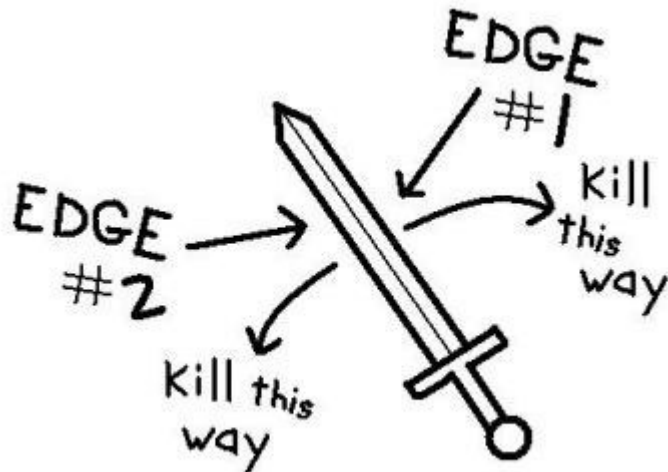


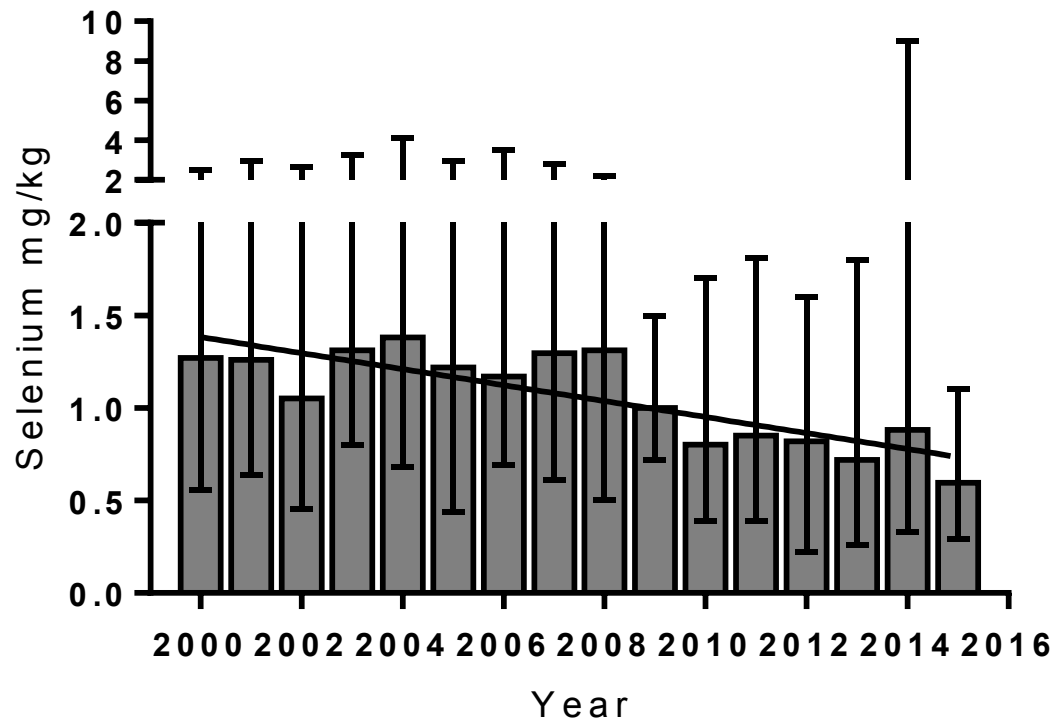
Evaluation of the maximum limit for selenium in feed to salmon

Ørnsrud R., Amlund H., Olsvik P. A., Rasinger J. D., Sundal T. K., Hamre, K., Hillestad M., Buttle L., Lundebye A.-K., & Berntssen M.



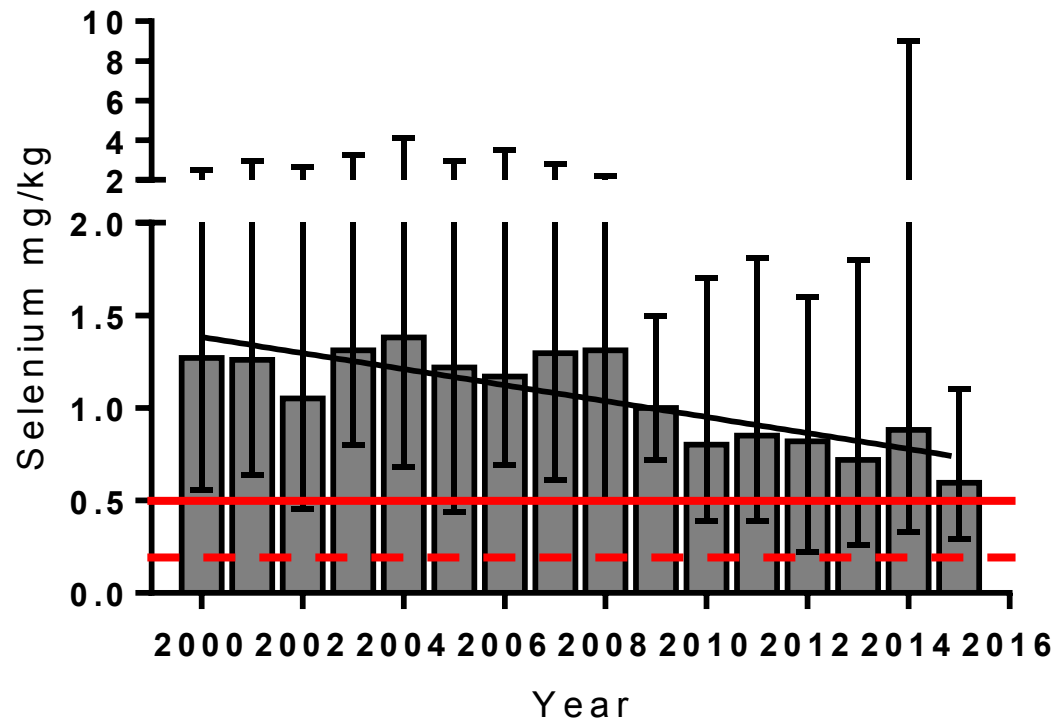
Evaluation of the maximum limits for selenium in Atlantic salmon feeds

- Farmed Atlantic salmon have traditionally been fed diets based on marine ingredients with sufficient Se levels
- As ingredients of plant origin replace marine ingredients, Se concentrations in salmon feeds decrease

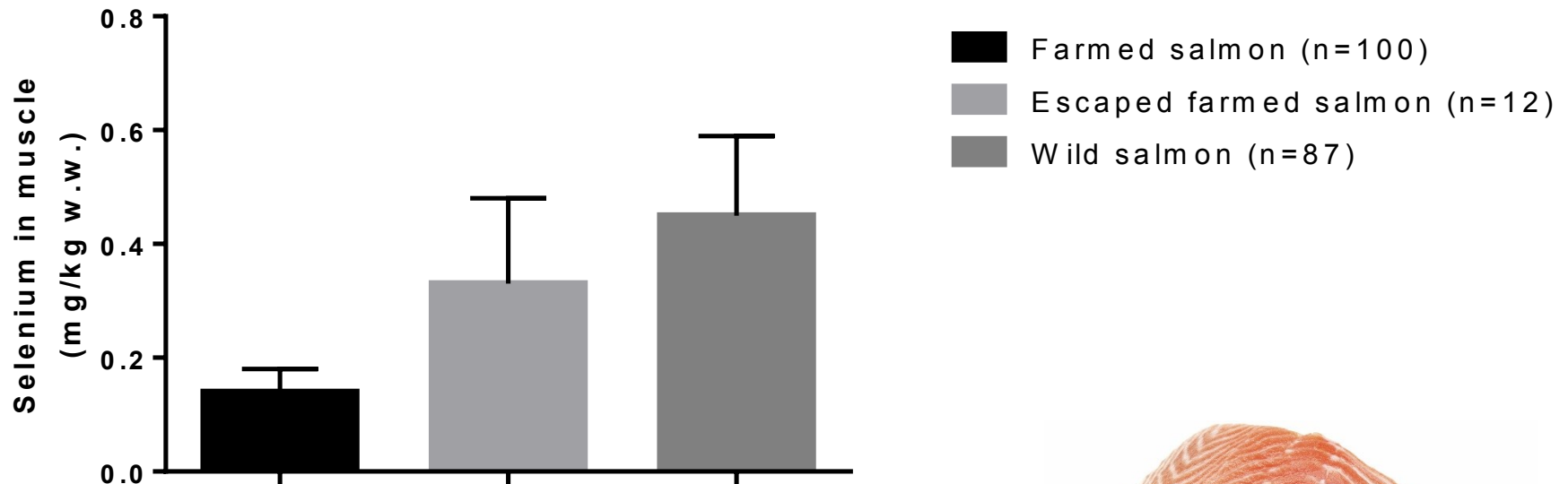


Evaluation of the maximum limits for selenium in Atlantic salmon feeds

- EU feed regulations on total selenium (Se) in fish feed
 - 0.5 mg total Se/kg feed
 - 0.2 mg organic Se/kg feed



Selenium in salmon muscle



Aim of study

- In order to produce a robust salmon, future aquafeeds may need to be supplemented with Se to compensate for reduced levels
- In order to establish a scientifically-based maximum limit for Se in feed for salmonids, a risk assessment on the dietary toxicity of both organic and inorganic forms of Se in Atlantic salmon is required
- Prior to a risk assessment, appropriate toxic mode of action and markers of toxic exposures have to be established.
- The aim of this study was to establish biomarkers of dietary Se toxicity in Atlantic salmon

	Negative control	Low inorganic Se	High inorganic Se	Low organic Se	High organic Se	Positive control
Fish meal ¹	8.0	8.0	8.0	8.0	8.0	15.0
SA Krill	2.0	2.0	2.0	2.0	2.0	7.0
Soy protein concentrate	18.0	18.0	18.0	18.0	18.5	10.0
Wheat Gluten	18.0	18.0	18.0	18.0	19.0	18.0
Maize Gluten	4.0	4.0	4.0	4.0	5.0	4.0
Pea Protein 50	6.1	6.1	6.1	6.1	2.6	
Pea Protein 72						4.0
Wheat	6.0	6.0	6.0	6.0	6.0	8.0
Peas	6.4	6.4	6.4	6.4	7.2	7.0
Fish Oil ²	8.3	8.3	8.3	8.3	7.9	8.0
Rapeseed Oil	16.0	16.0	16.0	16.0	16.8	14.9
Monosodium-phosphate	2.8	2.8	2.8	2.8	2.8	2.1
Micro-nutrients ³	4.0	4.0	4.0	4.0	4.0	2.3
<u>Selenite</u>		0.0022	0.028			
<u>Selenomethionine</u> (as a Se-yeast)				0.05	0.63	

South American Superprime fish meal¹, North Atlantic Capelin oil², mineral and vitamin premixture³

All of the diets contained 0.05 mg/kg yttrium

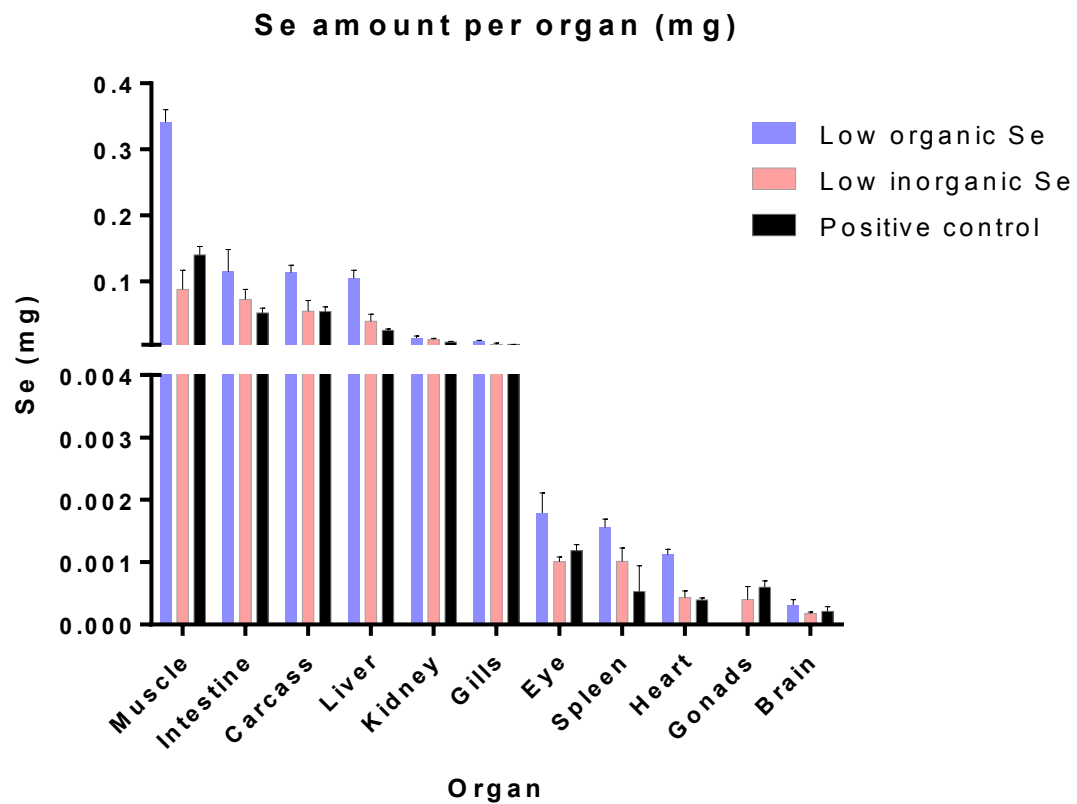
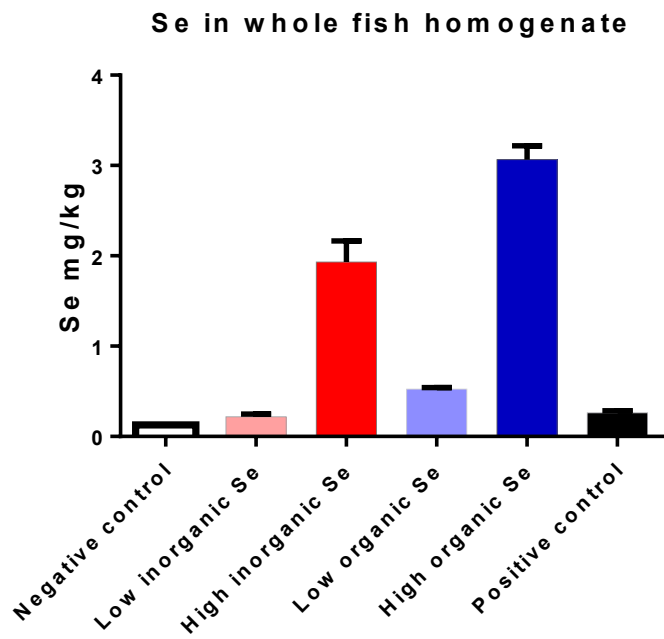
	Negative control	Low inorganic Se	High inorganic Se	Low organic Se	High organic Se	Positive control
Se measured (mg/kg dw)	0.35 ± 0.02	1.10 ± 0.03	15.0 ± 0.5	2.1 ± 0.1	15.0 ± 0.2	0.89 ± 0.03
Fat (g/100g ww)	23.6 ± 0.4	25.1 ± 0.3	25 ± 1	25 ± 1	26 ± 1	27.6 ± 0.3
Protein (g/100g ww)	43 ± 1	44.3 ± 0.4	45 ± 1	45 ± 1	44 ± 1	45 ± 1
Ash (g/100g ww)	5.81 ± 0.01	5.7 ± 0.1	5.75 ± 0.02	5.7 ± 0.1	5.7 ± 0.1	6.09 ± 0.02
Energy (J/g ww)	23088 ± 322	23426 ± 217	23767 ± 40	23340 ± 285	23788 ± 104	24213 ± 246
Dry matter (g/100g)	92.60 ± 0.01	93.37 ± 0.02	94.0 ± 0.3	93.491 ± 0.002	94.1 ± 0.1	94.7 ± 0.1
TBARS* (nmol/g ww)	8.23 ± 0.02	10 ± 1	11.0 ± 0.3	4 ± 1	12.0 ± 0.2	13.97 ± 0.01
Alpha-tocopherol (mg/kg ww)	278 ± 35	308 ± 4	329 ± 1	310 ± 2	335 ± 1	317 ± 3

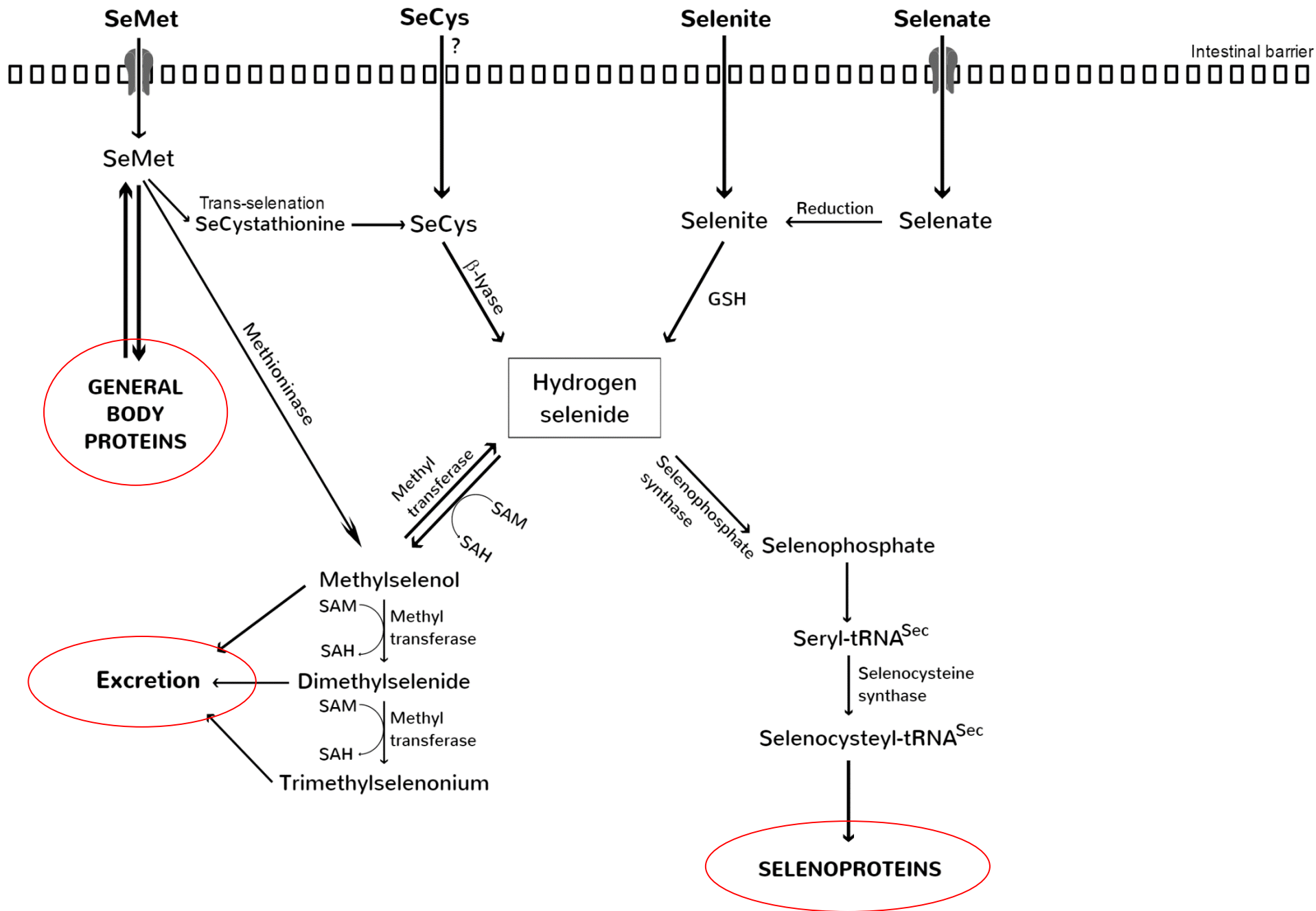
* Thiobarbituric acid-reactive substances, dw; dry weight, ww; wet weight

Experimental procedures

- Atlantic salmon (*Salmo salar* L.) were fed the experimental diets in triplicate tanks for 12 weeks in seawater
- Starting weight was ~570 g
- Metabolic screening was carried out in liver samples (3 fish per tank, 3 tanks per treatment) by Metabolon Inc, USA

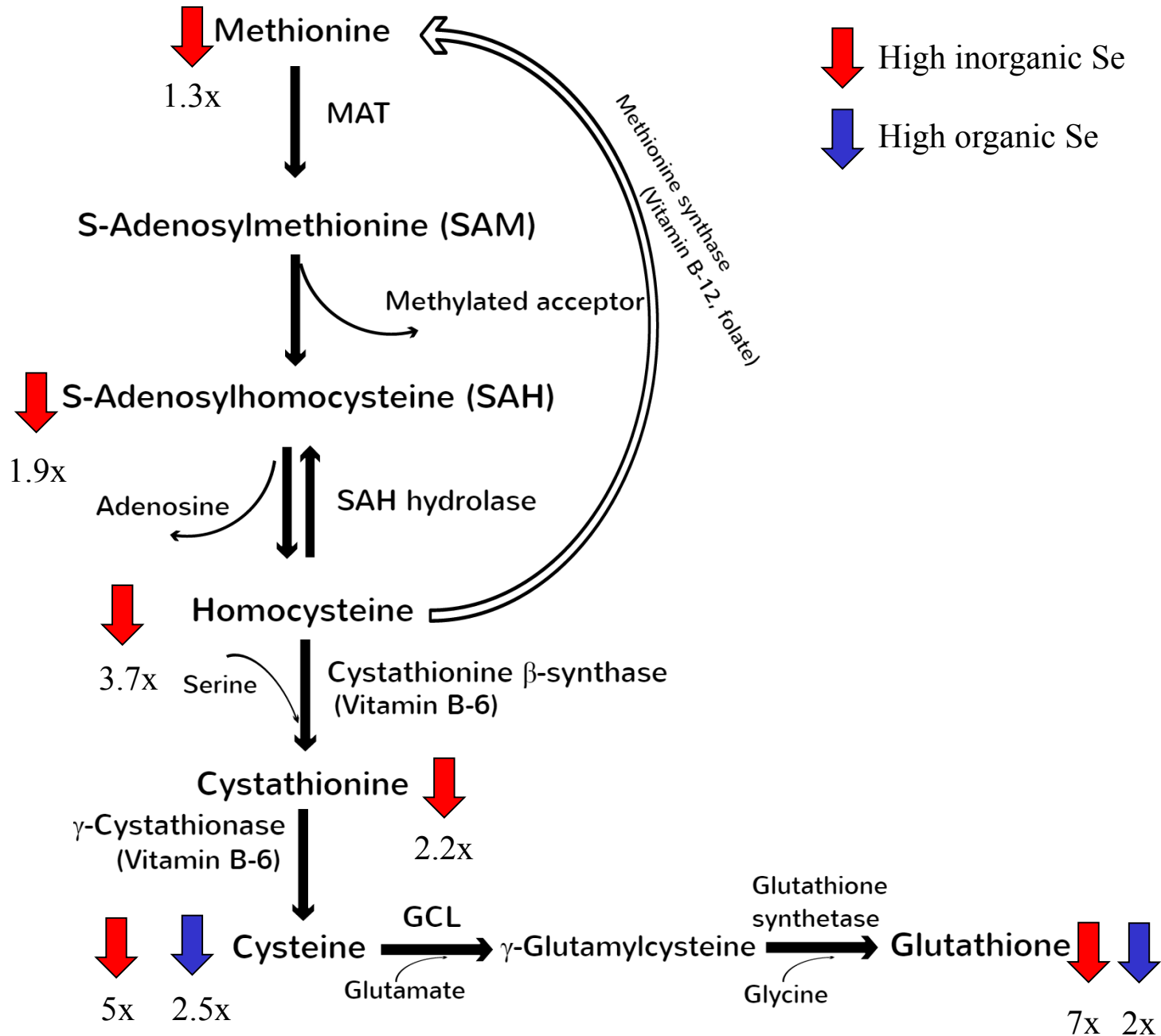
Higher Se deposition in fish receiving high Se diets and organic form





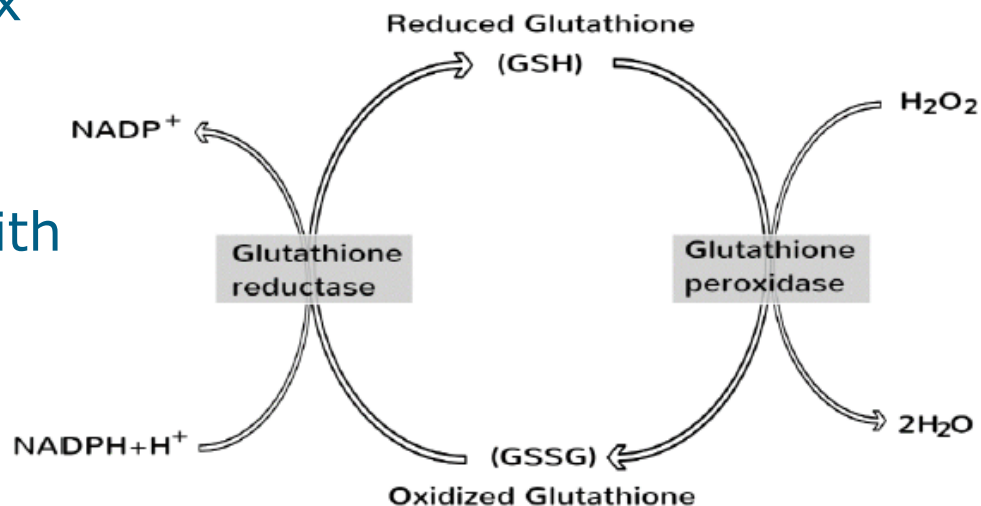
High inorganic Se diet: depletion of intermediates in the SAM cycle, cysteine and GSH

High organic Se diet: depletion of cysteine and GSH only



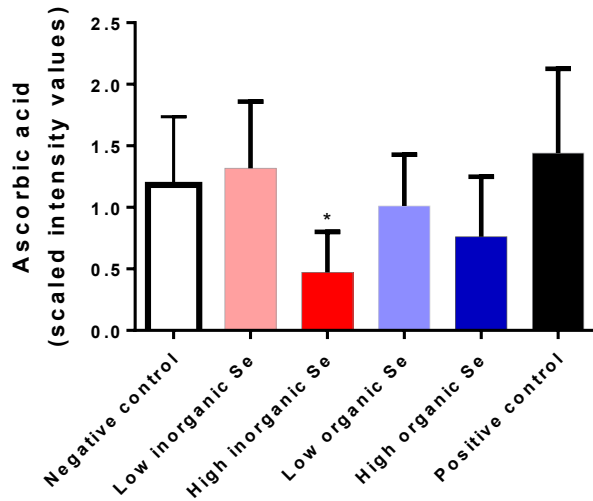
Glutathione (GSH)

- GSH is an important endogenous antioxidant
- GSH may contribute both directly and indirectly in the scavenging of ROS
- GSH is a co-factor for many enzymes, including GSHpx
- GSH may form adducts with reactive intermediates

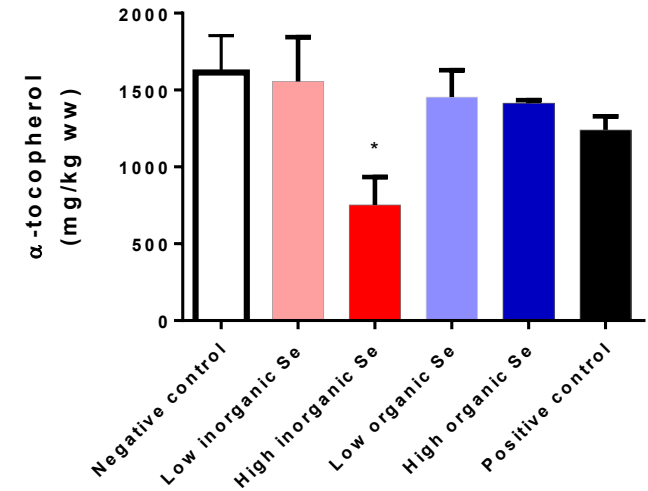


High selenite intake leads to oxidative stress

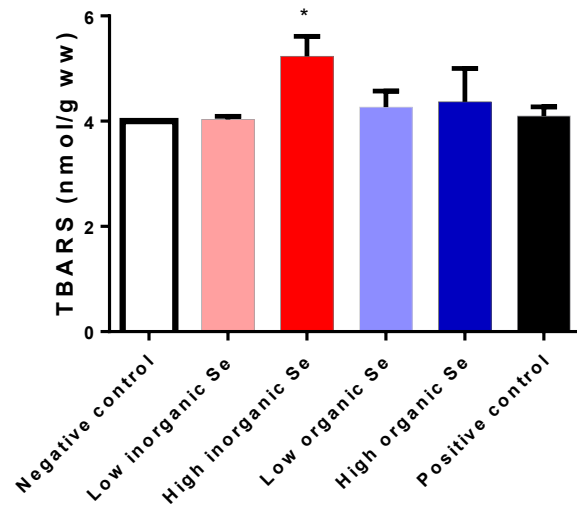
Vitamin C in liver

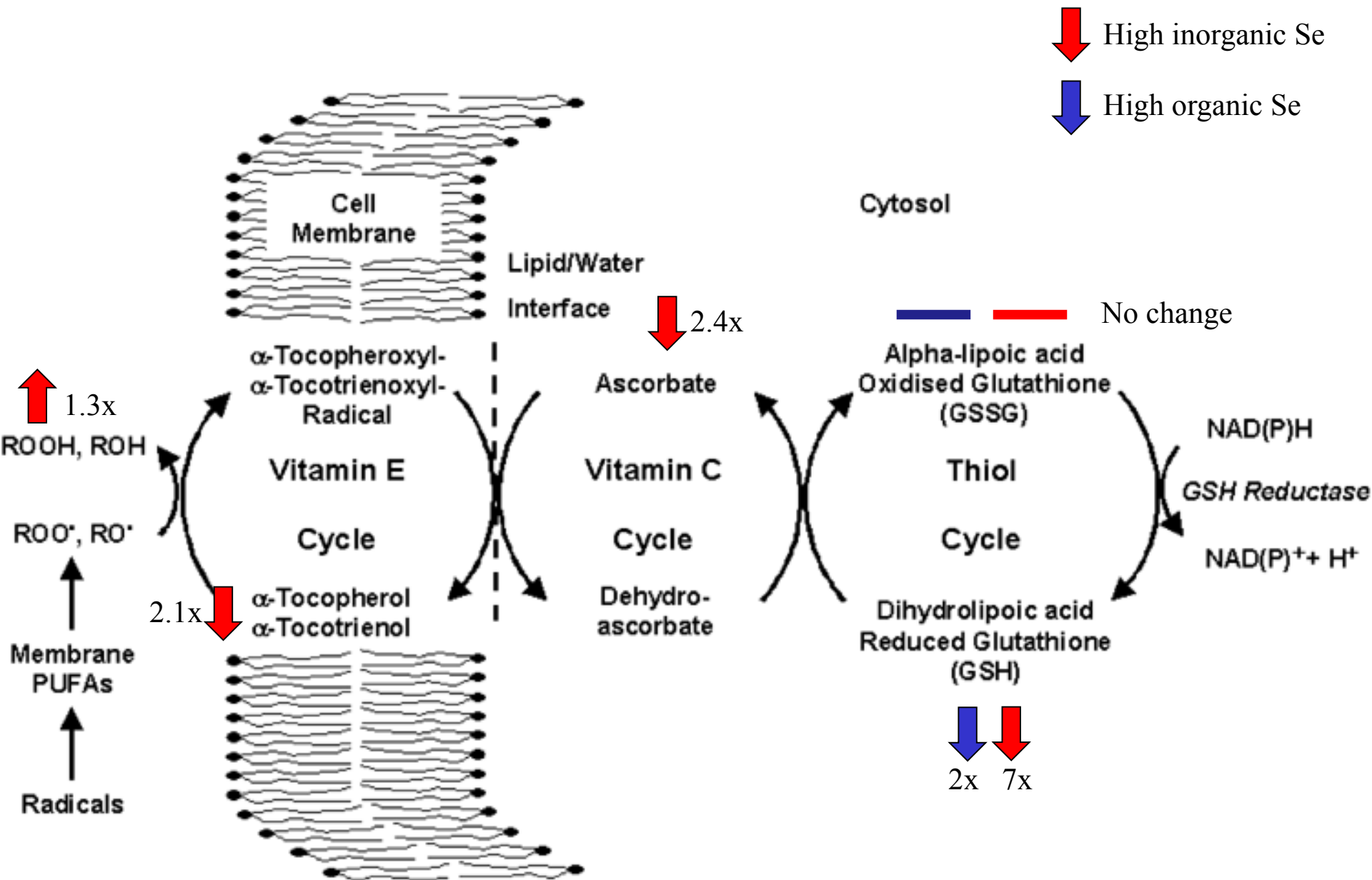


Vitamin E (α -tocopherol) in liver



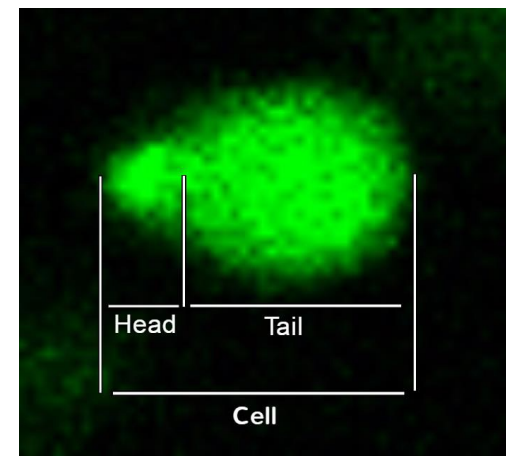
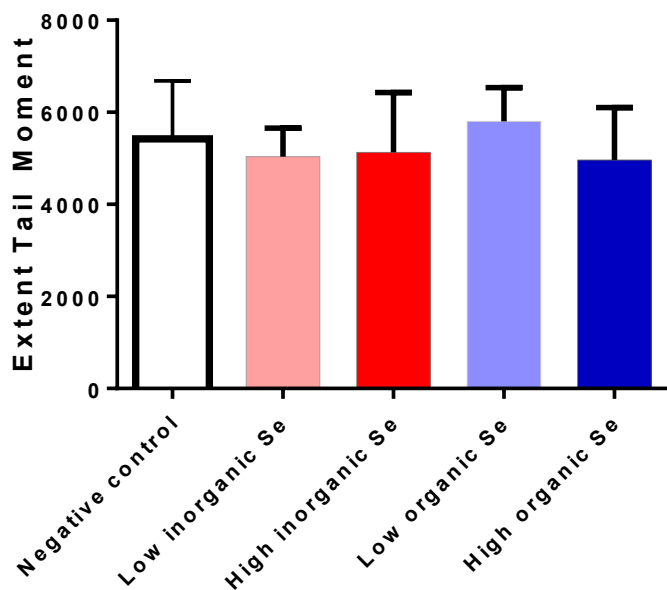
TBARS in liver





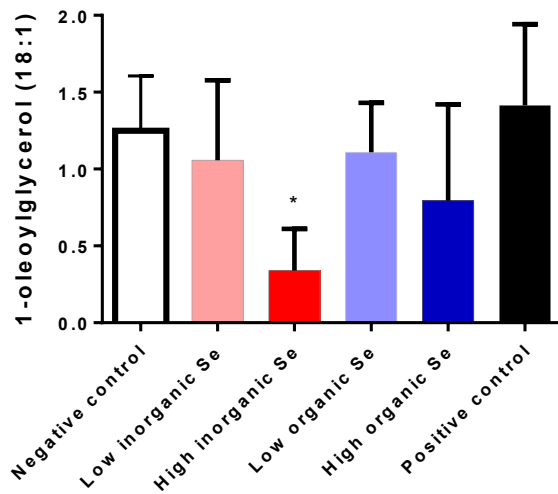
Reduced antioxidant capacity did not lead to oxidative damage

- No sign of DNA damage using the Comet assay

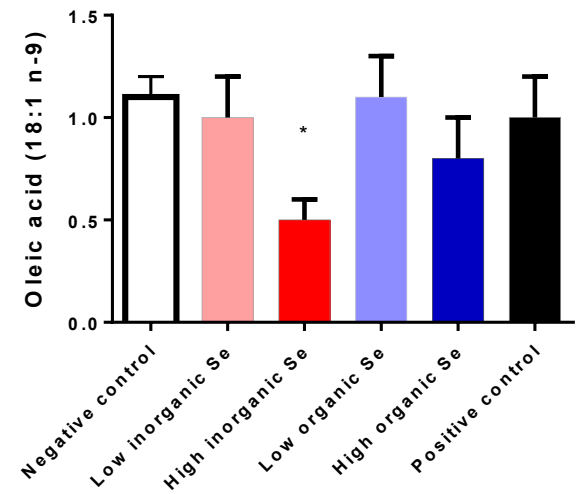


High selenite intake led to reduced concentration of intermediates in the energy metabolism

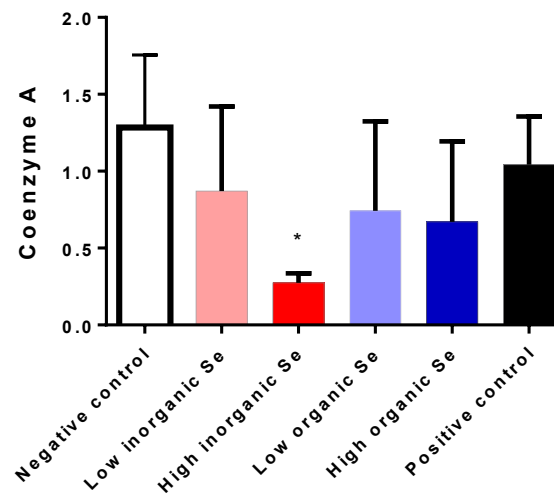
Monoacylglycerol in liver



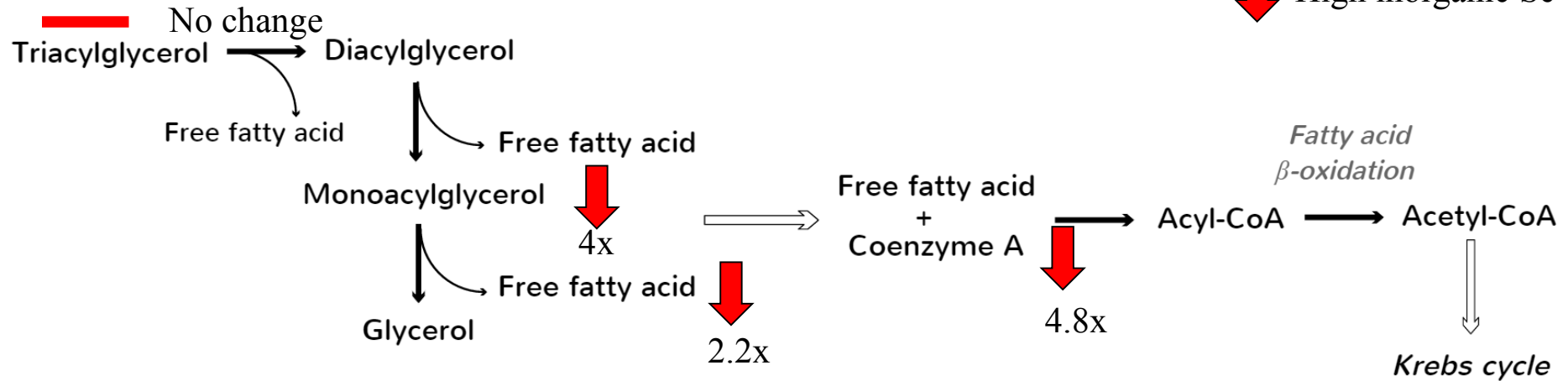
Free fatty acids in liver



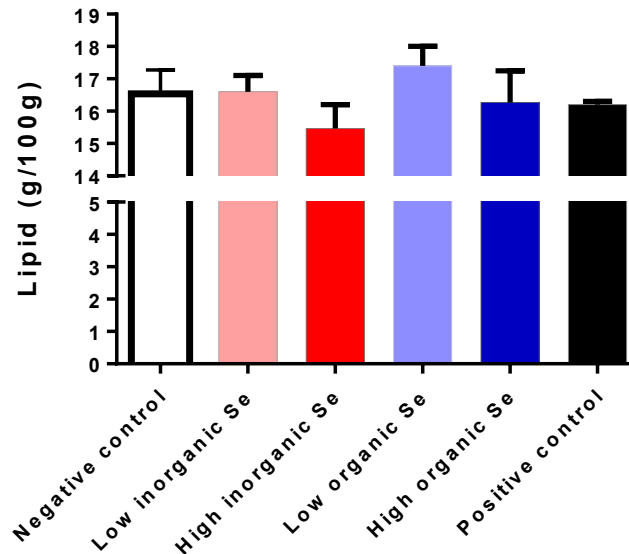
Coenzyme A in liver



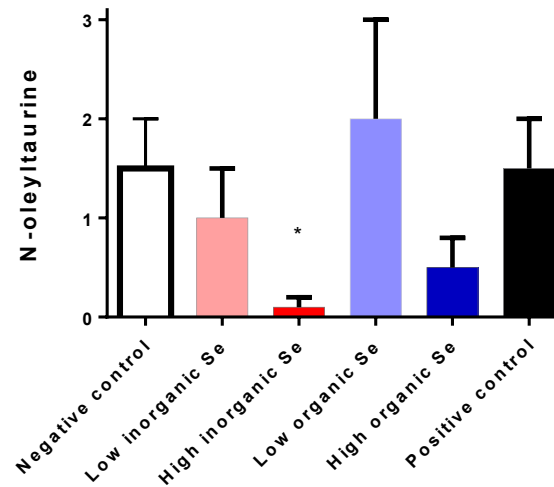
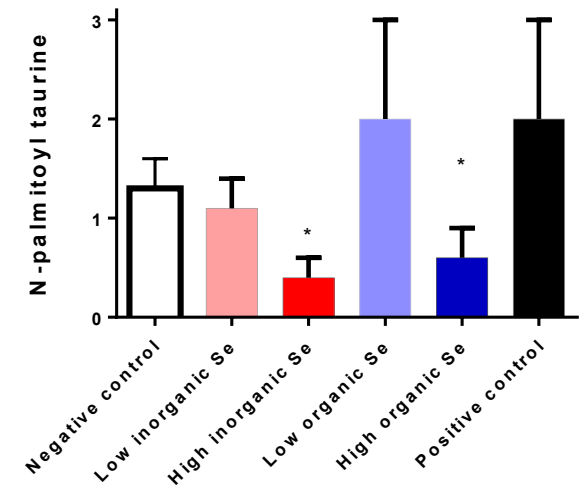
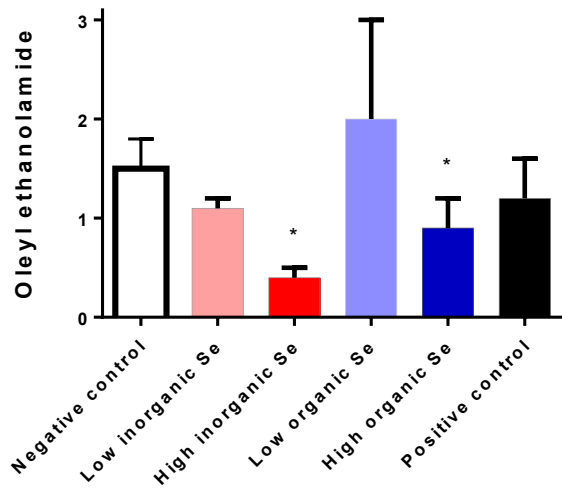
Does Se toxicity lead to increased energy expenditure?



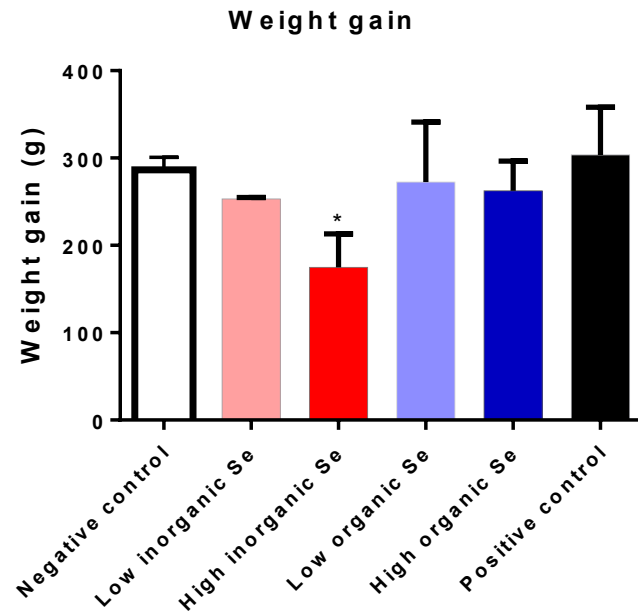
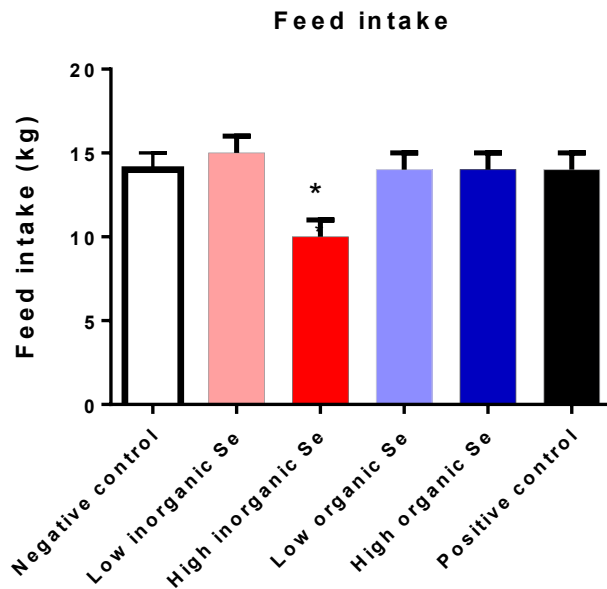
Lipids in whole fish homogenate



The endocannabinoid system –a factor in the regulation of appetite



High selenite intake led to decreased feed intake and weight gain



Conclusions

- High concentrations of Se leads to pro-oxidative conditions
- Markers of oxidative stress are suitable early markers of Se toxicity
- Inorganic Se is more potent than organic Se

Acknowledgements



FISKERI- OG HAVBRUKSNÆRINGENS
FORSKNINGSFOND



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