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1. SAMMENDRAG

Selen (Se) er et essensielt element som er viktig for dyrehelsen, også for fisk. I de senere år har fiskemel blitt erstattet med plantebaserte fôrråvarer som kan gi redusert seleninnhold i laksefôr. Den naturlige kilden til Se i laksefôr er marine råvarer, og det er relativt høye nivåer av fiskemel i laksefôr. Studier har vist at Se konsentrasjonen i oppdrettslaks er lavere når fisken får plantebaserte proteinråvarer enn når fisken får fôr med marine råvarer. Andre studier har foreslått at Se bør tilsettes til fiskefôr med høyt innslag av plantebaserte råvarer for å ivareta en robust fiskehelse og for å opprettholde Se konsentrasjonen i oppdrettsfisk.

EU har etablert øvre grenseverdier for viktige mineraler i dyrefôr for å sikre dyrevelferd, konsumentens helse og for å redusere miljøpåvirkningen. EU-forskriften og den aktuelle nasjonale forskrift om total Se i fiskefôr er på 0,5 mg/kg fôr og 0,2 mg/kg for organisk Se når det er tilsatt.

I tillegg til å være et essensielt element er Se toksisk i relativt lave konsentrasjoner, både for dyr og mennesker. Behovet for Se og den øvre grense for inntak av Se i laks er ikke fastsatt for laks, siden vitenskapelig informasjon mangler for å kunne gjennomføre en risikovurdering. Dette prosjektet skal levere kunnskap om øvre grense for inntak av Se fra laksefôr med hensyn på fiskehelse og konsumentens helse.

Delmål

- 1) Å etablere metoder for å måle ulike former av Se i laksefôr og -filét.
- 2) Å etablere terskelverdier for uønskede effekter av Se hos laks
- 3) Å etablere overføringsmodeller for Se fra fôr til den spiselige delen av laks.

Analysemetoder for organiske og uorganiske former av Se i fôr og muskel ble etablert i dette prosjektet. To ulike ekstraksjons- og målemetoder ble utviklet for å kunne måle organiske Se former som selenomethionine, og uorganiske former som selenitt og selenat. Slike analysemetoder er viktig for å kunne måle om Se konsentrasjonen og ulike former av Se i fôret er i overensstemmelse med regelverket.

Et fiskeforsøk for å etablere sensitivitet og toksisk virkemåte av Se ble gjennomført. Ved hjelp av avanserte screeningmetoder ble det lett etter tidlige effekter av Se toksisitet og resultatene viste at både organisk og uorganisk Se påvirket antioksidantsystemet og at oksidativt stress og endringer i lipidmetabolismen var den toksiske virkemåten til Se, med høyest toksisitet for uorganisk Se.

Et annet fiskeforsøk ble gjennomført for å etablere hvor mye uorganisk eller organisk Se laks kan tåle i fôret. Resultatene viste at laksen har en lavere sensitivitet for uorganisk Se og tåler mindre av denne enn av organisk Se. En trygg dose for uorganisk Se supplementering til plante basert fôr ble estimert til 1-2 mg total Se/kg og for organisk Se ble en trygg dose estimert til 3 mg Se total /kg laksefôr.

For å kunne forutsi hvor mye uorganisk og organisk Se man kan finne i filet basert på hvor mye som finnes i fôret ble det utviklet en matematisk fôr-til-fillet overføringsmodell. Denne modellen viste at organisk Se hadde en høyere opptaksrate og en lavere elimineringsrate enn uorganisk Se. Prediksjoner fra denne modellen og en tidligere EFSA risikovurdering ble så brukt til å vurdere hvilken effekt Se supplementering av laksefôr ville fått for konsumenten. Supplementering med 0,2 mg uorganisk Se/kg fôr er trygt for de mest sensitive konsumentene (småbarn). Supplementering med 0,2 mg organisk Se/kg fôr ville gitt et Se inntak et Se inntak som er høyere (14%-19%) enn den øvre grense for inntak hos småbarn.

SUMMARY

Selenium (Se) is an essential element for animals, including fish. The change from marine to plant feed ingredients will alter the nutritional composition of salmon feeds, reducing essential micro-nutrients such as Se that are naturally high in fish meal. Earlier studies have shown that Se concentrations in farmed Atlantic salmon muscle were lower when fed on plant protein replacement feeds compared to marine protein feeds. Several earlier studies on fish nutrition have suggested that Se should be supplemented to plant based feeds to restore or maintain Se levels in farmed fish as a Se source for consumers. In addition, Se supplementation to plant based feed for marine carnivorous fish has been suggested to be needed to cover animal nutrient requirement and ensure health robustness of the farmed fish.

In the European Union (EU), the maximum limit for total Se in animal feeds including fish feed has been set at 0.5 mg/kg feed. In addition, feeds can be supplemented with approved organic Se to a maximum authorised level of 0.2 mg/kg.

In addition to being an essential element, excess Se is known to be toxic at low concentrations and a narrow window exists between requirement and Se toxicity for most vertebrates, including farmed fish and humans consuming Se supplemented food producing animals.

This project aims to provide knowledge on the upper limits for Se in Atlantic salmon feed with regards to fish health and consumer safety.

Sub aims

- 1) Selenium speciation in salmon feeds and Atlantic salmon fillet
- 2) Establish threshold adverse effect levels for Se in Atlantic salmon feeds
- 3) Establish Se feed-to-fillet carry-over models and generate knowledge for risk assessment of Se for the consumer

Speciation analysis for Se in salmon feed and muscle were developed. Two different extraction methods were developed to measure organic Se species such as selenomethionine, and inorganic species such as selenite and selenite. Overall, the results showed that the methods can be used for assessing the Se species in the feeds, and hence be used to check compliance with feed legislation. Furthermore, the methods can be used to determine the Se species retained in tissues of salmon.

A fish feeding trial to establish the sensitivity and toxic mode of action of Se was performed. A wide scope metabolomics pathway profiling was performed, in addition to classical markers

of toxicity. The results showed that the toxic mode of action was oxidative stress and altered lipid metabolism for both inorganic and organic Se, with higher toxicity for inorganic Se.

A second fish feeding trial was performed to establish safe levels of inorganic and organic Se with regards to fish health. Fish fed high inorganic Se levels showed mortality, whereas fish fed organic Se showed no mortality. A safe Se concentration of Se in feed to Atlantic salmon was suggested as 1-2 mg inorganic total Se/kg or 3 mg organic total Se/kg feed.

To assess the transfer of inorganic and organic Se to the fillet of farmed Atlantic salmon, the uptake and elimination rate constants of the two Se forms were determined from the second feeding trial. The elimination and uptake rates were used to develop a mathematical model to predict levels of Se in fillet. Based on Feed-to fillet model predictions and earlier EFSA risk assessment of Se supplementation to food producing land animals;

Supplementing animal feed with 0.2 mg kg⁻¹ selenite would likely protect food safety for the most sensitive consumer group (toddlers). Supplementing feed to farmed animals, including salmon, with 0.2 mg kg⁻¹ SeMet would give a Se intake that is higher (14%-19%) than the upper limit set for toddlers

Scientific report

Details on the findings in the project have been submitted in a separate report and will not be repeated here.

Project organization

The project was organized as a project group consisting of researchers from NIFES and a project reference group consisting of sponsor representatives from the FHF, the Norwegian Seafood Federation, the salmon feed companies (EWOS Cargill, Biomar, Skretting, and Marine Harvest). Meetings were held with the project reference group at NIFES for the start-up meeting, and as telephone conferences every six months and as a final meeting discussing the main findings. The sponsors of the present project approved the study designs. The sponsors had no involvement in the collection, analysis or interpretation of data, or in the writing of the manuscript.

Main findings

- Methods for speciation analysis of Se were established
- The toxic mode of action and doses for the tolerance of inorganic and organic Se in Atlantic salmon was suggested
- The feed-to-fillet transfer of inorganic and organic Se in salmon fillet was described
- Based on Feed-to fillet model predictions and earlier EFSA risk assessment of Se supplementation to food producing land animals;
- Supplementing animal feed with 0.2 mg/kg inorganic Se would likely protect food safety for the most sensitive consumer group (toddlers).
- Supplementing feed to farmed animals, including salmon, with 0.2 mg/kg organic Se would give a Se intake that is higher (14%-19%) than the upper limit (UL) set for toddlers.

Deliverables

- Minutes from meetings in the project reference group.
- Administrative final report
- Scientific summary report (this report)
- Final scientific report consisting of several manuscripts
- Published manuscripts
- Workshop on selenium in fish feed

Publications:

Thesis for the degree Master of Science in Aquamedicine: Establishing biomarkers of dietary toxicity in Atlantic salmon (*Salmo Salar* L.), Tone Kristin Sundal, University of Bergen, June 2016

M.H.G. Berntssen, T.K. Sundal, P.A. Olsvik, H. Amlund, J.D. Rasinger, V. Sele, K. Hamre, M. Hillestad, L. Buttle, R. Ørnsrud (2017) Sensitivity and toxic mode of action of dietary organic and inorganic selenium in Atlantic salmon (*Salmo salar*) *Aquatic Toxicology* 192, 116-126

Sele, V., R. Ørnsrud, Sloth J.J., Berntssen M.H.G. & Amlund H. (2018). Selenium and selenium species in feeds and muscle tissue of Atlantic salmon. *Journal of Trace Elements in Medicine and Biology*, 47, 124-133.

Manuscripts delivered as part of the final report:

Berntssen, M.H.G., Betancor M., Hillestad M., Rasinger, J., Hamre, K., Sele, V., Amlund, H., Ørnsrud, R. Safe limits of selenomethionine and selenite supplementation to plant-based Atlantic salmon feeds (In preparation)

Berntssen, M.H.G., Lundebye, A.-K., Amlund, H., Sele, V., Ørnsrud, R. Modelling the feed-to-fillet transfer of selenite and selenomethionine supplements in plant based feeds to farmed Atlantic salmon (*Salmo salar*). (In preparation)

Presentations:

Veronika Sele, Jens J. Sloth, Robin Ørnsrud & Heidi Amlund (2016) The need for selenium speciation analysis of fish feed and fish tissue. *The 8th Nordic Conference on Plasma Spectrochemistry* June 5.-8. Loen, Norway (Poster)

Ørnsrud R., Amlund H., Olsvik P.A., Rasinger J.D., Sundal T.K., Hamre K., Hillestad M., Buttle L., Lundebye A.-K. & Berntssen M.H.G. (2016) Evaluation of the maximum limit for selenium in feed for salmonids. *International Symposium on Fish Nutrition and Feeding (ISFNF)*, June 5.-10., Sun Valley, Idaho, USA. (Oral and Poster)

Amlund H., Ørnsrud R., Olsvik P., Sele V., Rasinger J., Sundal T., Sloth J., Hamre, K., Hillestad M., Buttle L., Lundebye A.-K., & Berntssen M. (2016) Biomarkers of Se toxicity. *Programkonferansen HAVBRUK 2016*. Bodø, Norway, April 18-20

Veronika Sele, Magali Perez, Andrea Raab, Joerg Feldmann, Eva Krupp, Jens J. Sloth, Heidi Amlund, Robin Ørnsrud, Marc H.G. Berntssen and Josef D. Rasinger. (2017) Characterization of Atlantic salmon (*Salmo salar*) selenoproteins using bioinformatics and hyphenated analytical techniques, The 11th International Symposium on Selenium in Biology and Medicine, Stockholm 13. - 17. August

Veronika Sele, Robin Ørnsrud, Josef D. Rasinger, Jens J. Sloth, Marc H. G. Berntssen and Heidi Amlund (2017) Selenium supplementation of feed for Atlantic salmon. The 11th International Symposium on Selenium in Biology and Medicine, Stockholm 13. - 17. August

The workshop “Selenium in fish feed” was held at the Institute of Marine Research Friday 25th of May with representatives from the Food Safety Authorities, Biomar, Skretting, Marine Harvest, Ewos Cargill and the Institute of Marine Research

Bergen 30.05.2018

Robin Ørnsrud, prosjektleder