The influence of nutrition at the larval stages in marine fish

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1. Introduction

Marine fish larvae are very immature at hatching and undergo major developmental changes during the larval period. The most visible changes concern not only the morphology of the larvae, which acquire progressively the aspects of the juvenile stage, but also other crucial changes that occur at the tissular and cell levels (maturation of the digestive functions, onset of the immune system, settlement of metabolic pathways..). A description of most of these tissular and metabolic changes is reported in Zambonino-Infante et al. (2008).

Several parameters could influence these developmental processes and therefore could negatively affect the quality of the larvae with, in particular, the appearance of malformations. Fish deformities have the more detrimental effect on the consumers' image of aquaculture and, therefore, also on the market value of the juvenile fish.

Larval deformities are mostly induced at the hatchery stagge by several environmental factors (abiotic factors), diseases and some dietary components.

In the present work, we chose to investigate the potential influence of some dietary vitamins in the appearance of malformations. We evaluated the impact of the dose of the vitamin mix recommended by NRC93, and we more particularly studied three vitamins: Vitamin A (retinol acetate), vitamin D (1,25 dihydroxycalciferol), and vitamin C (ascobyl polyphosphate).

Two marine fish species were considered in the present work: European sea bass (*Dicentrarchus labrax*) and seabream (*Sparus aurata*). The recent development of an appropriate microparticulated compound diet (Cahu et al. 2003) allowed for more precise investigations of the influence of nutrients on fish larval morphogenesis than studies based on the use of live prey. Such inert feed was used in sea bass larvae experiments from mouth opening, with a total replacement of live prey, which resulted with good survival and growth. Seabream experiments were mainly based on the classical live prey feeding sequence (rotifers and Artemia). In order to better characterise malformations, sea bass and seabream juveniles were kept until they reached 2 g weight or 3 months age respectively..

2. Why dietary vitamins?

Marine fish larvae feeding sequence contained significant amounts of lipophilic vitamins like vitamin A and D, and hydrophilic vitamins like vitamin C. Besides their strict nutritional role, these vitamins can have physiological effects.

- Vitamin A is involved in night vision and is an antioxidant. It is associated with cell differentiation, and controls the expression of many genes involved in morphogenesis (it can be teratogenic)
- Vitamin D is an hormone that maintains calcium homeostasis and directly acts on bone cells.
- Vitamin C is hydrophilic, and is largely used in marine fish feeding sequence. It is an antioxidant, essential for collagen synthesis, and participates in several metabolic processes.

In consequence, we have considered essential to assess the impact of the dietary dose of these vitamin during the developmental process of marine fish larvae.

3. Effect of the dietary vitamin mix on larval morphogenesis

The vitamin mix used in larvae diets is 8 times more concentrated than the vitamin mix 762 (NRC 93 recommendations) established for juveniles. Is it appropriate?

Experimental set-up

Six replicated groups of sea bass larvae were reared at 20°C and fed, from day 6 until day 38 post-hatching, micro-particle diets incorporating 0.5%, 1.5%, 2.5%, 4 % (the standard level), 5% and 8% of the 2 times concentrated vitamin mix.

Results

The NRC standard vitamin mix incorporated into larvae feeds at 8 times the content recommended for juveniles gave the best results in terms of growth, survival, and also morphogenesis (Figure 1).

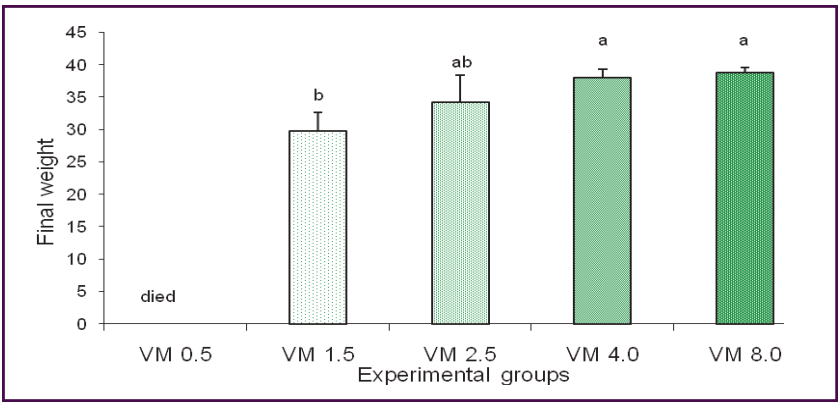

Figure 1 : Final weights of 38 days-old seabass larvae fed the different experimental diets containing graded levels of vitamin mix.

Conclusions

Our results showed that low dietary vitamin levels disrupted a temporal sequence of coordinated growth factor expression, involving different genes controlling the differentiation of osteoblasts ; part of the osteoblast is then converted into adipocytes and this led to the appearance of deformities. The results have been published (Mazurais et al. 2008).

Recommendations

The standard vitamin mix level induced a significant percentage of head and column deformities (Figure 2) showing the need to further refine the proportions of certain vitamins (particularly those known to be involved in bone and collagen synthesis, i.e. Vitamin A, D and C).

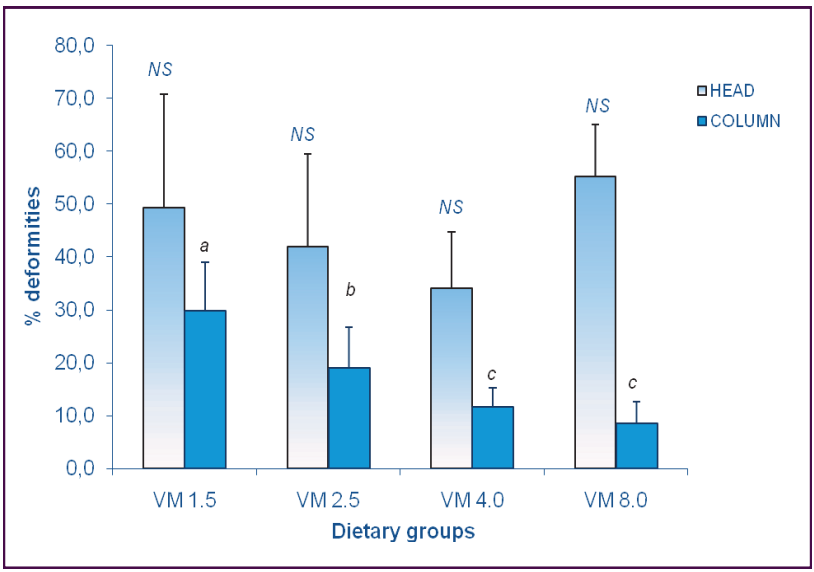

Figure 2 : Percentage of head and column deformities in 38 days-old sea bass larvae fed the different experimental diets containing graded levels of vitamin mix.

Avoid the incorporation of high HUFA levels in larvae diets (i.e. > 2g EPA+DHA/100g DW) during early stages to prevent the conversion of osteoblasts into adipocytes.

4. Effect of the dietary vitamin A on larval morphogenesis

Sea bass larvae:

Experimental set-up

Seven replicated groups of sea bass larvae were reared at 20°C and fed, from day 6 until day 42 post-hatching, microparticulated diets incorporating 0, 5, 10, 15, 25, 35 and 70 mg retinol acetate (RET)/kg of the diet and corresponding to 0, 16600, 33200, 50000, 83300, 116600, 233300 IU/ kg diet.

Results

The best growth and survival were observed in the larvae groups fed RET 10 and RET 15 diets, the other groups exhibiting a significant lowering in growth and survival. An analysis of malformations indicates that the optimum levels of dietary retinol for reduced deformities incidence depend on the malformation type considered; the structures that develop earlier are less affected by the low vitamin A levels. The best compromise in retinol acetate (vit A) level has to be comprised between 5 and 15 mg/ kg diet, which is lower to the recommended level of vitamin A.

The better results in ossification were also observed for vit A comprised between 5 and 15 mg/ kg diet; this dietary vit A levels also represented the better compromise for reduced deformities incidence. Our gene results strongly suggested that Vitamin A may regulate ossification/mineralisation processes and probably influences patterning of different cell types. These data have been published (Mazurais et al. 2009).

Seabream larvae:

Experimental set-up

3 groups of seabream larvae were fed with a classical live prey sequence, i.e. rotifers from day 4-19 and Artemia from day 20-34. Live preys were enriched or not with different levels of retinol. Group 1 received enriched rotifers, group 2 received enriched Artemia, group 3 received enriched rotifers and Artemia.

Results

High dietary vitamin A levels mainly affected the growth of seabream larvae at the earlier age, up to day 19, and induce a high percentage (50%) of cranial deformities (Figure 3).

At a later age, high dosage of vitamin A dramatically induced a high percentage of vertebral deformities (Figure 4).

Conclusions/recommendations:

Similar effects of the dietary levels of vitamin A were observed in sea bass and sea bream larvae. High dietary vitamin A levels strongly affect early larval development (before day 20); the effect is less marked after this stage. The dietary vitamin A level should be then changed during larval development in order to minimize the appearance of deformities: The optimal dose for the early developmental stage should be ~ 15 µg/g DW until day 20 post-hatching. A higher dose ~ 45 µg/g DW could be used afterwards.

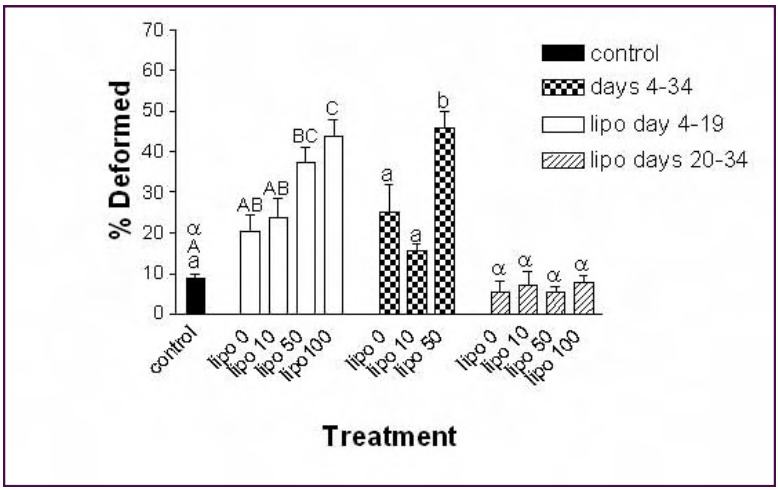

Figure 3 : Percentage of cranial deformations in 120 days-old seabream fed the different experimental feeding sequences during the larval period.

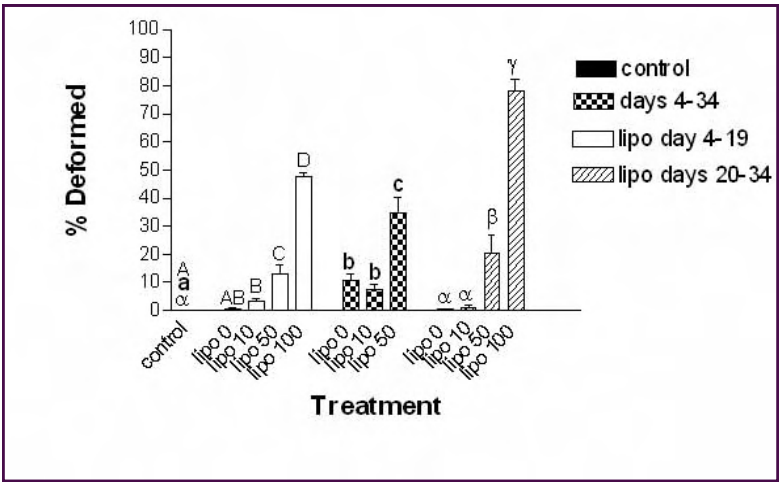

Figure 4 : Percentage of vertebral deformations in 120 days-old seabream fed the different experimental feeding sequences during the larval period.

5. Effects of the dietary vitamin D and C on larval morphogenesis

Experimental set-up

Four doses of dietary vitamin D: 11.2 (VD-0), 27.60 (VD-1), 42 (VD-2) and 120 (VD-3) IU VD_3 per gram of diet, and six doses of vitamin C: 0, 5, 15, 30, 50, 400 mg vit C/Kg diet were tested from day 6 until day 45 post-hatching.

Results

The lower dietary dose of vitamin D induced a poor mineralization, with many types of deformities (pugheadness, vertebral and caudal deformities). A disruption of intestinal calcium absorption was noted (lower expression of TRPV-6 gene). The 2 highest vitamin D doses induced a delay in the larvae mineralization process and the appearance of vertebral deformities (Figure 5).

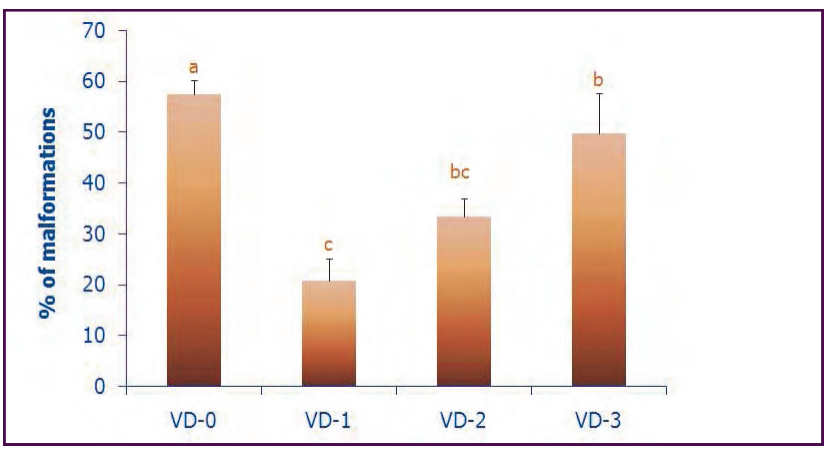

Figure 5: Percentage of deformities in 45 days-old seabass larvae the different experimental diets containing graded levels of vitamin D.

Larvae fed VIC0 and VIC5 did not survive after day 30 post-hatching. Dietary doses in vitamin C lower than 30mg/kg diet were associated with a poor mineralization in larvae, deformities in the head (pugheadness) and vertebral (one vertebra loss) areas. The highest dose of vitamin C also induced a poor mineralization and deformities in the head and vertebral (supernumerary vertebra) areas. The abnormalities observed with the low and high vitamin C levels were associated with a disruption of intestinal vitamin C transport (lower expression of SVCT-1 gene) and with a modulation of genes involved in lipid metabolism.

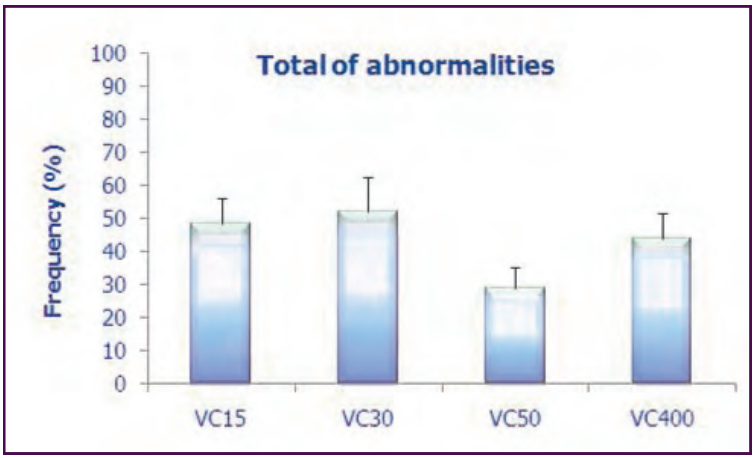

Figure 6: Percentage of deformities in 45 days-old seabass larvae the different experimental diets containing graded levels of vitamin C.

Conclusions

Low vitamin D levels in feeds could represent a risk for larval development; however this risk is unlikely considering the amount of vitamin D present in the live prey enrichments or in larval feed incorporating marine products.

The level of vitamin C in the feeds has to be accurately defined concurrently to the dietary lipids, in order to prevent a poor mineralization linked to a disruption of bone formation induced by the metabolism of lipids.

Cited Literature

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