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1. Introduction

Skeletal deformities have been recognized as a recurring problem in salmonids, especially in rainbow trout. A wide range of risk factors for development of skeletal abnormalities have been identified, including infections, toxins, genetics, environmental and nutritional factors. Inadequate feeding, especially during early development, has been reported to induce malformations in fish. Almost all nutrients can affect skeletal development of fish. However, three focus groups of nutritional factors have been identified for rainbow trout:

- Minerals, particularly phosphorus and calcium, as these key minerals are directly involved in the development and maintenance of the skeletal system and their availability in fish feeds can vary depending on the formulation with plant or marine ingredients.
- Vitamin A, as this vitamin plays a central role in many essential biological processes such as vision, immunity, growth and development. Vitamin A is also required for reproduction. However it can be teratogenic through its active metabolite, retinoic acid.
- Lipids, as significant amounts are present in skeletal tissues including collagen.

Polyunsaturated fatty acids which are characteristic of fish tissues are particularly susceptible to lipid peroxidation which is known to cause several pathologies including the development of malformations. Phospholipids also have been reported to influence skeletal development and mineralization.

2. Impact of dietary minerals on rainbow trout development

Two feeding trials were performed to assess:

1. the influence of dietary calcium and phosphorus on bone formation and mineralization in rainbow trout,
2. the influence of dietary phosphorus deficiency on bone formation and mineralization in rainbow trout fry and the impact on the later development,
3. the influence of triploidisation on the impact of dietary phosphorus deficiency in rainbow trout fry.

Experimental set-up

Feeding trial 1

Six semi-purified diets were tested on swim-up rainbow trout fry for 12 weeks at water temperature of 17°C. The basal diet A contained only phosphorus supplied by casein (0.5%) and other diets were supplemented with 0.4, 0.8, 1.2 and 1.6% of highly available phosphorus supplied as a mixture of sodium and potassium phosphates. These five diets were supplemented with 1% available calcium whereas another diet F, supplemented with 0.8% phosphorus (considered as the control level), was calcium-free.

Feeding trial 2

Half of the eggs from a common batch from 8 rainbow trout females and 6 males was triploidised by hyperbare pressure. The two groups, diploid and triploid rainbow trout fry, were then fed from first-feeding onwards the two semi-purified casein based diets C and A supplemented or not with 0.8% P. The feeding trial with the two experimental diets lasted 12 weeks and then the four groups of rainbow trout fry were fed a common commercial diet containing 1% P for another 12-week period.

Results

Feeding trial 1

There was no effect of dietary phosphorus or calcium level on growth.

A lower survival was observed in rainbow trout fry fed the high phosphorus diet E (Figure 1) whereas a lower skeletal mineralization was seen in rainbow trout fry fed the diet A with a low phosphorus level (Figure 2). This group of fish also displayed higher incidence of abnormalities with irregular placement of vertebrae with twisted arches at later developmental stages. So, both dietary deficiency and excess of phosphorus are detrimental to rainbow trout development.

Concerning the impact of dietary calcium level, the results were less obvious. However, a lowered or delayed skeletal mineralization with also a higher incidence of skeletal abnormalities such as kyphosis or fused vertebrae was observed in fish fed the low calcium diet F.

The impact of nutritional components on rainbow trout

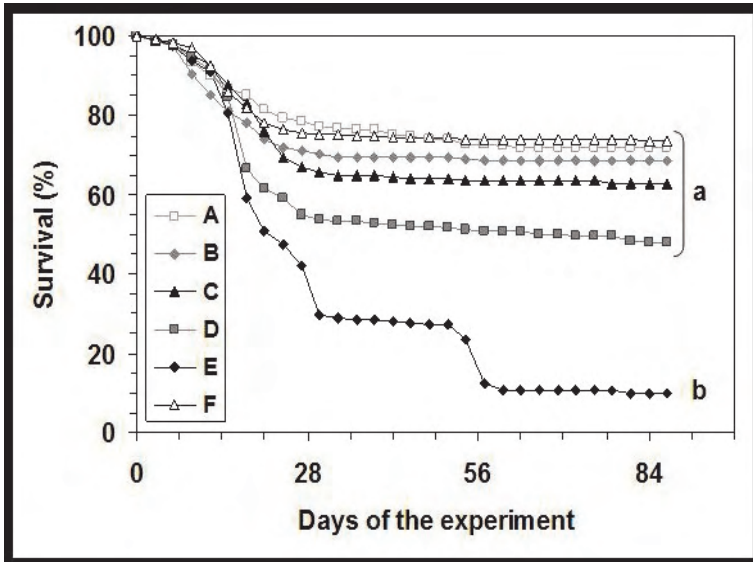


Figure 1. Survival of rainbow trout fry fed the six diets supplemented with different levels of phosphorus (0, 0.4, 0.8, 1.2 and 1.6%) and calcium (0 and 1%).

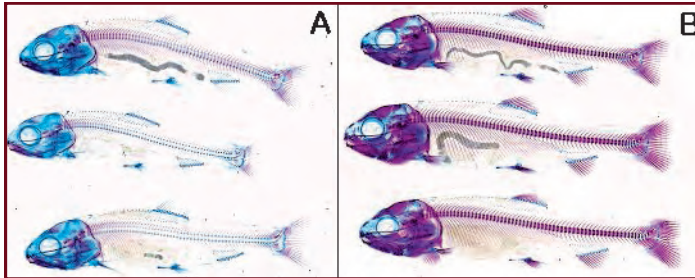


Figure 2. Rainbow trout fry sampled at day 28 of the experiment, fixed in 10% formalin and stained with Alcian blue for cartilage and Alizarin red for calcified bone and fed the P-deficient diet A (A) or the P-control diet F (B).

Feeding trial 2

No difference of survival and growth was noticed between the four groups. At the end of the first part of the trial, rainbow trout fry fed the phosphorus-deficient diet A displayed lower whole body phosphorus content (Figure 3) and were less ossified. There was no difference between diploid and triploid fish. At the end of the second part of the trial, the whole body phosphorus content of fish fed diet A was restored (Figure 3) and no external malformation was detected.

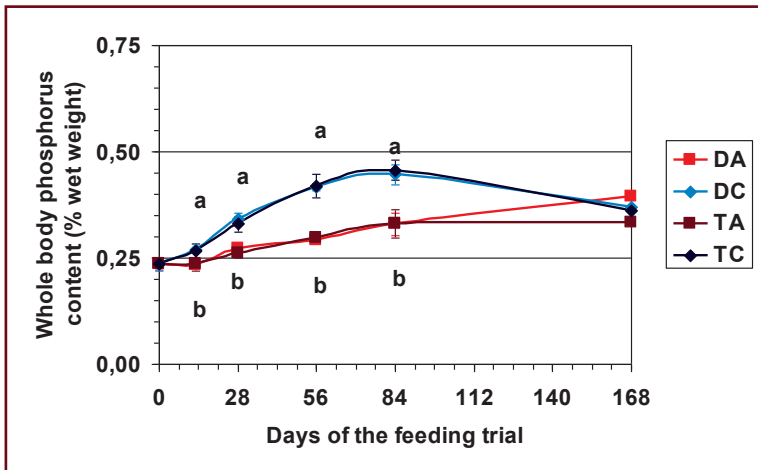


Figure 3. Whole body phosphorus content of the four groups of rainbow trout fry. DA: diploid fish fed the P-deficient diet A, DC: diploid fish fed the P-control diet C, TA: triploid fish fed the P-deficient diet A, TC: triploid fish fed the P-control diet C.

Conclusions

This study highlights the importance of dietary mineral supply for adequate skeletal mineralization. A special attention should be paid to the level and availability of phosphorus in the formulation of diets for normal vertebral development during early ontogeny of rainbow trout. The dietary available phosphorus requirement is estimated to be approximately 1% diet for adequate bone mineralization of rainbow trout fry.

3. Effect of feeding vitamin A to rainbow trout broodstock on skeletal development of the offspring

The experiment was set up to assess the influence of vitamin A level in broodstock diets on rainbow trout fry development. Vitamin A or retinol is essential for reproduction but high levels appear to be detrimental for larval development of many fish species.

Experimental set-up

Three groups of 15 rainbow trout broodstock females and 5 males each, individually tagged, were reared for 6 months before spawning at water temperature of 7°C.

The impact of nutritional components on rainbow trout

They were fed practical diets A1, A2 and A3 supplemented with different levels of vitamin A supplied as retinyl acetate: 0, 20 and 200 IU/g diet (1 IU = 0.3 μ g retinol), leading to a dietary level of 20, 40 and 200 IU/g diet. The first two levels correspond to the common levels found in commercial feeds for broodstock.

Results

The best fecundity, early growth and potential of muscle development were noticed in the group fed the highest level of vitamin A (Figure 4). Eggs from this group contained higher levels of retinyl palmitate, a storage form of vitamin A. However, no difference of retinoic acid level, the active metabolite of vitamin A involved in morphogenesis and early development, was recorded between eggs obtained from the 3 different groups. Likewise, no external malformation of fry originating from the 3 groups (from swim-up stage to the end of the 3-month feeding trial) was detected, only a small delay in mineralization processes was recorded in rainbow trout fry originating from the group fed the highest level of vitamin A.

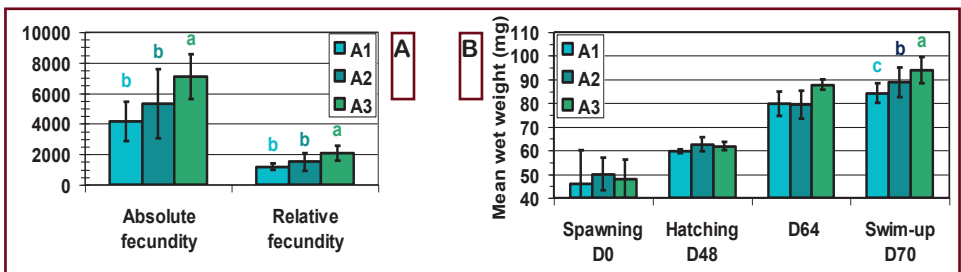


Figure 4. Fecundity of rainbow trout broodstock fed diets A1, A2 and A3 with different levels of vitamin A (A) and early growth of offspring from rainbow trout broodstock fed diets A1, A2 and A3 with different

Conclusion/Recommendations

For rainbow trout, quite high levels of vitamin A are recommended for broodstock nutrition (around 200 IU/g diet). The supplementation of 20 IU/g diet that is usually used in commercial diets for salmonids might not be enough to fulfill the vitamin A requirement of rainbow trout.

High dietary levels of vitamin A are beneficial for reproduction and early growth and no effect on skeletal development was noticed in comparison to other fish species. This difference might be due to the fact that the level of retinoic acid, the active metabolite of vitamin A, appears to be well controlled in eggs of rainbow trout.

4. Impact of dietary lipids on rainbow trout development

The experiment was set up to assess the impact of dietary lipids on rainbow trout development as previous studies in several fish species, especially in marine fish, have highlighted the influence of dietary lipids on the incidence and development of malformations. This study focused on the influence of the dietary phospholipid content and level of lipid peroxidation.

Experimental setup

Six semi-purified diets were tested on rainbow trout fry for 4 weeks at water temperature of 17°C at two different developmental stages: either from first-feeding onwards (trial 1) or 8 weeks after this stage (trial 2). Diets D1, D2, D3 and D4 contained 6% phospholipid supplied either as soybean lecithin or egg lecithin. Diets D5 and D6 were phospholipid-free and contained 6% soybean oil. Diets D2, D4 and D6 were supplemented with 12% oxidised fish oil whereas diets D1, D3 and D5 contained fresh fish oil.

Results

Dietary supplementation with oxidized lipid resulted in depressed growth in both early and late developmental stages of rainbow trout fry. In trial 1 with first-feeding fry, an improved growth was noticed in fish fed diets with phospholipids.

No external malformation was noticed. The skeletal development of fish was not affected by dietary oxidized lipid whereas a lower mineralization was noticed in rainbow trout fry fed diet D5 without phospholipid in trial 1 with swim-up fry.

Dietary control of antioxidant enzymes and vitamins was low in 4-week rainbow trout fry whereas increased activities of antioxidant enzymes and decreased vitamin E contents were noted in 12-week rainbow trout fry fed oxidized lipid compared to fish fed fresh lipid. This resulted in higher content of lipid peroxidation products in 4-week rainbow trout fed oxidized lipid compared to fish fed fresh lipid.

Conclusions

The results suggest that compared to late developmental stages, early stages are more susceptible to dietary oxidative stress, possibly due to lower response of endogenous antioxidant defense system.

This study has highlighted the importance of the control of lipid peroxidation in fish feeds for normal growth of rainbow trout fry. A correct supply of antioxidants (such as vitamin E or C) should be provided in fish feeds to protect polyunsaturated fatty acids from lipid peroxidation.

The results of this study have also highlighted the importance of dietary phospholipid supply for early growth and adequate skeletal mineralisation.