

Farmed Salmon and Farmed Rainbow Trout - Excellent Sources of Vitamin D?

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Abstract

Fatty fish are generally stated as having high vitamin D content and among these are salmon and trout. In the aquaculture industry of salmonids the two main species produced are *Salmo salar* (Atlantic salmon) and *Oncorhynchus mykiss* (rainbow trout). Published data have shown lower content of vitamin D in farmed than in wild species, but generally data on vitamin D in farmed salmon and rainbow trout are scarce. In commercial production facilities we aimed to study the variation of vitamin D in farmed salmon and rainbow trout prepared for sale to consumer. Thirteen organically produced salmon and 18 rainbow trout were sampled within the range 0.7-4.0 kg of gutted weight. All fish were ready for consumption, and analysed for content of vitamin D₃, 25-hydroxyvitamin D₃, and fat.

Mean vitamin D₃ content in salmon and rainbow trout was 1.6 ± 0.5 , and 5.0 ± 2.3 µg/100 g, respectively. Compared to vitamin D₃, the content of 25-hydroxyvitamin D₃ was 11% and 3%, respectively. In farmed salmon a linear relationship with vitamin D₃ being dependent on weight ($P < 0.05$) as well as to fat content ($P < 0.05$), while no similar relationship was found for farmed rainbow trout. Despite this, both species exhibit a linear correlation between fat and gutted weight ($P < 0.001$).

The results indicate that there is a difference in the storage of vitamin D between the two salmonids, as 25-hydroxyvitamin D₃ amounted more to the vitamin D activity than in rainbow trout. The low content of vitamin D in i.e. found in the salmonids is challenging farmed salmon and farmed trout as an essential vitamin D source.

Keywords: Vitamin D; Salmon; Trout; Aquaculture

Introduction

Vitamin D is a fat-soluble vitamin that humans produce in skin when exposed to UV light (290-315 nm), and obtain through dietary intake. The vitamin D content of food is composed of the parent vitamin D, cholecalciferol (vitD₃) and ergocalciferol (vitD₂), and the metabolites, 25-hydroxyvitamin D₃ (25OHD₃) and 25-hydroxyvitamin D₂ (25OHD₂). There are several high risk groups associated with vitamin D deficiency: individuals who avoid sun exposure, people with dark-pigmented skin, older people and those in residential care or nursing homes [1,2]. Dietary intake is essential all year round for people in high risk groups and during winter for people living at latitudes above 35°. A recent review of dietary intake of vitamin D in adults and children in Europe shows that intake of vitamin D is generally inadequate compared to recommendations [3]. Fatty fish e.g. salmon is generally stated as having high vitamin D content, which for wild salmon has been reported to 8-55 µg vitD/100 g [4], but it has also been reported that the content of vitamin D in farmed salmon is lower than in wild salmon [5]. In fish, vitamin D is essential to preserve calcium and phosphate homeostasis and the level rely on the intake in feed [6].

In the aquacultural production salmon and trout is the largest single commodity by value [7]. The dominant farmed salmon and farmed trout are the Atlantic salmon *Salmo salar* (*S. salar*) and the Pacific Ocean species of wild salmon *Oncorhynchus mykiss* (*O. Mykiss*),

respectively; *O. Mykiss* is also named rainbow trout [8,9]. In terms of volume, *S. salar* is the most important aquacultured salmon species, as it covers >90% of the farmed salmon market and >50% of the total global salmon market [8]. The main producers of farmed salmon are Chile, Norway, Scotland and Canada, [8], while the main producers of farmed rainbow trout are Iran, Chile, Turkey and Norway [10].

The dietary requirement of vitD₃ for rainbow trout is 40-60 µg/kg feed [11], while for *S. salar* the estimated required amount is 60 µg vitD₃/kg feed [12]. In Europe there are maximum limits for the addition of vitamin D to feed, namely 75 µg/kg feed [13]. Fish are very tolerant of high contents of vitD₃ in feed [6], e.g. no effect of excessive amount of vitD₃, up to 25000 µg/kg feed, for *O. mykiss* has been demonstrated [14].

In nine different species of fresh and salt water fish fattiness had no significant effect on content of vitamin D [15]. Similar results were obtained for herring (*Clupea harengus*) and mackerel (*Scomber scombrus*), as content of fat through the year varied from 2-32% and 11-35%, respectively, while content of vitamin D showed no association to fattiness [16]. Data on vitamin D in farmed salmon and trout are scarce. In commercial production facilities, we aimed to study the variation of vitamin D in farmed salmon and rainbow trout prepared for sale to consumer. The hypothesis was that the content of vitamin D depends on fat content and weight of the farmed fish.

Materials and Methods

Fish samples

Salmo salar-farmed salmon: Thirteen farmed salmon (0.7 kg to 3.9 kg gutted weight) were included in the study. The salmon fry hatched between Month 1-6, were put to sea in Month 12-16, and were harvested in Month 29, at an age of between two years and two years five months. The salmon were farmed by Murphy's Irish Seafood (Bantry, Ireland) using the organic feeding regiment Emerald Organic Feed (Skretting, Wincham, UK). One whole fillet from each of the 13 salmon was homogenized.

Oncorhynchus mykiss-farmed rainbow trout: Eighteen rainbow trout (0.9 kg to 3.9 kg gutted weight) were included in the study. The fry hatched in Month 1, were put to sea in Month 25, and were harvested in Month 32 at an age of between two years six months and two years seven months. The trout were farmed by Musholm A/S, Reersø, Denmark. During the period at sea (from week 15 to harvest), Aller Superior (Aller Aqua A/S, Christiansfeld, DK) and Efico Enviro 939 (BioMar A/S, Brande, DK), were used for feeding. For practical reasons the Norwegian Qualtiy Cut (NQC) [17] was sampled for each of the 18 rainbow trout and homogenised.

Quality control materials for the analyses of vitamin D: Certified reference materials, Milk powder (CRM421, IRMM, Geel, B), and proficiency testing materials, Milk Powder (FAPAS 2184, FAPAS, York, UK). In-house reference material, Control- salmon, is homogenised whole fillet of wild Atlantic salmon, divided into 50 individual 10 g portions and stored at max -20°C.

Homogenisation: All individual fish sampled were homogenised in a blender (Osterizer, Hohn Oster, WI, USA or 1094 Tecator, Höganäs, Sweden) for 30-60 seconds until homogenous. As a precaution against oxidation, the homogeniser instrument was purged with nitrogen before the homogenisation process was initiated. Homogenised samples were stored in plastic bags at maximum -20°C until analysis, which took place within 6 months.

Analytical methods

Vitamin D: The amounts of vitD3 and 25OHD3 in fish were detected and quantified by use of HPLC-UV/PDA [18,19] or liquid chromatography combined with triple quadrupole mass spectrometry and electrospray ionisation (LC-MS/MS-ESI) [20]. The equipment used being HPLC-UV/PDA from Waters (Alliance 2695; UV-detector 2487; PDA-detector 2996, Milford, MA) and LC-MS/MS from Agilent (LC 1200 and MS 6460, Agilent Technologies, Santa Clara, CA). The trueness of the methods was secured by acceptable results for the analyses of a certified reference material and participation in proficiency test scheme. The consistency of the methods during the studies was checked by analysis of Control-salmon. The HPLC-UV/DAD method (n=8) found 15.4 µg vitD3/100g ± 1.5%, and 0.268 µg 25OHD3/100 g ± 11%, which were similar values to the LC-MS/MS- method (n=18) being 15.2 µg vitD3/100 g ± 5.3% and 0.266 µg 25OHD3/100 g ± 5.8%. All analyses were conducted in a laboratory accredited to performing the analyses according to [21].

Fat content: The content of fat in the fish was determined gravimetrically by a modified Schmid-Bondzynski-Ratslaff (SBR) procedure [22].

Statistical tests: Linear regression and t-test with the probability level of P=0.05 was used. Excel 2010 was used for all calculations and all results are given as average ± standard deviation (x ± SD).

Results

The fish sampled were divided into the five weight categories usually used by rainbow trout farmers being 0.7-1.4 kg, 1.4-1.9 kg, 1.9-2.5 kg, 2.5-3.0 kg, and 3.0-4.0 kg (personal communication, Musholm A/S, Reersø, Denmark). The mean of gutted weight, content of fat, vitD3, and 25OHD3 in salmon and in rainbow trout are given in Table 1.

Farmed salmon (<i>S. salar</i>)					Farmed rainbow trout (<i>O. mykiss</i>)				
Weight kg	Fat g/100 g	Vitamin D3 µg/100 g	25OHD3 µg/100 g	Sample number	Weight kg	Fat g/100 g	Vitamin D3 µg/100 g	25OHD3 µg/100 g	Sample number
0.9 ± 0.3	2.9 ± 0.9	1.4 ± 0.5	0.10 ± 0.03	4	0.9 ± 0.0	7.6 ± 2.7	4.2 ± 1.3	0.09 ± 0.04	3
1.7 ± 0.2	5.5 ± 2.0	1.1 ± 0.4	0.14 ± 0.01	2	1.5 ± 0.2	7.6 ± 1.7	6.2 ± 3.2	0.09 ± 0.04	6
2.2 ± 0.2	4.9 ± 0.7	1.4 ± 0.6	0.14 ± 0.06	2	2.3 ± 0.1	7.5 ± 0.4	3.3 ± 2.1	0.09 ± 0.05	3
2.8 ± 0.2	8.5 ± 1.0	1.6 ± 0.1	0.26 ± 0.02	2	2.9 ± 0.0	10.9 ± 0.3	5.5 ± 0.5	0.11 ± 0.01	3
3.6 ± 0.3	7.9 ± 0.7	2.3 ± 0.3	0.24 ± 0.04	3	3.5 ± 0.5	12.0 ± 2.5	4.3 ± 1.1	0.17 ± 0.01	3
2.1 ± 1.1	5.6 ± 2.5	1.6 ± 0.5	0.17 ± 0.07	13	2.1 ± 0.9	8.9 ± 2.5	5.0 ± 2.3	0.10 ± 0.04	18

Table 1: Farmed salmon and farmed rainbow trout divided into five weight classes – content of vitamin D3, 25OHD3, and fat (mean ± SD).

In farmed salmon there was a linear correlation with fat being dependent on gutted weight (P<0.001). Additionally, content of vitD3 depends on gutted weight (P<0.05) and on content of fat (P<0.05). As for vitD3 content of 25OHD3 was found to depend on gutted weight (P<0.01) and content of fat (P<0.0001). The distribution of 25OHD3 amounted 11.0% ± 4.6% of the content of vitD3.

In farmed trout there was a linear correlation with fat being dependent on gutted weight (P<0.001). No association was found between vitD3 and gutted weight or vitD3 and fat, while for 25OHD3 there was an association with gutted weight (P<0.01), but not with fat content. The relative amount of 25OHD3 compared to vitD3 was 2.4% ± 1.1%.

Discussion

The farmed salmon and farmed rainbow trout were sampled at similar weights and ages. The analyses for farmed salmon were based on homogenization of the whole fillets, while for farmed trout the

NQC was used. NQC is used in the assessment of vitamin D in fish for food composition data [17], and is a more practical approach in the homogenisation procedure. However, the difference in subsampling for homogenisation is a limitation in the interpretation of the results.

Salmon, farmed (<i>S. salar</i>)			Salmon, farmed, organic (<i>S. salar</i>)			Trout, farmed (<i>O. mykiss</i>)			Trout, farmed, organic (<i>O. mykiss</i>)			Year	Ref
Vitamin D3		25OHD3	Vitamin D3		25OHD3	Vitamin D3		25OHD3	Vitamin D3		25OHD3		
Mean	(R) ± SD1	Mean2	Mean	(R) ± SD1	Mean2	Mean1	(R) ± SD2	Mean1	Mean1	(R) ± SD2	Mean1		
						13	(8-16)	na				1994	23
						7.5	(7.3-7.8)	0.14				1995	15
						19	(10-23)	na				1999	24
7.6	(4.2-9.1)	na	5.4	(4.2-6.6)	na	8.1	(3.8-10.7)	na	8.2	(7.9-8.5)	na	2006	25
10		0.49				6.9		0.22				2012	17
						7.0		0.18				2013	26
5.9	± 3.6	na				8.0	± 3.36					2015	27
5.8	(3.6-8.2)	nd				1.6	(1.4-1.8)	nd				2016	28
6.7		0.38										2017	29
			1.6	± 0.5	0.17	5.0	± 2.3	0.1				2017	*

¹(R)±SD: Range or ± standard deviation as provided in reference;

²na: not analyzed; nd: not detected;

*Data from this study.

Table 2: Farmed salmon (*S. salar*) and farmed rainbow trout (*O. mykiss*) – published data on content of vitamin D3 and 25OHD3 (µg/100 g).

The mean content of vitamin D3 in investigated salmon and rainbow trout was 1.6 ± 0.5 and 5.0 ± 2.3 µg/100 g, respectively. The level of vitD in salmon is surprisingly low, which might be due to the content of vitD in feed. We did not focus on feeding, as the sampled salmon and trout were taken from healthy stocks sold for consumption.

Data in the literature for content of vitamin D in farmed salmonids which include the species i.e. taxonomic name are shown in Table 2. Only vitD3 has been included in the majority of studies, as quantification of 25OHD3 has been an analytical challenge. Data for vitD content in raw samples of farmed salmon and farmed trout are scarce, and primarily analysed for food databanks [15,17,23-29]. The sampling system is therefore systematic to cover the market in the specific countries i.e. Denmark, Norway, United Kingdom, and the sampling and homogenization strategy make use of whole fillets as well as NQC. Our aim was different, but might be compared to the data established for food databanks. The content of vitamin D found in our study is currently sufficient to label these farmed fish with health claim “High content of vitamin D” in Europe [30]. However, it should be noticed that recommended dietary intake for vitamin D has increased from 5µg/day to 15µg/day over the last years [31,32]. The data in Table 2 indicate that content of vitamin D in farmed salmon and farmed trout seems to have decreased within the last 25 years. The change of feed based on fishmeal to vegetable feed [33] could cause such decrease. Fish products are essential for the dietary intake of vit D e.g.

in French diet the estimate is that 33% of the dietary intake of vitamin D derives from salmon [34]. An estimate which is based on the content of 15µg vitamin D/100 g salmon, which is almost 10 times higher than we find. The studies which did include quantification of vit D3 and 25OHD3 show that the relative amount of 25OHD3 compared to vitD3 is 5-11% for salmon and 2-3% for trout. We hypothesize that the distribution between vitD3 and 25OHD3 is species specific.

In the whole fillet from farmed salmon we found that content of fat increases in a linear matter with increase in weight, and vitD3 increased with increased content of fat. For the NQC in farmed trout similar correlation for content of fat and gutted weight occurred, but the content of vitD3 showed no association with fat. Whether this difference is species dependent or caused by the difference in subsampling has to be confirmed. However, if this is due to the subsampling of NQC the use of NQC could introduce a BIAS if used for analyses of vitD in fish for establishing data for food databanks. Previously other differences have been reported for *S. salar* and *O. mykiss* in studies aiming to increasing content of vitamin D in the two species by increasing vitD3 in the fish feed. Trout were fed 89-539 µg/kg for four months up to a weight of 618 g-1282 g and 7-14% fat [35], while salmon were fed 40-28680 µg/kg for 11 weeks to a weight of 450 g [36,37]. No effect was observed in trout, while content of vitamin D in salmon increased linearly with increased vitamin D in feed.

The limitation in our study is, apart from the differences in sampling strategy, that only one production cycle of each farmed

species was included. Furthermore, we did not focus on the content of vitamin D in feed, as the aim was to investigate the current production system, but the sampled salmon and trout were taken from healthy stocks sold for consumption.

Conclusion

The farmed produced salmon and the farmed rainbow trout were sampled at similar weights and ages. For analyses the whole filet was used for salmon while Norwegian Quality Cut was used for trout. The content of vitamin D₃ was 1.1-2.3 µg/100 g and 3.3-6.2 µg/100 g, respectively. In farmed salmon a relationship was found for vitamin D₃ to gutted weight as well as to fat content, while no similar relationship was found for farmed rainbow trout. Despite this, both species exhibit a linear correlation between fat and gutted weight. Compared to vitamin D₃, the content of 25-hydroxyvitamin D₃ was 11% and 3%, respectively. The low content of vitamin D found in the salmonids is challenging farmed salmon and farmed trout as essential vitamin D sources.

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Conflict of Interest

None of the authors have conflicts of interest.

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