news
FP6 Collective Research Projects
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FineFish

Improving sustainability of European fish aquaculture by control of malformations

In this issue

Training & extension

FineFish will organise in 2009 species-specific courses for cod, rainbow trout and Atlantic salmon. Both trainings of cod and salmon will take place in Norway, the training for trout in Denmark. Two courses will be organised for seabass and seabream together in Greece and Portugal.

Each training course will take about 2-3 days and will consist of three modules. 1) Common: Methodology with practical training and demonstration, 2) Species-specific: Results and recommendations on how to reduce malformations and if possible 3)Farm visit with hands-on training. More detailed information about the time and place of the different trainings will be soon available on the FineFish website.

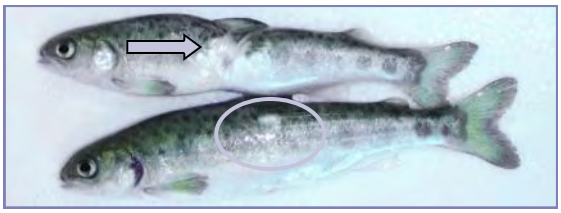
Extension

The project has received an 8 months extension induration, ending in June 2009. This gives the project the opportunity to record farm data on environmental factors and malformation rates during 2 full production years via the web-based monitoring system developed within the project.

New deformities coming up!

In the current yearclass of Atlantic salmon juveniles produced in Norway, a new malformation is a cause for concern, although the problem is associated to a limited number of fish groups.

The malformation is expressed as a stricture of the trunk of the body which develops as the fish grows. Early stages are manifested as a malpigmentation in the area behind the dorsal fin. In more severe cases, the abdominal fins are dislocated towards the dorsal fin, and in really severe cases the entire fish body has a stricture in the area of the dorsal fin.



Picture: Two examples of Atlantic salmon parr with strictures of the trunk. A: Stricture of the entire body and a dorsal dislocation of the abdominal fin. B: Mild case, with only a small malpigmented area (purple circle). Mean weight was about 10g

According to producers, this malformation has been observed from time to time, but never before systematic and only in low numbers. This year we have been contacted by producers that find this malformation in higher numbers, the worst case so far is one producer throwing out 15000 fry at vaccination.

The common interpretation of this malformation is some kind of mechanical injury during handling; or even healed wounds from bird attacks. However, we have now seen it in fish groups that have been very little handled and sorted, and a long time before any exposure for birds. The problem appears to be related to poor egg quality. In our samples, the most severe cases were systematically on the left side of the fish, suggesting that the development of the malformation may be related to direction of tank water current. There is some mortality, but most of clinically affected fish will be discarded during vaccination and handling.

Pathology of the spine generally reflects the severity of externally observed changes. In fish with only spots of malpigmentation, skeletal structures appear normal in radiographs. In severely affected fish, large parts of the spine is may be affected and the displacement of the fin is clearly visible. In fish with slightly abnormal shape, abnormality in the muscle tissue can be observed on radiography, but not necessarily on the vertebrae.

Picture: Ingrid Lein, Tinka, Grete Baeverfjord, Synnøve Helland and Kirsti Hjelde

AKVAFORSK merges into Nofima

At the first of January 2008, AKVAFORSK of Norway joined a number of other Norwegian research institutes within the blue-green sector. Nofima is the name of the new industry-oriented research institute that does research and development for aquaculture, fisheries and food industries. The research group consists of all activities at former AKVAFORSK, Matforsk, Fiskeriforskning and Norconserv.

Colophon

The FineFish project aims to generate new practical knowledge on how to reduce the incidence of malformations in the major fish species used in European aquaculture production and to apply this to the professional sector. FEAP (Federation of European Aquaculture Producers), ten major European hatcheries and eight leading European scientific institutions participate in this Collective Research Project.

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Restricted dietary levels of phosphorus and zinc induces specific skeletal deformities in juvenile Atlantic salmon

Grete Baeverfjord, Kirsti Hjelde, Synnøve Helland and Ståle Refstie

Among the causal factors for skeletal deformities which have been identified till now is mineral deficiency. In a previous project, a restricted level of digestible phosphorus in combination with low dietary zinc was shown to have long-term effects on skeletal development in Atlantic salmon.

”..the low P diet,.., induced a certain disturbance of bone mineralisation”

Beside that, recent analyses of fish reared in commercial production demonstrates that low whole body levels of phosphorus and zinc and Mg are frequent in juvenile fish. In the present study, an experiment addressing the supplementation of P, Zn and Mg in starter and juvenile diets was done. The aim was to identify any specific effects of the deficiency conditions.

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; 2) Low P diet; 3) Low Zn diet; and 4) Low Mg diet. The mineral levels in the experimental diets were comparable to commercial diets, i.e. in being within published requirement estimates but at levels expected to give subclinical deficiencies.

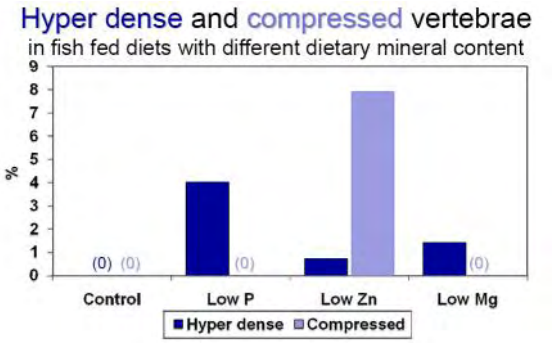
Duration of the study was 20 weeks. The fish were weighed and sampled for analysis at start, 1g size, 5g size, 15g size. At termination (approaching 30g size), individual weights and lengths of 50 fish per tank was recorded. At 5g and at termination, fish were sampled for radiography.

Results and discussion

Mortality throughout the experimental period was low, 5-10% per tank, and the overall growth rates were comparable to growth rates in commercial production. At termination, fish fed the low P and low Zn diets were significantly larger than those fed the control and low Mg diet. The low Zn fish had significantly higher condition factors than fish on the other diets.

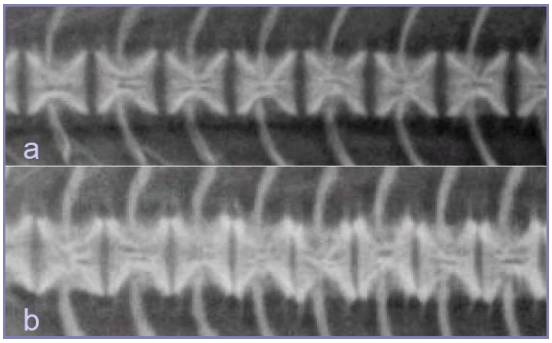
”analyses of commercially reared fish demonstrate that P and Zn may be low”

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 mg kg-1, compared to reference values of 40-60 mg kg-1. The Ca and P levels were reduced initially (1g and 5g samplings) in fish fed the low P and low Zn diets, but were not different from controls at 25g size. In the low Mg fish, Mg levels were higher at initial samplings, but equal in all diets at the final sampling.



Radiograhs

On radiographs, however, the low P groups had a significant number of fish with high density vertebrae. These vertebrae were seen also in low numbers in low Zn and low Mg fish, but not in controls. More specifically, it appeared that the low P diet, and to a lesser extent the low Zn and low Mg diets, induced a certain disturbance of bone mineralisation, sufficient to induce formation of hyper dense vertebrae. The high density vertebrae associated with subclinical P deficiency are comparable to the changes described by Helland et al (2006).



Picture: Detail of vertebrae in A. salmon parr (approx. 25g). a) Normal vertebra in fish fed control diet. b) Compressed vertebrae with lack of inter-vertebral space in fish fed low Zn diet.

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 intervertebral space, and compression of the vertebral bodies. The 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.

Thus, analyses of commercially reared fish demonstrate that P and Zn may be low, either one or both at the same time. Early-life subclinical hypo-mineralization was previously linked to development of late-onset platyspondylia, and these results will be helpful in providing more precise diagnostic tools related to this complex condition.

Long-term effects

A selection of fish from this study was tagged and reared beyond the experimental period in order to characterize any long term effects of the subclinical mineral deficiency during juvenile rearing. These fish will be examined again at the end of 2008.

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Effect dietary phospholipids ratios to seabream deformities

Mr. Eran Sandal (MSc student, NCM Israel) worked on the effect of dietary phosphatidylcholine (PC)/ phosphatidylinositol (PI) ratio fed to gilthead sea bream (Sparus aurata) larvae on the appearance of skeletal deformities during the juvenile and fry stages.

PC and PI are membrane phospholipids that are now widely believed to be essential dietary additives for optimal fish larval performance. PC generally represents the largest phospholipid fraction in the biological membrane and has been shown, when added to the diet, to improve growth in developing marine fish larvae by increasing synthesis of lipoproteins. This results in increased mobilization of digested lipids from the mucosa of the digestive tract to the body tissues. On the other hand, exceedingly high levels of dietary PC may induce skeletal deformity. In contrast, PI is found at much lower levels in the membrane but recent research has indicated that its presence in larval feeds may reduce skeletal deformity.

Experimental set-up

4 diets differing in their PC/PI ratios (3.1, 2.3, 1.6, and 1.3) were fed to 22-35 days post hatching (dph) larvae. After 35 dph, all fish were fed the same weaning and starter diets until 141 dph.

Results

Although in general cranial deformity was low, it was found that decreasing dietary PC/PI ratio during larval rearing significantly reduced cranial deformity (mainly the jaws). This reduction contributed to improved feeding efficiency on pellets in a later stage leading to significantly better growth .

On the other hand, feeding high dietary PI demonstrated a non-significant increase in skeletal deformity which was more marked in faster growing individuals compared to their slower growing cohorts. In fast growing larvae fed the increased dietary PI, there was a correlation between fish age and osteocalcin, a protein involved in bone formation. Interestingly, this was not the case in fast growing fish fed the low dietary PI.

(More information can be found on the website)

Conclusions

The results suggest that PI is involved in the regulation of osteocalcin synthesis which reduced jaw deformity. The results of this experiment showed that the most effective dietary PC/PI ratio for sea bream larval rearing was 1.3 in terms of growth, survival and reduced cranial deformity in the later developmental stages.

”decreasing dietary PC/PI ratio during larval rearing significantly reduced cranial deformity”

Effect of vitamin A on the deformities of the gilthead seabream fry

Mr. Boaz Ginsberg (MSc student, NCM, Israel) found that increasing dietary vitamin A significantly reduced growth and increased the appearance of deformities in the resultant fry. In fact, a correlation was found between developmental stage and the effect of vitamin A dose on deformity type.

Four different levels of vitamin A in rotifers (0, 3.3, 33.3 and 70.5 µg/g wwt) and Artemia nauplii (0, 3.9, 11.7 and 21.2 µg/g wwt) were fed separately and in combination to gilthead seabream larvae and tested the effect of this nutrient, as a function of developmental stage, on the appearance of deformities in fry at 67 and 115 days post hatching.

Results

Supplementing vitamin A during rotifer and Artemia feeding increased cranium and skeletal deformities in a dose-dependent manner, respectively . The dietary form of vitamin A used for enrichment in all of these studies was retinyl acetate, which was converted in the larvae to retinol in a dose-dependent manner in the rotifer studies, while retinyl acetate accumulated only in the highest vitamin A diets that later demonstrated the most deformities. However, in the Artemia trials this supplement was converted to retinol in a dose-dependent manner while both retinyl acetate and retinyl palmitate accumulated in the highest vitamin A diets. This latter result was also observed when retinyl acetate levels were fed throughout larval rearing (rotifers and Artemia).

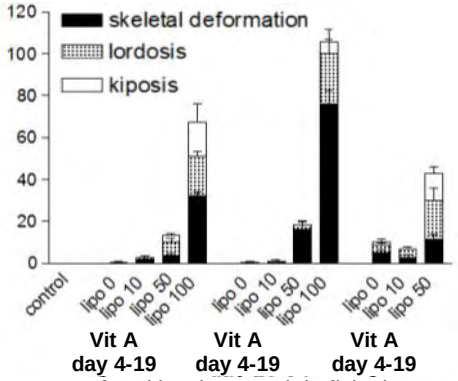

Figure: Deformities in 115 dph fish that were fed during larval rearing rotifers (4-19 dph) or Artemia (20-34 dph) or rotifers and Artemia (4-34 dph) that were enriched on 0, 10, 50 or 100 mg retinyl acetate/3 g of liposome suspension to the larvae.

The pattern of use and accumulation of these vitamin A isoforms, as a function of developmental stage, may be offering broad hints to determine the effective levels of this critical vitamin as a growth promoter during larval rearing and when dietary excess results in deformity at later developmental stages.

(More information can be found on the website)

Conclusion/Recommendations

Recommended levels of vitamin A in this study were 3.3 and 3.9 µg/g wwt in rotifers and Artemia, respectively, for best larval performance and reduced deformity in the resultant fry.

”Increased deformities were found in the two highest vitamin levels”

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Royal Veterinary College

The Royal Veterinary College, University of London (RVC) One of the College's key research strengths is 'Musculoskeletal pathobiology' that has received the highest grading in the UK's last two national Research Assessment Exercises.

The group has a wide technical expertise in molecular biology, histology, confocal microscopy and sophisticated imaging techniques.

Picture: Neil Stickland, Dulce Martins and Neal Anthwal

Regulating muscle growth to decrease deformities

Recent work from the Royal Veterinary College (RVC) and others indicates that skeletal deformities, such as lordosis, may be a consequence of a mismatch in the development of muscle and bone, with a period of early rapid muscle growth occurring when the bone which supports it has yet to develop sufficiently.

The proper growth and development of fish muscle is of prime importance in the production of fish for the food market.

"muscle growth does not occur in isolation"

It is well established that environmental and nutritional factors such as temperature and or dietary vitamin A content can alter the rate of development of fish muscle. However, since muscle growth does not occur in isolation, it is not always desirable to have sustained rapid muscle growth throughout the development of the larva.

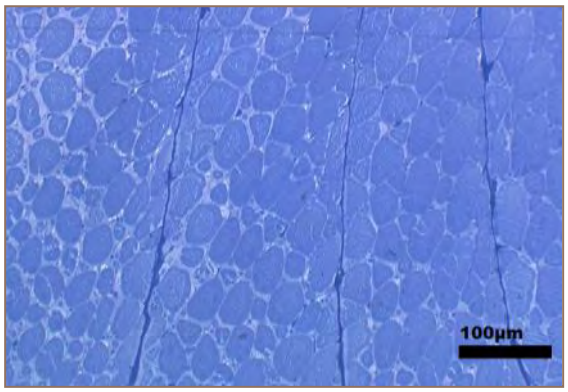
Controlling factors of muscle growth

The muscle regulatory factors are a group of expressed genes that control the initiation and growth of muscle fibres. One such factor, MyoD, is involved in the early proliferation of muscle cells. An elevated level of MyoD expression, measured using the qRT-PCR* technique, suggests periods of rapid muscle proliferation before a rapid muscle growth.

Other muscle regulatory factors include Myogenin, the expression of which follows MyoD and is involved in the later differentiation of muscle fibres, and Myostatin, which acts to regulate the action of MyoD and Myogenin.

The expression of these factors can be used as indicators of the potential growth of the fish, and hence whether the mismatch in the growth of muscle and bone will increase the incidences of skeletal deformities. Previous studies in Atlantic salmon have suggested that high expression of MyoD at about the time of hatch (rather than earlier) produces optimal posthatch growth and appears to correlate with a lower incidence of deformities.

**Quantitative reverse transcription polymerase chain reaction is a laboratory technique for amplifying and quantifying an expressed gene as encoded in a defined piece of a ribonucleic acid (RNA) molecule*



Picture: Histological section through the muscle of larval seabream showing the mosaic of large and small muscle fibres

Experimental setup and results

In experiments carried out by HCMR in Crete, Gilthead seabream were incubated at 16, 19 and 22Cº up until the fish reached a mean weight of 1g then allowed to grow through the larval stage at 19Cº.

Analysis was then carried out at the RVC. When sampled at the 30mm stage, those fish incubated at 19 Cº had a significantly higher bone to muscle ratio, suggestion less of a mismatch between the developing muscle and bone. These fish also had a greater proportion of small muscle fibres in the white muscle (which shows a mosaic of large and small fibres at this stage, see figure), indicative of muscle growth. The levels of MyoD expression in these fish was also greater than those incubated at 16 or 22 Cº. This suggests that, in gilthead sea bream larvae at the 30mm stage, a high expression of MyoD is indicative of good growth and probably fewer deformities.

Conclusions/Recommendations

The development of muscle and bone must occur in a coordinated manner, so that excessive rapid muscle development does not lead to skeletal deformities. A potentially useful indicator for the rate of muscle growth is the expression of the muscle regulatory factor MyoD (measured using the RT-PCR technique) and the desired level of expression at a particular time for the best growth is dependent on species.