Research Paper

Feed-to-Fillet Transfer of Selenite and Selenomethionine Additives to Plant-Based Feeds to Farmed Atlantic Salmon Fillet

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ABSTRACT

This study investigated the transfer kinetics of dietary selenite and selenomethionine (SeMet) to the fillet of farmed Atlantic salmon (*Salmo salar*). The uptake and elimination rate constants of the two selenium (Se) forms were determined in Atlantic salmon fed either selenite- or SeMet-supplemented diets followed by a depuration period. The fillet half-life of selenite and SeMet was 779 ± 188 and 339 ± 103 days, respectively. The elimination and uptake rates were used in a simple one-compartmental kinetic model to predict levels in fillet based on long-term (whole production cycle) feeding with given dietary Se levels. Model predictions for Atlantic salmon fed plant-based feeds low in natural Se and supplemented with either 0.2 mg of selenite or SeMet kg^{-1} gave a predicted fillet level of 0.042 and 0.058 mg Se kg^{-1} wet weight, respectively. Based on these predictions and the European Food Safety Authority risk assessment of Se feed supplementation for food-producing terrestrial farm animals, the supplementation with 0.2 mg of selenite kg^{-1} would likely be safe for the most sensitive group of consumers (toddlers). However, supplementing feed to farm animals, including salmon, with 0.2 mg of SeMet kg^{-1} would give a higher (114%) Se intake than the safe upper intake limit for toddlers.

HIGHLIGHTS

- The EU has restricted the use of Se additives in food-producing land animals to ensure food safety.
- A one-compartmental kinetic model is established that can predict fillet Se levels in farmed salmon.
- Adding 0.2 mg of inorganic Se per kg of salmon feed does not cause concern for food safety.
- Adding 0.2 mg of organic Se per kg of farmed animal (including salmon) feed causes the food safety limit to be exceeded.

Because of a rapid growth in aquaculture and limited access to marine resources, fish oil and fish meal in feeds for carnivorous marine species such as Atlantic salmon (*Salmo salar*) have increasingly been replaced with plant ingredients over the past decades *(43)*. Selenium (Se) is one of the essential minerals that there are higher levels of in fish meal than in plant feed ingredients *(4)*. A decline of Se in Norwegian-produced commercial salmon feed during the last decade has been attributed to the decreased inclusion of fish meal *(38)*. Se concentration in Atlantic salmon flesh was lower when fed on plant protein replacement feeds compared with marine protein feeds *(4)*. Se is a well-known essential trace element *(41)* that is active as part of functional selenoproteins *(24)* involved in physiological processes such as antioxidant defense (glutathione peroxidases) *(41)* and thyroid homeostasis (deiodinases) *(35)*. Of all food products, seafood has some of the highest natural background Se levels *(8, 41)*. Several studies on fish nutrition have recommended Se supplementation to plant-based feeds to restore or maintain Se levels in farmed fish as a Se source for consumers *(4, 30)*.

In addition to being an essential element, excess Se intake is known to be toxic for most vertebrates *(20)*, including humans *(31)*. The European Union (EU) has set a safe upper limit (UL) intake to guarantee consumer health *(7)*. Concern has been raised regarding excessive Se intake by consumers of food products of farmed terrestrial animals origin (eggs, milk, and meat) that have been reared on Se-supplemented feeds *(9)*. This refers in particular to the safety for children 1 to 3 years of age (toddlers), who have a recommended safe UL of intake of 60 μg day^{-1} *(7)*. Of the Se forms supplemented to feed for terrestial food-producing animals, the organic Se forms (e.g., Se-yeast or selenomethionine [SeMet]) have a higher feed-to-food transfer than the inorganic Se forms (e.g., selenite) *(9, 10)*. After risk assessment by the European Food Safety Authority (EFSA), the EU has set a specific UL for organic Se supplementation to animal feeds to ensure food safety *(9, 10)*. The EFSA risk assessment on Se feed supplementation has been performed

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for terrestrial food-producing animals only and is based on the food-specific (eggs, milk, and meat) feed-to-food transfer of organic and inorganic Se (9) and the estimated consumption of these food products in the general European population using the Comprehensive European Food Consumption Database (9, 12). The Se feed supplementation risk assessment does not include farmed fish, although dietary organic Se forms are also known to have a relative high muscle accumulation in farmed seafood species such as Atlantic salmon (3, 13, 14, 22, 26, 32). Currently, a feed-to-fillet transfer assessment of supplemented dietary Se throughout the whole Atlantic salmon food production chain (until slaughter size fish) is lacking, which is the first step of a risk assessment on feed Se supplementation in farmed seafood (10).

Several toxico-kinetic feed-to-food transfer models have been used to describe the fate of dietary contaminants and additives in food-producing animals, including Atlantic salmon fillet. Such models are valuable tools for predicting the levels of feed additive in salmon fillet when the fish is reared on different feed additive scenarios during an entire production cycle. Fish feed-to-fillet transfer models are based on uptake and elimination kinetics and vary from simple one-compartmental feed-to-fillet transfer models (5) to multicompartmental physiological-kinetic–based models (29). Although routes and rates of elimination from internal organs (e.g., liver and kidney) (17, 18) and whole body (16) have been assessed in salmonids, the fillet-specific uptake and elimination kinetics, which form the basis for feed-to-food transfer assessment, are lacking in Atlantic salmon.

The present study aimed to assess the feed-to-fillet transfer Se supplementation to Atlantic salmon feeds throughout the seafood production chain, thus expanding on earlier food safety risk assessments by the EFSA setting the UL for feed Se supplementation for food-producing terrestrial animals (10). The fillet Se uptake and elimination kinetics were assessed in Atlantic salmon fed on elevated levels of selenite or SeMet for 3 months followed by an extended (~3-month) depuration period. The fillet kinetics were used in a simple one-compartmental feed-to-food model that was used to predict fillet Se levels in farmed Atlantic salmon that were fed on different feed levels during an entire seawater food production cycle (>12 months). A consumer Se intake assessment was made on the contribution of consuming farmed seafood reared on Se-supplemented feed, expanding the earlier food safety risk assessment for farmed terrestrial food-producing animals. The present article is part of a series of articles that aim to assess the safety of Se supplementation to Atlantic salmon feeds. Previous publications addressed analytical methods for Se speciation in salmon feed and tissues (36), identification of main pathways of toxicity in Atlantic salmon (3), and establishment of safe limits for fish heath (1).

MATERIALS AND METHODS

Ethics statement. The experiment was approved by the Norwegian Food Safety Authority (National Animal Research Authority surveillance and application system [Forsøksdyrforvaltningens tilsyns- og søknadssystem] identification no. 9003) and performed in compliance with national and international ethical standards.

Experimental conditions and diets. The feeding trial was carried out at the Norwegian Institute of Food, Fisheries and Aquaculture Research (Sunndalsøra, Norway) between 15 November 2016 and 3 March 2017. Details regarding in the experimental setup and diet composition were published previously (1). In brief, the general basal diet had the following composition (percent, inclusion level of the feed ingredient to the total diet): wheat gluten (17%), maize gluten (10%), pea protein 50 (5%), pea protein >72 (5%), wheat (10.5%), fish oil (12.2%), rape seed oil (12.2%), fish meal (10%), soya protein concentrate (10%), and micronutrient mixture (8.1%). The diets were formulated based on commercial diets and feed ingredients that fulfilled the nutritional requirements of salmonids (27). The basal diet was supplemented with either inorganic Se (sodium selenite [Na_2SeO_3], DSM, Heerlen, The Netherlands) or organic Se (>98% L-SeMet, Excential Se4000, Minsups, Winsford, England) at a nominal concentration of 5 mg kg^{-1} (analyzed levels 5.4 ± 0.09 and 6.2 ± 0.2 mg kg^{-1} wet weight [ww] for selenite and SeMet, respectively, mean ± standard deviation [SD], $n = 3$). The experimental feeds were produced by a commercial fish feed producer (Biomr, Brande, Denmark).

The feeds were fed to 630 Atlantic salmon smolt (Salmobreed, 6 months, both sexes) in total, with an initial weight of 147 ± 4 g (mean ± SD, $n = 30$). The smolt were randomly distributed into nine tanks with 70 fish in each tank. Before the experiment, all fish were fed the control diet (background level of 0.45 mg kg^{-1} total Se) for 2 weeks to acclimate to the holding facilities. After the acclimatization period, fish were fed selenite- or SeMet-supplemented diets for 90 days. After the dietary selenite and SeMet exposure period, the fish were fed the control diets for a depuration period of 90 days. Six daily meals were provided with 4 h between the meals, at a feeding level of ~1.1% of body weight per day. Unconsumed feed pellets were collected and weighed once per day, to calculate feed intake, feed conversion, and Se exposure. To avoid possible leakage from feces or pellets to the water, a relatively high-water flowthrough was maintained of ~10 L min^{-1} per tank. During the accumulation period, three fish from each tank were sampled on days 0, 4, 8, 20, 45, 75, and 90. During the depuration period, three fish per tank were sampled on days 0, 2, 6, 12, 24, 72, and 90. Fish were randomly collected from the tanks, anesthetized in a bath of tricaine methanesulfonate (FINQUEL MS-222; Scanvacc, Hvam, Norway; ~60 mg L^{-1}), and sacrificed by a blow to the head. Fish were stored at –28°C and at the end of the experiment all fish were thawed, weighed, and filleted (whole fillet muscle with skin on the left side of the salmon, fillet weight per fish was registered); three fish per tank were pooled ($n = 3$ per sampling point), freeze-dried, and analyzed for Se. Muscle samples were weighed before and after freeze-drying to measure water content.

Se analyses of total Se and speciation. The earlier EFSA food safety assessment on Se feed supplementation to terrestrial food-producing animals is based on total Se levels; hence, the Se uptake and elimination kinetics and final food safety assessment is based on total Se levels. Se speciation of the experimental diets supplemented with either selenite or SeMet and muscle from salmon reared on these feeds at the end of the exposure period were assessed according the Se speciation method described by Sele et al. (36). In brief, for Se speciation analysis in feed and muscle tissue, anion-exchange high-performance liquid chromatography–inductively coupled plasma–mass spectrometry (HPLC-
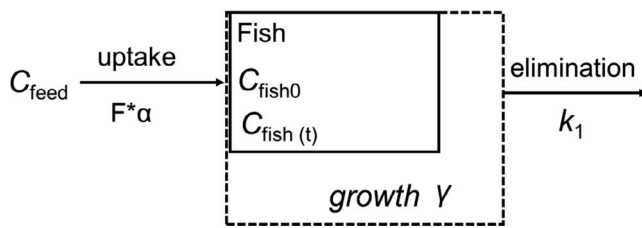
FIGURE 1. *Schematic representation of the feed-to-fillet transfer kinetics in Atlantic salmon:* C_{feed} *is concentration in feed; F is feeding rate; α is uptake rate;* C_{fish0} *is initial concentration in fish; γ is growth; and* K_1 *is elimination.*

ICP-MS) for analysis of selenite and selenite and cation-exchange HPLC-ICP-MS for analysis of SeMet were applied. In addition, reverse-phase HPLC-ICP-MS was applied for analysis of selenocysteine in fillet samples. The uptake and elimination kinetics over time were assessed on total Se levels, assuming the percentage of inorganic and organic Se in feed and muscle speciation at the end of the exposure period. Total Se levels were assessed in freeze-dried diets and fish tissues, and samples were digested using the microwave-acid decomposition method based on the method described by Berntssen et al. *(3),* modified from Julshamn et al. *(21).* Total Se concentration was determined in the digests by ICP-MS (iCAP-Q and FAST SC-4Q DX auto sampler, Thermo Fisher Scientific, Waltham, MA). A solution of internal standard (germanium, rhodium, and thulium, Thermo Fisher Scientific) was added on-line for correction of instrumental drift during the analysis. Oyster tissue (OT; CRM 1566 b, National Institute of Standards and Technology, Gaithersburg, MD) and lobster hepatopancreas (TORT-3, National Research Council Canada, Ottawa, Ontario, Canada) were used as reference materials for the Se analysis (Se value for OT and TORT-3 reference material is 2.06 ± 0.15 and 10.9 ± 1.0 mg kg^{-1} dry weight [dw], respectively). The analyzed total Se value of the reference material was 2.08 ± 0.10 and 11.1 ± 0.51 mg kg^{-1} dw for OT and TORT-3, respectively (mean $\pm$ SD, $n = 4$), and the method was found satisfactory when analysis of the reference material was within the 95% confidence limit. Samples were run in two batches of 58, with six procedural blanks, two reference materials (OT and TORT-3), and 24 samples with a duplicate per sample. The limit of quantification for total Se is 0.01 mg kg^{-1} ww.

Model description. The dietary selenite and SeMet uptake and elimination kinetics in Atlantic salmon fillet were assessed by a simple one-compartmental kinetic fillet model, as described for the feed-to-food transfer for Atlantic salmon *(2).* The simple one-compartment fillet model was derived from Sijm et al. *(37),* and the model was used to predict total Se fillet concentrations in fish fed with different feed supplementation levels of selenite and SeMet. The model concentrates only on the transfer of dietary Se in salmon muscle and does not include organs not used for food

consumption. The model shown in Figure 1 describes the feed-to-fillet transfer as the product of feed concentration (C_{feed}, mg of Se kg^{-1}), feeding rate (F, % body weight day^{-1}), uptake rate (α, mg of Se day^{-1}), initial concentration in the fish fillet (C_{fish0}, mg of Se kg^{-1}), growth dilution (γ, % body weight day^{-1}), and physiological elimination rate (K, day^{-1}).

With a compound-specific uptake and elimination rate constant for organic and inorganic Se at a certain feeding rate and dietary concentration (C_{feed}), the concentration of a chemical in a fish (C_{fish}) at a given time can be described as

$$C_{fish}(t) = \frac{\alpha F t}{K + \gamma} C_{feed} \left(1 - e^{-(K+\gamma)t}\right) + C_{fish0}{}^{-(K+\gamma)t} \quad (1)$$

The model equation parameters, based on the uptake and elimination kinetics from the feeding trial, are given in Table 1. The correctness of the model parameters is validation of comparing model predicted values with analyzed values in Figure 2.

Statistics. As detectable Se levels were present in the acclimatized fish and the low Se control diet, data from the selenite and SeMet dietary groups were corrected for fish from the control group to compensate for background Se levels. Growth rates were calculated by fitting fish weight to the following equation: ln fish weight = $a + b \times t$, where a is a constant, b the growth rate (g day^{-1}), and t is the time of the experiment. The elimination constant (k_{el}) was determined by fitting concentration data to a first-order decay curve: ln $C_{fillet} = a + k_{el}$. Elimination half-lives ($t_{1/2}$) are ln $2/k_{el}$. The weight of the fish increased from 147 to 468 g during the exposure period and from 468 to 953 g during the elimination period. This growth corresponds to a decrease in fillet Se concentration of ~220% during the exposure period and of ~100% during the elimination period. The Se muscle concentrations were corrected for this growth dilution by assessing the uptake and elimination rates as total fillet Se amount and not fillet concentrations.

The uptake rates were calculated by fitting (Statistica, Statsoft Inc., Tulsa, OK) the concentration data to the integrated form of the kinetic rate equation 1 for constant dietary exposure *(6):*

$$\alpha = \frac{C_{fish}(t) \cdot k_{el}}{F \cdot C_{feed}[1 - \exp(-k_{el} \cdot t)]} \quad (2)$$

where C_{feed} is the Se concentration (mg g^{-1} ww) in feed, α is the uptake rate constant, and F is feeding rate (g feed g^{-1} fish day^{-1}).

All statistics were performed using the program Statistica (Statsoft Inc.). Statistical differences in Se concentrations and amount between sampling points were assessed one-way analysis of variance, followed by Tukey's honestly significant difference post hoc test at a significance level of 0.05 *(44).*

Se input data and model scenarios. Different Se feed level scenarios were used to estimate fillet Se levels during an entire seawater food production cycle for Atlantic salmon. One set of feed level scenarios (scenarios 1 to 3) is based on the current basal

TABLE 1. *Dietary selenomethionine (SeMet) and selenite, uptake rate (α), half-life (*$t_{1/2}$*), and elimination (*k_2*) constants in Atlantic salmon (*Salmo salar*) fed SeMet- and selenite-supplemented feeds (6.2 and 5.4 mg kg*$^{-1}$ *wet weight, respectively) for 90 days followed by a 90-day elimination period[a]*

	Feed concn (mg kg^{-1})	α	$t_{1/2}$ (days)	k_2 (10^{-3} day^{-1})
SeMet	5.4	0.148 ± 0.016 A	779 ± 188 A	0.98 ± 0.39 A
Selenite	6.2	0.012 ± 0.001 B	339 ± 103 B	1.80 ± 0.45 B

[a] Values are means $\pm$ SD, $n = 3$ of three pooled fish, per time point. Values with the same letters are not significantly different ($P > 0.05$).
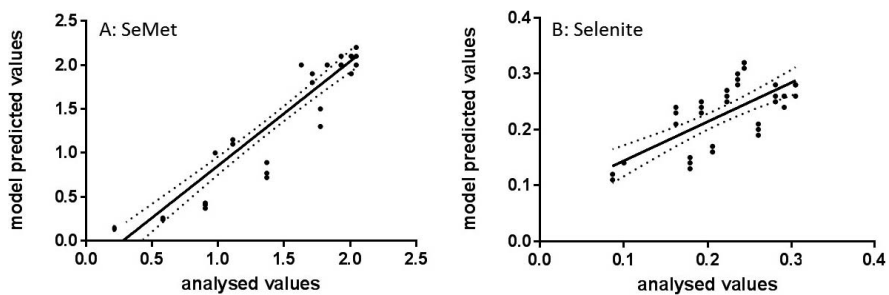
FIGURE 2. *Observed versus model-predicted fillet concentrations (milligrams per kilogram ww) of SeMet (A) and selenite (B) in Atlantic salmon fed on SeMet- or selenite-enriched diets, followed by a depuration period where fish were fed a control diet (n = 39 for both selenite and SeMet).*

Se levels in commercial salmon feeds supplemented with SeMet or selenite: scenario 1, average Se levels from Norwegian feed surveillance in 2016 (1.1 mg kg^{-1} ww) and scenarios 2 + 3, average Se levels from Norwegian feed surveillance supplemented with SeMet or selenite to a level of 0.2 mg kg^{-1} (1.1 + 0.2 = 1.3 mg kg^{-1} ww). The second set of scenarios (scenarios 4 to 6) is based on low background levels of Se (low fish meal and high plant meal inclusion levels) supplemented with SeMet or selenite: scenario 4, Se background levels for fish feed with high plant and low (10%) marine protein (0.4 mg kg^{-1} ww) and scenarios 5 + 6, Se levels for fish feed with high plant and low (10%) marine protein (0.4 mg kg^{-1} ww supplemented with SeMet or selenite at a supplementation level 0.2 mg kg^{-1} (0.4 + 0.2 = 0.6 mg kg^{-1} ww).

Surveillance data on both commercially available Norwegian produced Atlantic salmon feeds and Atlantic salmon fillet from market size Atlantic salmon sampled from commercial fish farms were used as input data to the feed-to-fillet transfer models. For long-term model prediction in the different feed scenarios, an average feeding rate of 0.78% of body weight day^{-1}, a growth rate of 0.64% day^{-1}, and a feeding duration of 13 months were used in the model scenarios, as could be expected in a commercial seawater food production cycle. The final growth phase started with a 100-g postmolt and ended with a market size weight of 3 kg (industrial model of Cargill EWOS innovation *(33)*) *(2)*. The average Atlantic salmon fillet level of 0.10 mg of Se kg^{-1} ww for a 100-g presmolt was used as the initial fillet concentration.

Farmed salmon food safety assessment. The EFSA has conducted a risk assessment of organic and inorganic Se feed additives for food-producing farm animals *(9)*. This risk assessment did not include the consumption of farmed seafood reared on Se-supplemented feeds. In the present study, the EFSA opinion is used as a basis and extended with data for farmed Atlantic salmon fed plant-based diets supplemented with L-SeMet or selenite to the level of organic Se that is currently authorized for food-producing animals (0.2 mg of Se kg^{-1}).

The following assumptions and input data were used as described in the EFSA opinion *(9)*:

(i) Toddlers are the most sensitive consumer group, with an upper tolerable limit for total Se intake of 60 μg day^{-1} *(7)*.

(ii) Toddlers have a background intake of 10 μg of Se day^{-1} from vegetables, fruits, and cereals *(9)*.

(iii) Se intake from food is based on the sum of estimated intake from consumption of food from food-producing animals fed Se-supplemented feeds.

(iv) As food intake data, toddlers consumption data for meat, milk, and dairy and eggs are used *(9, 12)*, and this is extended with the median value of the 95 percentile consumption data for Atlantic salmon consumption of 40 g day^{-1}, based on the updated (28 April 2018) EFSA Comprehensive European Food Consumption Database for consumers only *(12)*.

(v) Background Se levels in control (low or unsupplemented) food classes are based on the EFSA *(9)* opinion, and the predicted levels in Atlantic salmon fed Se-unsupplemented, plant-based feeds (this study).

(vi) Background levels are multiplied by a factor that expresses the relative increase in Se levels at a given supplementation level compared with the background Se level per food class (supplementation increase factor) *(9)*.

RESULTS AND DISCUSSION

Uptake and elimination kinetics and Se speciation. Details on fish health and aquaculture performance such as weight, length, condition factor, and liver somatic index have been published elsewhere *(1)*. No significant differences were observed in any of the aforementioned parameters among fish fed control diet and fish fed SeMet- or selenite-enriched diets during the 90-day feeding period. No mortality was observed in the control or the Se-exposed groups. Se speciation analyses of the experimental feeds confirmed that inorganic Se (selenite and selenate) was the dominant (~87%) Se species in selenite-supplemented feeds and that SeMet was the dominant Se species (~95%) in SeMet-supplemented feeds. Muscle Se speciation analyses in fish fed the experimental diets for 3 months showed that SeMet was the major fillet Se species (~91%) in salmon fed diets supplemented with SeMet. By contrast, for salmon reared on selenite-enriched feeds, inorganic Se was the dominant form in the muscle (~70%), with SeMet as the second dominant form (~28%).

Figure 3 gives the fillet Se concentration and the total amount of Se in fillet in Atlantic salmon fed SeMet- or selenite-supplemented, plant-based diets followed by control feed. Fish fed SeMet showed a significant increase in fillet concentrations at all subsequent sampling points except the last two sampling points of the accumulation period (between 75 and 90 days). Fish fed selenite had a significant increase between sampling point until day 20, after which an apparent steady-state condition was reached as fillet Se concentrations did not increase significantly between sampling days 20 and 90. By contrast, when expressed as total amount of Se per fillet, thus compensating for growth dilution, both SeMet- and selenite-exposed fish had a continuously significant increase at all sampling points of the exposure period. After the exposure to SeMet- and selenite-supplemented feed was terminated, total amount of SeMet fillet continued to increase for 6 days, whereas selenite levels decreased immediately after changing to the control diets. The total amount of fillet Se

FIGURE 3. *Fillet concentration (milligrams of Se per kilogram ww; A and B) and total amount (milligrams; C and D) of Se in Atlantic salmon fed SeMet (A and C)– and selenite (B and D)–supplemented feeds (6.2 and 5.4 mg kg^{-1}, respectively) for 90 days followed by a 90-day elimination period (mean ± SD, n = 3 of three pooled fish, per time point). Dashed vertical line represents the end of the exposure period.*
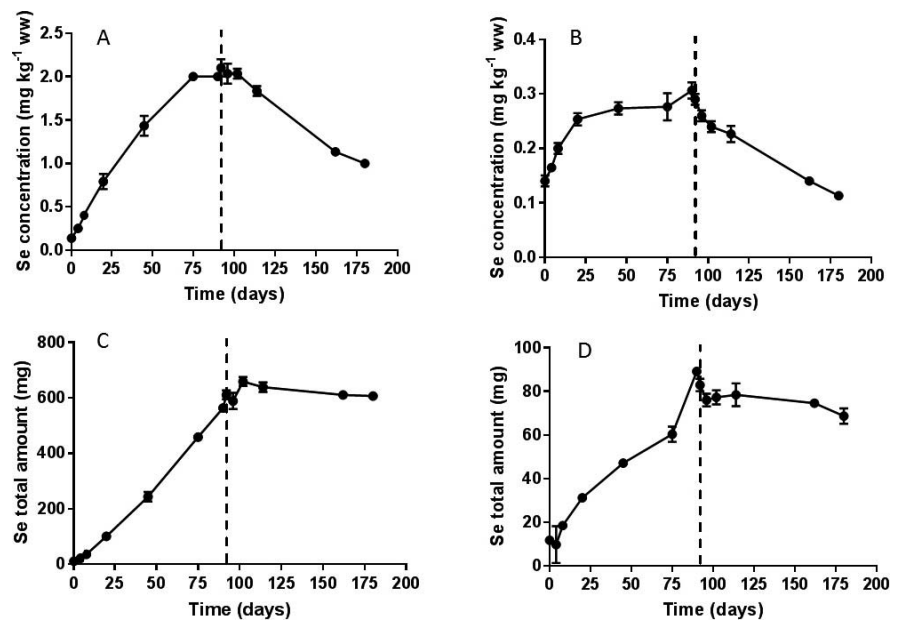
decreased significantly during the depuration period for both SeMet- and selenite-fed fish. However, for both exposure groups elimination was slow, and based on the estimated half-lives ($t_{1/2}$ of ~790 and 340 days for SeMet- or selenite-fed fish, respectively), no steady state in either SeMet or selenite accumulation is expected to be reached during a normal seawater production cycle of 12 to 16 months. The use of the presented one-compartmental transfer model allows predictions of Se fillet levels during an average production cycle. In addition, final fillet Se levels are not only determined by the level of dietary Se supplementation but also aquaculture production parameters such as relative feed intake and growth rates (2). The present model allows the use of commercially relevant feed intake and growth rate to provide a transfer assessment at different whole cycle feeding scenarios (see below). The use of a short (subchronic 10% of the life cycle, ~3 months) experimental exposure feeding trial, without uptake and elimination kinetics assessment, would not be appropriate to reflect Se fillet levels following a commercial seafood production cycle.

Table 1 gives the uptake and depuration rates for SeMet and selenite, which are the model equation parameters described in Figure 1 The uptake rate of SeMet was significantly higher, and elimination significantly ($P <$ 0.05) lower than those of selenite. Earlier trials showed a higher whole-body ^{75}Se-isotope uptake and lower elimination, resulting in a higher accumulation of SeMet compared with selenite (22). This difference in SeMet and selenite uptake and relative accumulation can be explained by differences in protein incorporation. Some of the absorbed Se from SeMet is metabolized to dihydrogen selenide to be used in Se pathways and specific selenoproteins, whereas another portion is nonspecifically incorporated into the general body proteins as a substitute for the common amino acid methionine (11). In contrast, selenite can only become part of the specific selenoprotein pool after a reaction with glutathione to form hydrogen selenide before being

incorporated in specific selenoproteins (39). Liver is the main organ for Se metabolism, and at excess intake of Se can be excreted (39). The nonspecific incorporation of SeMet into the general protein pool, as opposed to the specific incorporation of selenite in selenoproteins, is reflected by the higher muscle Se levels in fish fed organic Se compared with inorganic Se in the present and previous studies (3, 13, 14, 32). Also, for other food items such as brown rice, selenite supplementation caused an increase of Se in the Se-protein fraction of the rice (25).

Previous kinetic studies on dietary Se in fish have shown a high intestinal absorption with a higher uptake rate for SeMet than selenite (22, 23), and Se elimination is dominated by gill and urine with lesser routes by bile and mucus (23). The biological half-life of Se from tissues decreased with increased Se loading except in the liver, suggesting a rate-limiting metabolic transformation of Se for excretion in this organ (18). Hilton and Hodson (17) reported liver and kidney elimination $t_{1/2}$ values of 9 to 45 and 26 to 28 days, respectively, depending on Se feed concentrations. Similarly, in a long-term trial with cutthroat trout (*Oncorhynchus clarkii*), fed graded levels of SeMet, the whole-body elimination increased with increased initial body burden (16) and whole-body half-lives varied from $t_{1/2}$ of 518 to 84 days (16). Few studies have included the long-term muscle kinetics that reflect the selenite- and SeMet-specific kinetics that include their difference in protein incorporation. In the present study, the fillet half-lives in 6.2-mg Se kg^{-1} ww SeMet-fed fish were in the same order ($t_{1/2}$ of 779 days) as those in the study by Hardy et al. (16). Fish fed 5.2 mg kg^{-1} ww selenite had a lower half-life ($t_{1/2}$ of 339 days). The low elimination rate for both SeMet and selenite ($t_{1/2} > 11$ months) reflects the low release of Se from proteins in the fillet. The lower elimination of SeMet compared with selenite is likely the result of the larger unspecific protein pool in which SeMet is incorporated compared with the more limited selenoprotein pool for selenite.

Based on the growth-corrected uptake and elimination rates, we established a simple one-compartment transfer model and validated it with the analyzed values from the present trial. Figure 2 gives the observed and predicted concentrations for SeMet and selenite during the accumulation and elimination phases as calculated by equation 3. A significant ($P < 0.001$) linear correlation was discerned between observed and predicted fillet concentrations ($r^2 = 0.88$ and 0.65 for observed-predicted SeMet and selenite, respectively). Further model validation was made with the data from an earlier long-term (whole seawater production cycle) Se feeding trial in which Atlantic salmon were fed a high fish meal diet with high Se levels (1.1 to 1.0 mg kg^{-1} ww) or a low fish meal diet with lower Se levels (0.7 to 0.6 mg kg^{-1} ww) (4). Based on the reported feed concentrations, feeding rate, growth rate, and feeding duration, model-predicted Se levels were 0.098 and 0.053 mg kg^{-1} ww, respectively, whereas observed values were 0.096 and 0.050 mg kg^{-1} ww, respectively.

Model input data and model scenarios. The simple one-compartment model, based on uptake and elimination kinetics and aquaculture performance parameters (growth rate and feed intake), was used to estimate the Se fillet levels in market-size Atlantic salmon (3 kg) during an entire production cycle when fed with different SeMet and selenite supplementation levels to basal feeds with current (surveillance) Se levels (scenarios 1 to 3) or low background levels of Se (low fish meal and high plant meal inclusion levels; scenarios 4 to 6, Table 2). Surveillance data on both commercially available Norwegian-produced Atlantic salmon feeds and Atlantic salmon fillet from market-size Atlantic salmon sampled from commercial fish farms were used as input data to the whole life cycle feed-to-fillet transfer models. Other input data for a long-term (13-month) prediction were average feeding and a growth rates as could be expected in a commercial seawater food production cycle.

The whole life cycle predictions are compared with surveillance data on farmed salmon. Based on the average Se content in Norwegian-produced commercial salmon feed of 1.1 mg kg^{-1} ww (34), the model estimates Se Atlantic salmon fillet levels of 0.11 mg kg^{-1} ww (scenario 1). The average fillet level in Norwegian Atlantic salmon randomly sampled in 2016 was 0.13 mg kg^{-1} ww, with a minimum and a maximum concentration of 0.065 and 0.27 mg kg^{-1} ww ($n = 190$), respectively (19). Surveillance of commercial feed showed a large variation in total Se levels, with a minimum and a maximum concentration of 0.3 and 17 mg kg^{-1} ww, respectively. Surveillance of mineral mixes used in salmon feeds showed average levels of 24 mg kg^{-1} ww (minimum to maximum, 1 to 91 mg kg^{-1}, $n = 8$), thus indicating a general supplementation of Se to salmon feed (34). The current EU UL for total Se in animal feeds when supplemented with Se is 0.5 mg kg^{-1}, indicating commercial salmon feed typically exceeds the UL of supplementation. For organic Se, a supplementation level of 0.2 mg kg^{-1} is authorized. When supplementing 0.2 mg SeMet kg^{-1} to average commercial feed levels (1.1 mg kg^{-1}), the

TABLE 2. *Overview of model-predicted Atlantic salmon selenium (Se) fillet levels when fed on different feed scenarios (feed scenarios, number) during an entire salmon seafood production chain, based on a feed-to-fillet transfer model*[a]

Feed scenario (no.)	Feed concn (mg Se kg^{-1})	Predicted fillet concn (mg Se kg^{-1} ww)
Surveillance avg (1)	1.10	0.11
Surveillance avg + 0.2 SeMet (2)	1.30	0.14
Surveillance avg + 0.2 selenite (3)	1.30	0.12
High plant background (4)	0.40	0.039
High plant + 0.2 SeMet (5)	0.60	0.058
High plant + 0.2 selenite (6)	0.60	0.042

[a] The feed scenarios include salmon Se fillet levels when fed on (1) average Se feed concentration currently found in salmon feed (surveillance), (2) average current Se feed levels supplemented with 0.2 mg kg^{-1} SeMet (surveillance + 0.2 SeMet), (3) or 0.2 mg kg^{-1} selenite (surveillance + 0.2 selenite), (4) Se feed concentration in high plant-based feed (high plant), (5) Se feed levels in high plant-based feed supplemented with 0.2 mg kg^{-1} SeMet (high plant + 0.2 SeMet), or (6) 0.2 mg kg^{-1} selenite (high plant + 0.2 selenite). For details, see main text.

predicted fillet concentrations increased by 16% to 0.14 mg kg^{-1} ww (scenario 2 compared with scenario 1), whereas supplementing with 0.2 mg of selenite kg^{-1}, the predicted fillet concentrations increased by 2.7% to 0.12 mg kg^{-1} ww (scenario 3 compared with scenario 1). Supplementing selenite or SeMet at 0.2 mg kg^{-1} feed to a plant-based diet with a low background Se level (0.4 mg kg^{-1} ww) increased predicted Atlantic salmon fillet concentrations by 33 and 13% to a level of 0.058 and 0.042 mg kg^{-1}, respectively (scenarios 5 or 6 compared with scenario 4, respectively).

Food safety. Excess Se intake is known to cause harmful effects (31), and Se supplementation of food should be carefully considered since this could increase the risk of Se toxicity (28). At concentrations higher than those necessary for fulfilling requirements, Se can cause selenosis (15), type 2 diabetes (20), and endocrine disruption by impairing synthesis of thyroid hormones (28) and can be genotoxic and carcinogenic (20, 41). At a biochemical level, Se can induce oxidative stress (by upregulation of antioxidant proteins) and redox cycling of auto-oxidizable Se metabolites; cause glutathione depletion, protein synthesis inhibition, depletion of S-adenosyl-methionine (cofactor for selenide methylation), and general replacement of sulfur; and cause reactions with critical sulfhydryl groups of proteins and cofactors (reviewed in European Commission (7) and Jablonska and Vinceti (20)).

Earlier risk assessments have established an UL for Se intake of 300 μg day^{-1} for adults (7) and 60 μg day^{-1} for toddlers (7). Young children (toddlers) are the most vulnerable group with regard to possible excessive Se intake resulting from adding Se to animal feed (9) and Se intake that may be near or above the UL (8). Seafood has some of the highest natural background levels of Se

TABLE 3. *Estimated intake of Se by toddlers from food products from farm animals fed diets supplemented with selenite or SeMet (0.2 mg kg^{-1}) (based on the EFSA (9) assessment), with the addition of Atlantic salmon fillet[a]*

Food	Amt consumed (kg)	Increase factor Se	Control food (mg kg^{-1} ww)	Supplemented food (mg kg^{-1} ww)	Total Se intake (mg)
Selenite supplemented (0.2 mg kg^{-1})					
Meat	0.090	1.30	0.107	0.139	0.013
Milk	1.050	1.45	0.010	0.015	0.015
Eggs	0.035	2.60	0.074	0.192	0.007
Salmon fillet	0.040	1.10	0.039	0.043	0.002
Total food from farmed animals					0.037
Background intake from cereals, etc.					0.010
Total intake					0.047
% of UL					78
SeMet supplemented (0.2 mg kg^{-1})					
Meat	0.09	2.20	0.107	0.235	0.021
Milk	1.05	2.36	0.010	0.024	0.025
Eggs	0.035	3.84	0.074	0.282	0.010
Salmon fillet	0.040	1.480	0.039	0.058	0.002
Total from farmed animals					0.059
Background intake from cereals, etc.					0.010
Total intake					0.069
% of UL					114
Avg Se level in commercial Atlantic salmon fillet					
Meat	0.09	2.20	0.11	0.24	0.021
Milk	1.05	2.36	0.01	0.02	0.025
Eggs	0.035	3.84	0.07	0.28	0.010
Salmon fillet	0.040			0.13	0.005
Total food from farmed animals					0.061
Background intake from cereals, etc.					0.010
Total intake					0.071
% of UL					119

[a] Estimated total Se intake per food product is based on (i) consumption data from the Comprehensive European Food Consumption Database *(12)* 95th percentile for toddlers (amount consumed, kg), (ii) the estimated Se deposition in farmed animals fed on Se-supplemented feed from experimental trials (increase factor Se), (iii) the Se level in control groups of food-producing animals when fed on unsupplemented Se diets (control food, mg kg^{-1} wet weight [ww]), and (iv) a background Se intake of 10 µg day^{-1} from cereals and fruits (background intake) *(9)*. The estimated total Se intake is compared with the percentage of the safe upper limit (% of UL) established for toddlers (60 µg day^{-1}).

compared with other food *(8, 28, 40)*. For example, sardines have average levels of 0.57 mg Se kg^{-1} ww, whereas apples are reported to contain 0.0045 mg Se kg^{-1} ww *(28)*. However, in a European food consumption study on children, seafood was not the main source of Se exposure. By contrast, the food groups cereals, vegetables, fresh meat, and milk and dairy drinks were the dominant sources of Se intake for most EU countries. This was not due to high levels of Se present in these food groups but to their high consumption *(8)*. However, for some national studies (e.g., in Italy and Spain), fish was a dominant source of Se intake for 1- to 2-year-old children *(8)*. Because toddlers have a Se intake near or above the UL, a reassessment of the consequences for the consumer from feeding organic Se (Sel-Plex) to terrestrial farmed animals was performed. The EFSA reported that supplementation of 0.2 organic Se mg kg^{-1} to animal feed was safe with regard to the elevated levels reported in meat, eggs, and milk *(9)*.

The EFSA *(9)* assessment of Sel-Plex feed supplementation for food-producing animals did not include farmed seafood fed Se-supplemented feeds. The present study used the EFSA risk assessment and added the intake of farmed seafood (Atlantic salmon) reared on selenite- or SeMet-supplemented feed. The Comprehensive European Food Consumption Database *(12)* was used for the assessment of seafood intake by toddlers, and in particular Atlantic salmon. The median value (*n* = 17) of the 95 percentile consumption data from seven EU countries for fish fillet for toddlers is 45 g day^{-1}, and the median value of 95 percentile consumption data for Atlantic salmon alone is 40 g day^{-1} *(12)* (*n* = 17 from seven EU countries). In the EFSA *(9)* assessment of Sel-Plex feed supplementation, an "increase factor" that expresses the relative increase at a given Se feed supplementation level compared with the background Se concentration per food class was used. In the present study, the Atlantic salmon SeMet increase factor was based on model predictions of the increase in fillet levels when 0.2 mg SeMet kg^{-1} ww was supplemented to fish reared on plant-based feeds (scenario 5 versus scenario 4; Table 2), giving a SeMet increase factor of 1.61 for Atlantic salmon fillet. This estimated increase factor for Atlantic salmon fillet is lower than for meat products (2.2) used in the EFSA

opinion *(9)* (Table 3). The relatively higher increase factor can in part be explained by the higher relative growth rate of fish compared with terrestrial farm animals as seen from the lower feed conversion factor (amount of feed needed for growth of 1 kg) for fish compared with, for example, cows (1.1 versus 3.2, respectively).

Table 3 summarizes the predicted Se intake estimated in the earlier EFSA opinion on Sel-Plex supplementation and adds the Se intake from Atlantic salmon fed on plant-based feeds supplemented with 0.2 mg of SeMet or selenite kg^{-1}. Based on the 0.2 mg of SeMet kg^{-1} supplementation (current authorized level) to feed for food-producing terrestrial animals (producing meat, milk, and eggs) alone, the estimated total intake was 66 µg of Se day^{-1}, exceeding the UL for toddlers (60 µg day^{-1}) by 10% *(9)*. The EFSA-predicted Se intake estimates given in Table 3 is based on (i) consumption data from the Comprehensive European Food Consumption Database *(12)* 95th percentile for toddlers, (ii) the Se level in control groups of food-producing animals when fed on unsupplemented Se diets, (iii) the estimated Se deposition in farmed animals fed on Se-supplemented feed from experimental trials, and (iv) a background Se intake of 10 µg day^{-1} from cereals and fruits *(9)*. Adding the intake of Se from the consumption farmed Atlantic salmon fed a plant-based diet supplemented with 0.2 mg of SeMet kg^{-1} to the Se intake for toddlers *(9)* (Table 3), the estimated total Se intake is 68 µg day^{-1}, exceeding the UL by 14%. When adding consumption of farmed Atlantic salmon fed a plant-based feed supplemented with 0.2 mg of selenite kg^{-1} to the consumption of eggs, milk, and meat, from farm animals also fed on selenite-supplemented feed, the estimated total Se intake would be 47 µg day^{-1}, corresponding to 78% of the UL (Table 3).

In conclusion, based on the feed-to-fillet transfer prediction models for farmed Atlantic salmon and earlier EFSA risk assessment of Se supplementation to animal feed used in food-producing terrestrial farm animals, supplementation with 0.2 mg of selenite kg^{-1} feed would likely be safe for the the most sensitive group of consumers (toddlers). However, supplementing 0.2 mg of SeMet kg^{-1} to food-producing animals, including Atlantic salmon, leads to a Se intake for toddlers that exceeds the safe upper Se intake limit by 14%.

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REFERENCES

1. Berntssen, M. H. G., M. Betancor, M. J. Caballero, M. Hillestad, J. Rasinger, K. Hamre, V. Sele, H. Amlund, and R. Ornsrud. 2018. Safe limits of selenomethionine and selenite supplementation to plant-based Atlantic salmon feeds. *Aquaculture* 495:617–630.
2. Berntssen, M. H. G., M. Sanden, H. Helge, and Ø. Lie. 2016. Modelling scenarios on feed-to-fillet transfer of dioxins and dioxin-like PCBs in future feeds to farmed Atlantic salmon (*Salmo salar*). *Chemosphere* 163:413–241.
3. Berntssen, M. H. G., T. K. Sundal, P. A. Olsvik, H. Amlund, J. D. Rasinger, V. Sele, K. Hamre, M. Hillestad, L. Buttle, and R. Ornsrud. 2017. Sensitivity and toxic mode of action of dietary organic and inorganic selenium in Atlantic salmon (*Salmo salar*). *Aquat. Toxicol.* 192:116–126.
4. Betancor, M. B., T. M. C. Dam, J. Walton, T. Morken, P. J. Campbell, and D. R. Tocher. 2016. Modulation of selenium tissue distribution and selenoprotein expression in Atlantic salmon (*Salmo salar* L.) fed diets with graded levels of plant ingredients. *Br. J. Nutr.* 115:1325–1338.
5. Brambilla, G., E. Dellatte, I. Fochi, N. Iacovella, R. Miniero, and A. di Domenico. 2007. Depletion of selected polychlorinated biphenyl, dibenzodioxin, and dibenzofuran congeners in farmed rainbow trout (*Oncorhynchus mykiss*): a hint for safer fish farming. *Chemosphere* 66:1019–1030.
6. Bruggeman, W. A., L. Martron, D. Kooiman, and O. Hutzinger. 1981. Accumulation and elimination kinetics of dichlorobiphenyls, trichlorobiphenyls and tetrachlorobiphenyls by goldfish after dietary and aqueous exposure. *Chemosphere* 10:811–832.
7. Euoropean Commission. 2000. Opinion of the Scientific Committee on Food on the tolerable upper intake level of selenium. Available at: http://ec.europa.eu/food/fs/sc/scf/out80g_en.pdf.
8. European Food Safety Authority. 2010. Long-term dietary exposure to selenium in young children living in different European countries (EXPOCHI). Available at: http://www.efsa.europa.eu/en/scdocs/scdoc/56e.htm.
9. European Food Safety Authority. 2011. Scientific opinion on safety and efficacy of Sel-Plex® (organic form of selenium produced by *Saccharomyces cerevisiae* CNCM I-3060) for all species. *EFSA J.* 9:2110.
10. European Food Safety Authority. 2013. Scientific opinion on the safety and efficacy of L-selenomethionine as feed additive for all animal species. *EFSA J.* 11:3219.
11. European Food Safety Authority. 2014. Scientific opinion on dietary reference values for selenium1. *EFSA J.* 12:3846.
12. European Food Safety Authority. 2018. The EFSA Comprehensive European Food Consumption Database. Available at: http://www.efsa.europa.eu/en/food-consumption/comprehensive-database.
13. Fontagne-Dicharry, S., S. Godin, H. K. Liu, P. A. J. Prabhu, B. Bouyssiere, M. Bueno, P. Tacon, F. Medale, and S. J. Kaushik. 2015. Influence of the forms and levels of dietary selenium on antioxidant status and oxidative stress-related parameters in rainbow trout (*Oncorhynchus mykiss*) fry. *Br. J. Nutr.* 113:1876–1887.
14. Godin, S., S. Fontagne-Dicharry, M. Bueno, P. Tacon, P. A. J. Prabhu, S. Kaushi, F. Medale, and B. Bouyssieret. 2015. Influence of dietary selenium species on selenoamino acid levels in rainbow trout. *J. Agric. Food Chem.* 63:6484–6492.
15. Goldhaber, S. B. 2003. Trace element risk assessment: essentiality vs. toxicity. *Regul. Toxicol. Pharmacol.* 38:232–242.
16. Hardy, R. W., L. L. Oram, and G. Moller. 2010. Effects of dietary selenomethionine on cutthroat trout (*Oncorhynchus clarki bouvieri*) growth and reproductive performance over a life cycle. *Arch. Environ. Contam. Toxicol.* 58:237–245.
17. Hilton, J. W., and P. V. Hodson. 1983. Effect of increased dietary carbohydrate on selenium metabolism and toxicity in rainbow-trout (*Salmo-gairdneri*). *J. Nutr.* 113:1241–1248.
18. Hilton, J. W., P. V. Hodson, and S. J. Slinger. 1982. Absorption, distribution, half-life and possible routes of elimination of dietary selenium in juvenile rainbow trout (*Salmo gairdneri*). *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.* 71:49–55.
19. Institue or Marine Research. 2017. Seafood data. Avialable at: https://sjomatdata.nifes.no/#search/.
20. Jablonska, E., and M. Vinceti. 2015. Selenium and human health: witnessing a copernican revolution? *J. Environ. Sci. Health Part C Environ. Carcinog. Ecotoxicol. Rev.* 33:328–368.
21. Julshamn, K., A. Maage, H. S. Norli, K. H. Grobecker, L. Jorhem, and P. Fecher. 2007. Determination of arsenic, cadmium, mercury, and lead by inductively coupled plasma/mass spectrometry in foods after pressure digestion: NMKL1 interlaboratory study. *J. AOAC Int.* 90:844–856.

22. Kleinow, K. M., and A. S. Brooks. 1986. Selenium-compounds in the fathead minnow (*Pimephales promelas*). 1. Uptake, distribution, and elimination of orally-administered selenate, selenite and l-selenomethionine. *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol*. 83:61–69.

23. Kleinow, K. M., and A. S. Brooks. 1986. Selenium-compounds in the fathead minnow (*Pimephales promelas*). 2. Quantitative approach to gastrointestinal absorption, routes of elimination and influence of dietary pretreatment. *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol*. 83:71–76.

24. Kryukov, G. V., and V. N. Gladyshev. 2000. Selenium metabolism in zebrafish: multiplicity of selenoprotein genes and expression of a protein containing 17 selenocysteine residues. *Genes Cells* 5:1049–1060.

25. Liu, K. L., F. S. Chen, Y. Zhao, Z. X. Gu, and H. S. Yang. 2011. Selenium accumulation in protein fractions during germination of Se-enriched brown rice and molecular weights distribution of Se-containing proteins. *Food Chem*. 127:1526–1531.

26. Lorentzen, M., A. Maage, and K. Julshamn. 1994. Effects of dietary selenite or selenomethionine on tissue selenium levels of Atlantic salmon (*Salmo salar*). *Aquaculture* 121:359–367.

27. National Research Council. 2011. Nutrient requirements of fish and shrimp. The National Academies Press, Washington, DC.

28. Navarro-Alarcon, M., and C. Cabrera-Vique. 2008. Selenium in food and the human body: a review. *Sci. Total Environ*. 400:115–141.

29. Nichols, J. W., P. N. Fitzsimmons, and F. W. Whiteman. 2004. A physiologically based toxicokinetic model for dietary uptake of hydrophobic organic compounds by fish. II. Simulation of chronic exposure scenarios. *Toxicol. Sci*. 77:219–229.

30. Pacitti, D., M. M. Lawan, J. Feldmann, J. Sweetman, T. Wang, S. A. M. Martin, and C. J. Secombes. 2016. Impact of selenium supplementation on fish antiviral responses: a whole transcriptomic analysis in rainbow trout (*Oncorhynchus mykiss*) fed supranutritional levels of Sel-Plex®. *BMC Genomics* 17:116.

31. Rayman, M. P. 2012. Selenium and human health. *Lancet* 379:1256–1268.

32. Rider, S. A., S. J. Davies, A. N. Jha, A. A. Fisher, J. Knight, and J. W. Sweetman. 2009. Supra-nutritional dietary intake of selenite and selenium yeast in normal and stressed rainbow trout (*Oncorhynchus mykiss*): implications on selenium status and health responses. *Aquaculture* 295:282–291.

33. Ruohonen, K. 2015. Personal communication.

34. Sanden, M., G. I. Hemre, A. Maage, B. T. Lunestad, M. Espe, K. K. Lie, A. K. Lundebye, H. Amlund, R. Waagbø, and R. Ørnsrud. 2017. Programme for surveillance of fish feedsm yeqalry report for samples collected in 2016. NIFES report in Norwegian. Available at: https://nifes.hi.no/report/overvakning-fiskefor-2017/.

35. Schweizer, U., and C. Steegborn. 2015. New insights into the structure and mechanism of iodothyronine deiodinases. *J. Mol. Endocrinol*. 55:R37–R52.

36. Sele, V., R. Ornsrud, J. J. Sloth, M. H. G. Berntssen, and H. Amlund. 2018. Selenium and selenium species in feeds and muscle tissue of Atlantic salmon. *J. Trace Elem. Med. Biol*. 47:124–133.

37. Sijm, D., W. Seinen, and A. Opperhuizen. 1992. Life-cycle biomagnification study in fish. *Environ. Sci. Technol*. 26:2162–2174.

38. Sissener, N. H., K. Julshamn, M. Espe, B. T. Lunestad, G. I. Hemre, R. Waagbo, and A. Mage. 2013. Surveillance of selected nutrients, additives and undesirables in commercial Norwegian fish feeds in the years 2000–2010. *Aquac. Nutr*. 19:555–572.

39. Suzuki, K. T. 2005. Metabolomics of selenium: Se metabolites based on speciation studies. *J. Health Sci*. 51:107–114.

40. Toppo, S., S. Vanin, V. Bosello, and S. C. E. Tosatto. 2008. Evolutionary and structural insights into the multifaceted glutathione peroxidase (Gpx) superfamily. *Antioxid. Redox Signal*. 10:1501–1513.

41. Ullah, H., G. Liu, B. Yousaf, M. U. Ali, Q. Abbas, M. A. M. Munir, and M. M. Mian. 2018. Developmental selenium exposure and health risk in daily foodstuffs: a systematic review and meta-analysis. *Ecotoxicol. Environ. Saf*. 149:291–306.

42. Valdiglesias, V., E. Pasaro, J. Mendez, and B. Laffon. 2010. In vitro evaluation of selenium genotoxic, cytotoxic, and protective effects: a review. *Arch. Toxicol*. 84:337–351.

43. Ytrestoyl, T., T. S. Aas, and T. Asgard. 2015. Utilisation of feed resources in production of Atlantic salmon (*Salmo salar*) in Norway. *Aquaculture* 448:365–374.

44. Zar, J. H. 1984. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, NJ.