Aquaculture 495 (2018) 617–630

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Safe limits of selenomethionine and selenite supplementation to plant-based Atlantic salmon feeds

Berntssen M.H.G.[a,*], Betancor M.[b], Caballero M.J.[c], Hillestad M.[d], Rasinger J.[a], Hamre K.[a], Sele V.[a], Amlund H.[a], Ørnsrud R.[a]

[a] Institute of Marine Research (IMR), P.O 1870, Nordnes, N-5817, Bergen, Norway
[b] Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling FK94LA, United Kingdom
[c] Department of Morphology, Veterinary School, University of Las Palmas de Gran Canaria, Trasmontana s/n, 35416 Arucas, Las Palmas, Spain
[d] BioMar AS, Trondheim, Norway

ARTICLE INFO

Keywords:
Atlantic salmon
Selenite
Selenomethionine
Toxicity
Bench mark dose

ABSTRACT

The use of plant-based feeds warrants the supplementation with selenium (Se) to cover the requirement for Atlantic salmon. Depending on its chemical form, Se is a trace element with a narrow range between requirement and toxicity for most vertebrates. Information on safe upper limit for Atlantic salmon feed supplementation is lacking. Atlantic salmon (147 g) were fed a low natural background organic Se diet (0.45 mg Se kg^{-1}, wet weight (ww)) fortified with 5 graded levels of inorganic sodium selenite (0.45, 5.4, 11.0, 29.4, or 60.0 mg kg^{-1} ww) or organic selenomethionine (SeMet) (0.45, 6.2, 16.2, 21, or 39 mg kg^{-1} ww), in triplicate for 3 months. Excess Se supplementation was assessed by targeted biomarkers of Se toxicity pathways (e.g. markers of oxidative stress and lipid metabolism), as well as general adverse effect parameters (plasma biochemistry, hematology, liver histopathology, and growth). Safe limits were set by model-fitting the effect data in a dose-response (lower bound) bench mark dose (BMDL) evaluation. Fish fed the two highest selenite levels showed mortality while fish fed SeMet had no mortality. Fish fed 5.4–11 mg selenite kg^{-1} feed showed significantly (ANOVA, Tukey's *t*-test, $p < .05$) increased liver oxidative stress, as seen from altered hepatic GSH and vitamin E levels, and liver damage as seen from increased plasma ALAT and liver histopathology such as degeneration and focal necrosis. Fish fed SeMet mainly showed liver pathology and kidney dysfunction as seen from altered plasma creatinine and total plasma proteins in fish fed ≥ 21 mg kg^{-1}, compared to control. For selenite exposed fish, a safe feed limit (BMDL) was set at 1–2 mg kg^{-1} ww feed (daily dose 0.01–0.02 mg kg BW^{-1} day^{-1}), based on plasma ALAT increase, liver vitamin E depletion, and liver histopathology. For SeMet fed fish, the safe feed limit was higher than for selenite with a BMDL of 2.8 mg kg^{-1} ww (dose 0.03 mg kg BW^{-1} day^{-1}), based on liver histopathology and plasma creatinine. In conclusion, with regards to fish health, Atlantic salmon seemed to tolerate the supplementation of selenite or SeMet to a level of total selenium of respectively 1–2 or 3 mg kg^{-1} feed, respectively, in a high plant-based salmon feed with background levels of 0.45 mg Se kg^{-1}.

1. Introduction

Due to a rapid growth in aquaculture and limited access to marine resources, fish oil and fish meal in feeds to carnivorous marine fish species such as Atlantic salmon (*Salmo salar*) have been replaced with plant ingredients the last decades (Ytrestoyl et al. 2015). The change from marine to plant feed ingredients will alter the nutritional composition of salmon feeds, reducing the levels of essential micro-nutrients that are naturally high in fish meal and oil such as vitamins and minerals (Sissener et al. 2013). Selenium (Se) is one of the essential minerals that is known to be higher in fish meal than plant ingredients (Betancor et al. 2016), although plant products can differ largely in Se content according to the Se concentrations in soil (Alfthan et al. 2015). The observed Se decline in Norwegian produced commercial salmon feed during the last decade has been attributed to the decreased use of fish meal (Sissener et al. 2013). Furthermore, the use of plant ingredients may reduce the bioavailability of minerals due to presence of phytates (Denstadli et al. 2006). Selenium concentration in Atlantic salmon flesh was lower when fed on plant protein replacement feeds compared to marine protein feeds (Betancor et al. 2016). Several studies have indicated the need for Se supplementation in plant-based feed to marine carnivorous fish (Fontagne-Dicharry et al. 2015; Godin et al.

* Corresponding author.
E-mail address: marc.berntssen@nifes.no (M.H.G. Berntssen).

https://doi.org/10.1016/j.aquaculture.2018.06.041
Received 4 May 2018; Received in revised form 15 June 2018; Accepted 16 June 2018
Available online 19 June 2018

2015; Pacitti et al. 2015; Ilham et al., 2016). Of the mineral supplements used, the organic forms, seleno-methionine (SeMet) or Se-yeast forms have a higher bioavailability than inorganic selenite forms (Rider et al. 2009; Fontagne-Dicharry et al. 2015). Studies in Atlantic salmon indicate that the natural Se levels in plant based diets cover requirement (Hamre et al. 2016). Higher Se requirements, and hence need for possible supplementation, might occur during the early life stages of fish (Bell et al. 1985) or during handling stress (Rider et al. 2009). In the EU, feeds can be supplemented with organic (e.g. selenized yeasts) Se to a maximum authorised level of 0·2 mg kg^{-1} (Regulations (EU) No 427/2013; 445/2013; 121/2014; 847/2014 and 2015/489). The maximum limit for total Se in animal feeds, including fish feed, has been set at 0.5 mg kg^{-1} feed ((EC) No 1831/2003 and amendments).

Selenium has a narrow range between its toxic and its beneficial effects (Wang and Lovell 1997; Teh et al. 2004; Han et al. 2011; Lee et al. 2016). Supplementation of aquafeeds with SeMet or selenite hence requires toxicological assessment to set safe upper limits that protect fish health (Berntssen et al. 2017). Several studies have given an overview on adverse effect levels in several fish species exposed to both excess dietary inorganic and organic Se (Lemly 1993a; Hamilton 2004; Zee et al. 2016a). A wide range of effect concentrations have been reported that differ between fish species and life stages, hence species-specific adverse effects of Se supplementation is important to consider. Several studies have performed graded dose-response with organic or inorganic Se for salmonids, including chinook salmon (*Oncorhynchus tshawytscha*) (Hamilton et al., 1990), cutthroat trout (*Oncorhynchus clarkii*) (Hardy et al. 2010), and rainbow trout (*Oncorhynchus mykiss*) (Hilton et al. 1980; Hamilton et al. 1990; Hamilton 2004; Palace et al. 2004; Rider et al. 2009; Hunt et al. 2011; Wiseman et al. 2011a; Wiseman et al. 2011b; Knight et al. 2016; Pacitti et al. 2016b). However, few studies have assessed selenite and SeMet supplementation in Atlantic salmon (Lorentzen et al. 1994; Berntssen et al. 2017), which is one of the main farmed salmonid species.

Oxidative stress has been identified as a main toxic action (MOA) for excess dietary Se exposures (Palace et al. 2004; Miller et al. 2007; Han et al. 2010; Choi et al. 2015; Hursky and Pietrock 2015; Lee et al. 2015; Hauser-Davis et al. 2016). Also for dietary Se exposed Atlantic salmon, oxidative stress was a main driver for both high selenite and SeMet-yeast toxicity, with a higher toxicity for selenite compared to SeMet-yeast (Berntssen et al. 2017). In contrast, for white sturgeon (*Acipenser transmontanus*) (Zee et al. 2016a; Zee et al. 2016b) oxidative stress was not the main cause of dietary SeMet toxicity, and juvenile rainbow trout fed organic Se showed no oxidative stress while growth and liver lipids were reduced (Knight et al. 2016). Recent wide-scope pathway assessments, by use of metabolomics, have shown that disturbance in lipid metabolism could be an additional MOA for inorganic and organic Se toxicity (Berntssen et al. 2017). Earlier wide-scope pathway assessments by transcriptomics confirmed that disturbed liver lipid synthesis and metabolism was a central mechanism in dietary organic Se exposed rainbow trout (Knight et al. 2016; Pacitti et al. 2016a).

Assessment of biochemical markers in the central pathways of dietary selenite and SeMet toxicity can be used to assess early effects of excess selenite and SeMet exposures. Atlantic salmon fed sublethal selenite and SeMet levels (15 mg kg^{-1}), showed reduced vitamin E, formation of peroxidative products, and reduction in glutathione as markers of oxidative stress, while altered lipid composition were used as markers of disturbed lipid metabolism (Berntssen et al. 2017). The use of biomarkers in central pathways of toxicity, are valuable in sub-chronic studies (10% of life cycle) where chronic whole-body adverse effects are expected to occur only after prolonged (life-cycle) exposure. Final adverse effect outcomes of dietary selenite and SeMet in fish, include decreased egg viability (Schultz and Hermanutz 1990), reduced neurological and immunological functions (Choi et al. 2015), reduced growth (De Riu et al. 2014; Zee et al. 2016a; Berntssen et al. 2017), reducedenergy stores (De Riu et al. 2014; Zee et al. 2016a), pathological effects on kidney and liver (Hicks et al. 1984; Teh et al. 2004; Tashjian et al. 2006; Zee et al. 2016a), pathological effects on heart and ovaries, as well as skeleton/cranial deformation (Lemly, 2002; Hamilton, 2003; Hamilton, 2004).

Traditionally, animal health safe dietary levels of feed supplements in toxicological studies are assessed by establishing a no observed adverse effect level (NOAEL) based on a (sub)-chronic dose-response study with graded levels of the supplement (Teh et al. 2004). The European food safety agency (EFSA), recently evaluated the methods to assess safe feed levels, and advised to use bench mark dose (BMD) models instead of NOAEL to establish safe levels of supplements or contaminants (EFSA 2017b). In addition, a guidance document was published in which the difference between adverse effect, biomarkers of exposure or effect, and mode of action (MOA) were defined (EFSA 2017a). In general, dose-response adverse effects (i.e. reduced growth, histopathology) are weighed in the BMD with a benchmark response (BMR) of 5%, while for biomarker of effect or exposure (i.e. plasma enzymes and organ oxidative stress) a higher (20%) BMR is used. For histopathology data (i.e. degeneration and focal necrosis) a BMR of 10% is used with an extra risk factor assessment (EFSA 2017b). The present study assessed the safe limits of selenite and SeMet supplementation to plant based feed with regards to the health of Atlantic salmon. The present paper uses an integrated feed safety assessment on several levels of biological organization. These include the use of specific targeted biomarkers of Se toxicity mode of action (e.g. markers of oxidative stress and lipid metabolism) as well as general adverse effect parameters (plasma biochemistry, hematology, histopathology, and growth) of Se toxicity. The safe levels are assessed in a common EFSA dose-response bench mark dose regression model applied to all parameters.

2. Material and methods

2.1. Ethic statement

The experiment was approved by the Norwegian National Animal Research Authority (Mattilsynet; FOTS ID: 9003) and performed in compliance with national and international ethical standards.

2.2. Experimental conditions

The feeding trial was carried out at NOFIMA (Sunndalsøra, Norway) between the 15th of November 2016 and the 3th of March 2017. A total of 1890 Atlantic salmon smolt (*Salmo salar*, L., Salmobreed, 6 months, both genders) were randomly distributed into 27 tanks (1.4 m^2 and ca 840 L volume) with 70 fish in each tank with an initial weight of 147 ± 4 g (mean ± SD, $n = 30$). Prior to the experiment, all fish were fed a control diet (see diet description under) during a 2-week acclimation to holding facilities. Thereafter, randomly selected tanks received one of nine experimental diets for 3 months, in triplicate. The feeding regime was based on automatic feeders under a photoperiod regime with 24 h light. Six daily meals were provided with 4 h between the meals, to a level approximating 1% of body weight per day. The feeding rate was adjusted for growth biomass increase, which was assessed by measured average weight gain of the sampled fish per sampling point. Fish were routinely monitored for nutritional performance and appetite throughout the experiment. Unconsumed feed pellets were collected and weighed once per day, and feed intake, feed conversion and Se exposure were calculated. To avoid possible leakage from feces or pellets to the water, a relative high water flow-through was maintained of 11 L min^{-1} per tank. Water Se levels were monitored by routine water samples of 50 ml, which were taken from each tank and acidified with nitric acid 65% HNO3 (Suprapur, Merck, Germany) in a final concentration of 5.2% for Se analysis. Environmental parameters in tanks were measured five times a week, showing a salinity of 27 ± 0.3‰, temperature of 8.0 ± 0.3 °C, and oxygen levels of 85 ± 4% at the outlet.