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Introduction

Previous research has identified restricted dietary level of digestible phosphorus (P) as a causal factor for vertebral deformities in Atlantic salmon. In these experiments, effects on skeletal development were seen with dietary levels of P which were in line with or higher than publishes dietary requirements, indicating that requirement estimates in this species were due for revision. The most prominent effects from a long term subclinical P deficiency were a thick and distorted lower jaw, a break in the tail axis, and compressed and short vertebrae, in particular in the tail (Figure 1 a, b & c). These were, however, long term effects, which were expressed primarily in big fish (>1kg), even though some of the affected fish were exposed to the restricted P diets only in juvenile stages (0-20g).



Figure 1a . Pathology of long term, subclinical P deficiency in A. salmon: a) Thick and distorted lower jaw ("screamer disease") in harvest size salmon,

In early juvenile stages, Atlantic salmon grows very fast. Under commercial conditions daily growth rates (SGR) is commonly 5-7% per day, combined with a low feed conversion ratio (FCR, kg feed fed/per kg gain in body weight). A FCR of 0.6 is not uncommon, and means that 600g feed is required to produce 1 kg of fish. Thus, the nutritional quality of the feed becomes a critical factor. Recent research has demonstrated that P bioavailability from common fishmeal sources may be low and unpredictable, and the intro-

duction of vegetable meals in feeds for juvenile fish represents an additional challenge, as soybean meal and some of the other vegetable meals contain phytic acid which may impair mineral absorption. Thus, the P content of the feed per se is an inadequate indicator for dietary supply, and the most reliable reference is mineral content of fish.

Mineral content of salmon is commonly expressed as whole body content on a wet weight basis. Whole body analyses are the easiest to standardize, and for juvenile salmon there are reliable reference values to compare with (Shearer et al., 1994). A juvenile salmon with normal mineralization of skeletal structures should contain 4000 mg kg⁻¹ or more of both Ca and P, and the level of Ca should be equal to or higher than the level of P. In fish from commercial production, low levels of P are frequently found, indicating that not all batches of commercial diets supply adequate amounts of P.

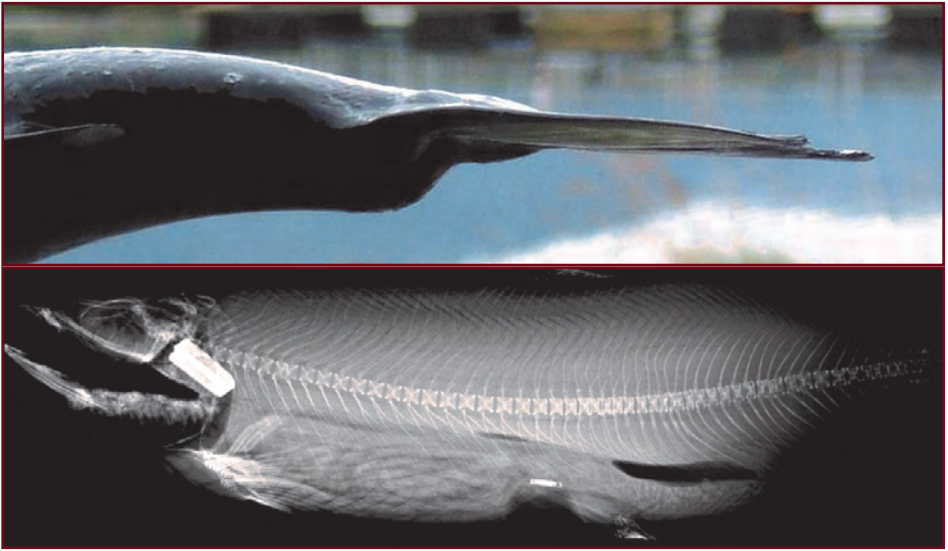


Figure 1b & 1c . Pathology of long term, subclinical P deficiency in *A. salmon*: b) broken tail axis induced by low P in juvenile rearing, c) X-ray image of 4 kg salmon fed a diet deficient in P and Zn in freshwater rearing.

Low zinc (Zn) was identified as an additional risk factor for impaired skeletal mineralization. Analyses of commercially reared fish demonstrated that whole body levels for this element commonly were low, with values typically seen in the 30-40 mg kg⁻¹ range compared to reference values of 50-60 mg kg⁻¹, and with values below 30 mg kg⁻¹ occurring sporadically. In previous experiments, the effects of low Zn levels were examined in combination with low P, and low Zn was found to aggravate the symptoms of P deficiency. In the FineFish project, an experiment was done addressing the supplementation of P, Zn and Mg in starter and juvenile diets more specifically. Mg was included as an experimental factor in addition to P and Zn, due to observed variable levels of this element on whole body analyses both in experimental groups and in commercially reared fish.

Material and methods

There were four experimental diets with differing dietary mineral contents, and each diet was fed to triplicate groups of fish. The diets were:

1. Control diet (1,6-1,7g P kg⁻¹, 190-200mg Zn kg⁻¹, 2,4g Mg kg⁻¹)
2. Low P diet (1,3g P kg⁻¹)
3. Low Zn diet (85mg Zn kg⁻¹)
4. Low Mg diet (1,8-1,9g Mg kg⁻¹)

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Each diet was formulated into a series of particle sizes suitable from first feeding up to fish size >30g.

Each group consisted of 400 first feeding fry. The fish were reared in circular fibre-glass tanks ($\approx 0.5\text{m}$) with flow-through water supply, and each tank was fitted with an overhead light source. The fish were reared under continuous light supply, and feeding was 24 hours per day, in excess. The fish were weighed and sampled at start (0,2g), 1g size, 5g size and 15g size. At termination, approaching 30g size, individual weights and lengths of 50 fish per tank were recorded.

Feeds and fish were analysed for mineral contents. At 5g and at termination, fish were sampled for radiography. The X-ray equipment used was a semi-digital system, using a mammography X-ray source, and reusable image plates for mammography with a final image resolution of 20 pixels/mm.

Duration of the feeding trial was 20 weeks. Following termination of the experimental treatments (30g size), a selection of fish from each tank was tagged, all fish were mixed in a pooled group, and subsequently reared through smoltification and transferred to a sea cage. Following 8 months seawater rearing, the fish were killed and examined again at approximately 2 kg size, to be able to describe any long term effects from different dietary treatments in early life.

Results

Mortality throughout first feeding and the rest of the experimental period was low, 5-10% per tank, and no differences were noted between dietary treatment. Overall growth rates were comparable to growth tables (Club N, Skretting AS). At the end of the experimental period (30g size), fish fed the low P and low Zn diets weighed significantly more than those fed the control and low Mg diet. The low Zn fish had significantly higher condition factors than fish on the other diets.

The mineral content of the fish showed relatively small differences in elemental composition of fish, except the low Zn fish, which were down to levels $<20\text{ mg kg}^{-1}$. The low P fish were low in P and Ca at 1g size, but not particularly so at later samplings. On radiographs, however, both the low P and the low Zn fish displayed significant changes from normal skeletal morphology. The low P groups had a significant number of fish with high density (HD) vertebrae (Helland et al., 2006). The low Zn fish had a high number of fish displaying a specific condition in which the larger part of the spinal column was affected. These spines were characterized by a lack of inter-vertebral space, and compression of the vertebral bodies. No specific effects were seen in the low Mg fish.

The long term effects recorded at 2 kg size were prominent; in fish with juvenile Zn deficiency, vertebrae were narrow and distorted. There were, however, few external signs of the impaired skeletal development in the same fish. Fish diagnosed with HD vertebrae at seawater transfer followed one of two developmental patterns; approximately half of the HD vertebrae were not identifiable at 2 kg size, whereas the other half had developed into fusions. There were no differences in weight between the dietary groups at the final sampling.

Discussion

The experiment was successful in distinguishing between the specific effects of a subclinical P deficiency and a corresponding Zn deficiency. Analyses of commercially reared fish demonstrate that these two elements may be low, either one or both at the same time. Thus it is helpful for future diagnostics to be able to identify early signs and to distinguish between the subclinical pathology related to these elements.

The high density vertebrae associated with subclinical P deficiency are comparable to the changes described by Helland et al (2006), who hypothesised that this pathology could be read as an early sign of subadequate mineralization. The level of P deficiency observed in this experiment was, however, very moderate, compared to the vertebral pathology induced in other experiments with low P diets, and the typical platyspondylia in big fish associated with juvenile P deficiency was not observed.

The Zn-deficiency induced compressed vertebrae lacking intervertebral space may be compared to the “short body dwarfism” described by Murai and Andrews (1978) in catfish, in response to riboflavin deficiency. The results from this study indicate that this type of pathology relate to Zn deficiency specifically also in Atlantic salmon, and may be used as a diagnostic indicator.

The results confirm the importance of proper mineralization of skeletal structures in the early juvenile stages for prevention of skeletal deformities.

References

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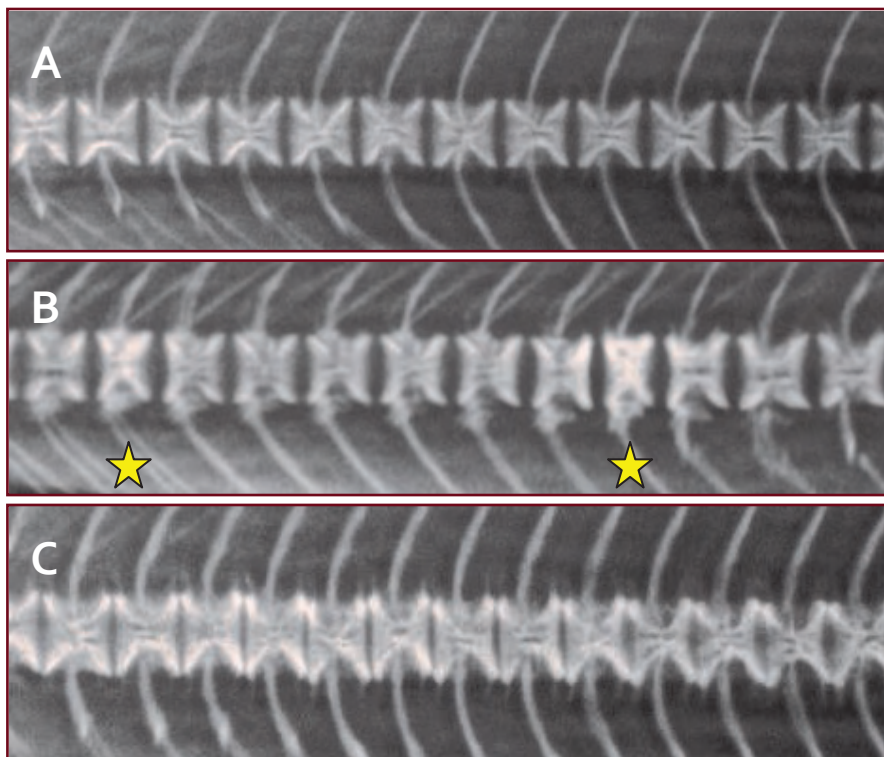


Figure 2. Detail of X-ray, A. salmon parr at 30 g size, following an experimental period with diets differing in dietary mineral content: a) normal vertebrae (control), b) high density (HD) vertebrae (*) in fish fed low P diet, c) compressed vertebrae in fish fed low Zn diet.

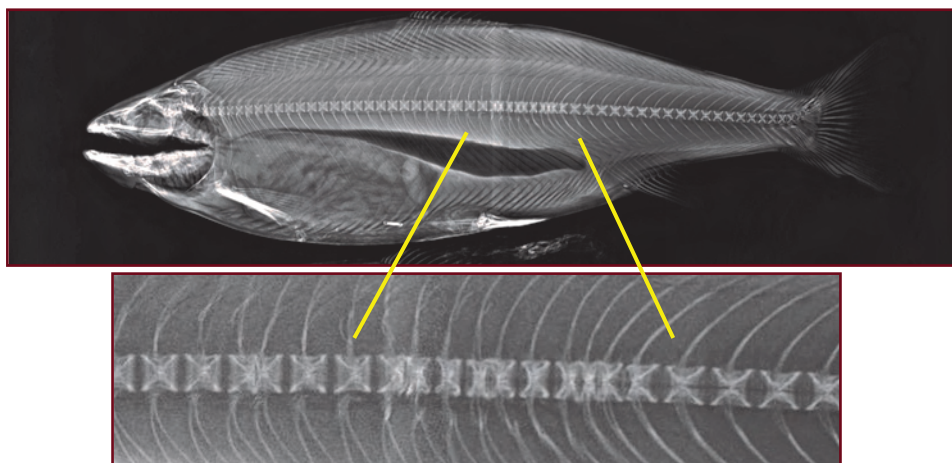


Figure 3. Zn-deficiency induced vertebral pathology in A. salmon. Exposure to deficient diet from first feeding to 30g size. X-ray at 2 kg, following 8 months in seawater on commercial diet.