brief, dried homogenized sample ( $1.0\pm 0.5$  g) was weighed into 100 mL Duran glass bottle, saponified with ethanolic potassium hydroxide (140 g KOH : 310 mL EtOH : 50 mL H<sub>2</sub>O) in a shaking water bath (GYROTORY, Model G76), and extracted with hexane. The extracted solution was evaporated using a rotary evaporator (BUCHI R-114 Rotary Vap System) and purified by solid-phase extraction using silica column (Cleanert® SLE 500 mg/3 mL). The eluted fraction was injected

(250  $\mu\text{L}$ ) and then separated by HPLC system (Agilent 1260 Series). Equipment consists of Vacuum Degasser (G1322A), Isocratic pump (G1310A), Thermostat plate Compartment, Diode Array Detector (DAD-MWD/G1365B), Autosampler (ALS/THERM, G1330B), a 250  $\mu\text{L}$  injector loop, and reversed-phase C18 column (4.6 x 250 mm, 5  $\mu\text{m}$ ). The mobile phase and flow rate are shown in **Table 2**. Vitamin D<sub>2</sub> was added as the internal standard in each sample before saponification for the quantification of vitamin D<sub>3</sub>. The ratios of three concentrations of vitamin D<sub>3</sub> to constant concentration of vitamin D<sub>2</sub> were used for the preparation of standard curve. Vitamin D<sub>3</sub> in the unknown samples was estimated from the standard curve and reported as  $\mu\text{g}$  per 100g fresh sample.

##### **Quality control system of vitamin D analysis**

Method validation, which included limit of detection (LOD), limit of quantitation (LOQ), accuracy, precision, intermediate precision, internal quality control system and interlaboratory performance study were conducted. Fortified vitamin D<sub>3</sub> milk powder was used as in-house quality control (QC) sample. Each sample was analyzed in duplicate along with the QC sample. The vitamin D values of QC samples for each batch of analysis must be within mean  $\pm 2$  standard deviation (SD) of the assigned values. When the vitamin D value falls outside the mean  $\pm 2$  SD value, the analysis of unknown sample was repeated.



**Table 2** Flow rate and gradient concentrations of mobile phase components for vitamin D determination by HPLC

Running time (min)	Flow rate (ml/min)	% Gradient concentrations of mobile phase		
		Acetonitrile	Methanol	Ethyl acetate
0.0	0.7	91.0	9.0	0.0
15.0	0.7	91.0	9.0	0.0
15.5	2.5	0.0	0.0	100.0
15.5	2.5	0.0	0.0	100.0
17.0	2.5	0.0	0.0	100.0
17.5	2.5	91.0	9.0	0.0
19.0	2.5	91.0	9.0	0.0
20.0	2.5	91.0	9.0	0.0
20.0	0.7	91.0	9.0	0.0

1) Checking accuracy:

A. Standard Reference Material (SRM): NIST 1849a, Infant/Adult Nutritional Formula I (milk-based), from National Institute of Standards & Technology, Gaithersburg, USA with vitamin D<sub>3</sub> level of 0.111±0.017 mg/kg was analyzed (three replicates, twice: n=6). The results were evaluated against the SRM certified vitamin D<sub>3</sub> value.

B. Percent recovery: Vitamin D<sub>3</sub> fortified milk powder was used as test material for percent recovery study. Standard vitamin D<sub>3</sub> (180 µg/mL) was diluted to a concentration of 2.88 µg/mL and 200 µL (0.576 µg) of the diluted solution was added into about 0.5 g of fortified milk powder. Vitamin D<sub>3</sub> in the test material and the spiked test material was analyzed. Percent recovery of vitamin D<sub>3</sub> was then calculated.

2) Checking precision and intermediate precision: Vitamin D<sub>3</sub> fortified milk powder was used as test material. For checking precision (repeatability, RSD<sub>r</sub>), vitamin D in 10 samples

of test materials was analyzed, in duplicate, whereas for intermediate precision (reproducibility, RSD<sub>ir</sub>), vitamin D<sub>3</sub> was analyzed together with samples in every set. The values of mean±SD<sub>r</sub>, %RSD<sub>r</sub>, mean±SD<sub>ir</sub>, and %RSD<sub>ir</sub> were calculated. The RSD<sub>r</sub> and RSD<sub>ir</sub> values of this study were compared with those (RSD<sub>r</sub> and RSD<sub>ir</sub> were estimated from the values reported in the AOAC method no. 995.05)<sup>23</sup> from a collaborative study of infant formula powder which were used as Horwitz's predicted RSD. Based on the Horwitz ratio, the accepted values of RSD<sub>r</sub>/pRSD and RSD<sub>ir</sub>/pRSD were 0.3-1.3 which indicates good intermediate precision.

3) Interlaboratory study: Selected fish species with low, medium and high levels of vitamin D were analyzed by LC-MS/MS at the National Measurement Institute (NMI), Australia and the data were statistically compared (t-test) with the results of this study.





## Results and discussion

### Method validation of vitamin D analysis

The accuracy of the vitamin D analysis by HPLC was checked by analysis of SRM NIST 1849a (a milk-based, hybrid infant/adult nutritional powder) and percent recovery. The result showed vitamin D value of  $0.123 \pm 0.015$  mg/kg, which was close to the certified value of  $0.111 \pm 0.017$  mg/kg in the standard reference material - SRM, NIST 1849a. The percent recovery obtained was  $99.8 \pm 2.9\%$  (97-103%) which is in the acceptable range of 80-115%<sup>24</sup>. These results indicated the accuracy performance of the analyst who conducted the vitamin D analysis.

For precision, repeatability (daily precision) and reproducibility (intermediate precision) were checked by using fortified D<sub>3</sub> milk powder as in-house quality control (QC) sample. The vitamin D levels obtained from repeatability and reproducibility were  $5.2 \pm 0.2$  µg/100g (%RSD<sub>r</sub> = 3.8) and  $5.2 \pm 0.5$  µg/100g (%RSD<sub>ir</sub> = 9.6), respectively. The ratio of RSD<sub>r</sub> and RSD<sub>ir</sub> compared to the predicted relative standard deviation, pRSD<sub>R</sub> of 12.0 (estimated from the RSD<sub>R</sub> value presented in the AOAC guideline, 2016)<sup>24</sup> was  $3.8/12 = 0.3$  and  $9.6/12 = 0.8$ , respectively. Both values are within the HORRAT accepted criteria of 0.3-1.3 RSD<sub>R</sub> and the results indicated good performance of analyst's precision.

The collaborative study on method performance of vitamin D analyzes: HPLC-UV detector vs liquid chromatography-mass spectrometer, LC-MS/MS, used by the National

Measurement Institute (NMI) Australia, showed no statistically significant difference ( $p = 0.139$ ) at low, medium and high levels of vitamin D (Table 3). Other forms of vitamin D (25-Hydroxy vitamin D<sub>2</sub>, 25-Hydroxy vitamin D<sub>3</sub> and vitamin D<sub>2</sub>) were found in negligible amounts (less than 0.2 µg /100g) in all selected fish by LC-MS/MS whereas vitamin D<sub>2</sub> was found lower than the detection limit (LOD = 0.62 µg /100g) by HPLC-UV detector used in this study.

In conclusion, all information obtained from method validation and analytical quality testing revealed that the vitamin D<sub>3</sub> analysis in fish has been reliable in terms of accuracy and precision. Besides, the vitamin D values at various levels were well comparable to those obtained from the highest metrological technique (LC-MS/MS).

### Moisture and vitamin D content in fish

Moisture content of fish samples are shown in Table 4. Moisture content in raw fish ranged from 68 to 76 and 72 to 77 g per 100g FW of freshwater and marine fish, respectively.

Vitamin D content of raw freshwater fish ranged from  $2.42 \pm 1.4$  to  $48.5 \pm 26.5$  µg per 100 g FW and was higher than that of raw marine fish ( $2.94 \pm 2.1$  to  $4.69 \pm 0.8$  µg per 100g FW). Three kinds of freshwater fish namely common silver barb, red Nile tilapia, and Nile tilapia contained extraordinarily high levels of vitamin D ( $48.5 \pm 26.5$ ,  $31.0 \pm 7.7$  and  $19.8 \pm 3.5$  µg per 100g FW, respectively). Marine fish contained considerably lower vitamin D content than that of freshwater fish.





**Table 3** Comparison of two methods, LC-MS/MS and HPLC-UV for quantitation of vitamin D content in selected fish

English name	Vitamin D by NMI Australia ( $\mu\text{g}/100\text{g}$ dry matter, DM)				Vitamin D in this study	% Diff.
	25-Hydroxy Vitamin D <sub>2</sub>	25-Hydroxy Vitamin D <sub>3</sub>	Vitamin D <sub>2</sub>	Vitamin D <sub>3</sub>	( $\mu\text{g}/100\text{g}$ DM)	
Common silver bard	<0.2	<0.2	1.7	197	193	-1.8
Nile tilapia	<0.2	<0.2	<0.2	86	102	18.6
Spanish mackerel	0.1	<0.1	<0.1	12	14	17.6
Short-bodied mackerel	<0.05	<0.05	2.5	11	13	13.7

**Table 4** Moisture and vitamin D contents of freshwater and marine fish (Mean  $\pm$  SD)

Fish name	Moisture	Total vitamin D	Total vitamin D
	(g/100g)	( $\mu\text{g}/100\text{g}$ ) fresh weight	( $\mu\text{g}/100\text{g}$ ) Dry matter
Striped snake-head	74 $\pm$ 0.4	5.7 $\pm$ 2.6	22.0 $\pm$ 9.9
Walking catfish	68 $\pm$ 0.6	2.4 $\pm$ 1.4	7.58 $\pm$ 4.2
Nile tilapia	76 $\pm$ 1.8	19.8 $\pm$ 3.5	81.0 $\pm$ 9.9
Nile tilapia (Red)	73 $\pm$ 0.4	31.0 $\pm$ 7.7	113.0 $\pm$ 26.0
Common silver barb	74 $\pm$ 3.2	48.5 $\pm$ 26.5	180.0 $\pm$ 76.0
Giant sea perch	74 $\pm$ 3.0	3.3 $\pm$ 2.8	13.3 $\pm$ 11.1
Grey mullet	77 $\pm$ 0.6	4.7 $\pm$ 0.8	20.7 $\pm$ 3.3
Black-banded trevally	72 $\pm$ 1.8	3.0 $\pm$ 1.3	10.6 $\pm$ 3.9
Short-bodied mackerel	76 $\pm$ 1.8	2.9 $\pm$ 2.1	12.1 $\pm$ 8.7
Indo-Pacific king mackerels	75 $\pm$ 2.3	3.2 $\pm$ 0.3	12.7 $\pm$ 2.0

This can be explained by the fact that fish with high levels of vitamin D lived in the limnetic zone (the layer that receives sufficient sunlight) compared to other species living in profundal (a deep zone of an inland body of freestanding water) and benthic zone (the ecological region

at the lowest level of a body of water). This is the first report of vitamin D in Thai fish, and there are no prior studies for comparison among species. Several reports have described the vitamin D<sub>3</sub> content in different fish species. A previous study in Canada reported that 3



different fish species namely mahi-mahi, canned pink salmon and tilapia had average vitamin D levels of 1.11 (0.24–2.24), 22.3 (12.7–43.5), and 45.3 (17.9–75.3)  $\mu\text{g}$  per 100g FW, respectively<sup>25</sup>. In our study, Nile tilapia had a vitamin D content of  $19.8 \pm 3.5$   $\mu\text{g}$  per 100g FW, which was lower than the previous study (45.3 (17.9–75.3)  $\mu\text{g}$  per 100g FW). The difference in vitamin D content may be due to different species. In another report, vitamin D<sub>3</sub> content of 8 different fish ranged between 0.5 and 35.6  $\mu\text{g}/100\text{g}$ <sup>26</sup>. Besides, vitamin D levels in the skin of different kinds of fish namely trevally, Atlantic salmon, yellowfin tuna, bream, blackfish, whiting, rainbow trout were found in a range of 1.8 to 30  $\mu\text{g}/100\text{g}$ <sup>27</sup>. In general, vitamin level can vary in different parts of the same tissue and among animals collected at different times and locations. Indeed, geographic availability, seasonality, and physiological state/maturity are known to affect variability in nutrient composition, especially vitamins<sup>17</sup>. It has been assumed that oily fish such as salmon, mackerel and bluefish, are excellent sources of vitamin D<sub>3</sub><sup>28</sup>. Our study found that oily fish such as striped snake-head, walking catfish, and black-banded trevally contain low level of vitamin D<sub>3</sub>. A previously conducted study assumed that diet could be a factor that causes differences in cholecalciferol contents of fish<sup>29</sup>. Based on this information, fish might get vitamin D<sub>3</sub> from zooplanktons, as these organisms acquire vitamin D through solar irradiation. Also, fish that live close to the

water surface might produce higher vitamin D through sunlight exposure.

#### **Estimation of vitamin D as % Thai recommended daily intake (Thai RDI)**

The Food and Drug Administration has established the prescriptions of Thai Recommended Daily Intake for ages 6 years and up (Thai RDI) for setting reference values for calculating displayed nutrition values on nutrition labels. However, reference values in Thai RDI are mean values for general Thais. The reference serving size of fish is 55 grams, and the amount of vitamin D recommended for consumption in Thai RDI is 5  $\mu\text{g}$  per day<sup>30</sup>. Vitamin D in one serving of fish and percentage of the amount recommended for consumption in one day (% Thai RDI) are shown in **Table 5**. Consuming one serving of fish contributed 1.33 to 26.69  $\mu\text{g}$  of vitamin D, which is equivalent to 27 – 534 % of Thai RDI. Freshwater fish provided higher vitamin D content than that of marine fish. Vitamin D content in freshwater fish ranged from 2.42  $\mu\text{g}$  per 100g FW in walking catfish (27% Thai RDI) to 48.53  $\mu\text{g}$  per 100g FW in common silver barb (534% Thai RDI) whereas those of marine fish ranged from 2.94  $\mu\text{g}$  per 100g FW in short-bodied mackerel (32% Thai RDI) to 4.69  $\mu\text{g}$  per 100g FW in mullet (52% Thai RDI). The wide ranges of vitamin D were probably due to the differences in vitamin D content of different species of fish.



**Table 5** Vitamin D in freshwater and marine fish accounted for a percentage of the amount of nutrients recommended for consumption in one day (% Thai RDI)

Fish name	Total vitamin D ( $\mu\text{g}/100\text{g FW}$ )	Vitamin D <sub>3</sub> /Serving (55 g)	% Thai RDI
Striped snake-head	5.69	3.13	63
Walking catfish	2.42	1.33	27
Nile tilapia	19.82	10.90	218
Nile tilapia (Red)	31.04	17.07	341
Common silver barb	48.53	26.69	534
Giant sea perch	3.33	1.83	37
Gray mullet	4.69	2.58	52
Black-banded trevally	2.99	1.64	33
Short-bodied mackerel	2.94	1.62	32
Indo-Pacific king mackerels	3.15	1.73	35

### Conclusion

The objective of this study was to determine vitamin D content in selected commonly consumed freshwater and marine fish in Thailand. Moisture content in freshwater fish ranged from 68 to 76 g per 100g FW and 72 to 77 g per 100g FW in marine fish.

Vitamin D content of freshwater fish ranged from  $2.42 \pm 1.4$  to  $48.5 \pm 26.5$   $\mu\text{g}$  per 100g FW whereas that of marine fish ranged from  $2.94 \pm 2.1$  to  $4.69 \pm 0.8$   $\mu\text{g}$  per 100g FW. Among the tested freshwater fish, common silver barb, red Nile tilapia, and Nile tilapia contained high levels of vitamin D, while striped snake-head and walking catfish exhibited lower levels. Vitamin D content of marine fish was

lower than that of freshwater fish. A single serving of different fish contributed 1.3–26.7  $\mu\text{g}$  of vitamin D which equals to 27–534% of Thai RDI.

In conclusion, fish was demonstrated as an excellent source of vitamin D, especially freshwater fish, for all age groups. Freshwater fish is cheaper than marine fish and could be purchased throughout the year. Common silver barb exhibited the highest level of vitamin D content, but promoting this fish for consumption may be a problem due to the presence of numerous fish bones. Traditionally, Thai people boil common silver barb for a long time (> 4hrs) for easy consumption, which leads to the loss of vitamin D. Consumers may also be at risk of high sodium intake by eating salty and boiled



common silver barb (Ta-pain-tom-kem). Therefore, cooked tilapia can be promoted as a good source of vitamin D because it is inexpensive, delicious and had fewer fish bones. Consumers could meet their needs of vitamin D and lead a healthy lifestyle by eating fish.

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