EFFECT OF VITAMIN C INCORPORATION IN LIVE FOOD ON THE LARVICULTURE SUCCESS OF AQUACULTURE SPECIES

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ABSTRACT

A study was undertaken to upgrade the levels of vitamin C in Artemia and Brachionus through bicapsulation, and to investigate in this way the requirements for ascorbic acid (AA) during larviculture of fish and prawn. Ascorbyl palmitate (AP) was used as the vit C source because of its stable and lipophylic characteristics which allowed its incorporation in booster emulsions and its readily conversion into AA by Brachionus and Artemia. Under standard conditions very high levels of AA could be incorporated into the Artemia nauplii, supplementation of the enrichment emulsion with 20% AP (w/w) increased the AA-content up to 2000 ppm after 24 h enrichment. Also in Brachionus the AA-level changed in function of the concentration of AP applied. 20% AP in the diet enhanced the AA-content in the rotifers 10-fold over 3 days of culture.

Using three different enrichment levels in the live food (0%, 10% and 20%), no differences on growth nor survival could be observed for Macrobrachium rosenbergii under standard culture conditions. However, a significantly positive effect on the physiological condition of the postlarvae could be demonstrated when the vit C-boosted live food was administered.

For Claris gariepinus larvae fed vit C-boosted Artemia, supplemental dietary ascorbate resulted in a significantly positive effect on growth: the 20% AP group weighed 30% more than the control (0% AP) on the final day of the experiment. Evaluation of the physiological condition showed a significantly higher resistance of the larvae according to the dietary vit C level.

Dicentrarchus labrax larvae were successively fed rotifers (day 4-12) and enriched Artemia nauplii (day 13-46), supplemented with the same three vitamin C enrichment levels. No significant differences in production characteristics nor in stress resistance of the fish larvae could be observed, however, for all salinity stress tests the 20% AP group performed better.

Comparing the results for the two aquaculture fish species, with those reported earlier for the larvae of the prawn Macrobrachium, it appears that the requirements for vit C are species specific, and might even differ according to the culture conditions.

INTRODUCTION

One of the major improvements in the production of fish and shrimp larvae has been the optimization of the feeding strategy in function of the feeding behaviour and nutritional requirements of the predator larvae (Sorgeloos and Léger, 1992). Whereas in recent years lots of studies and methods were developed to determine nutritional requirements for fry and juveniles, studying larval needs is far more difficult. In this respect the development of a live food enrichment technique for the manipulation of the fatty acid profile in Artemia...
nauplii (Léger et al., 1987) has contributed considerably to the study of the needs for essential fatty acids (EFA) during the larval phases of fish and shrimp. Moreover, application of this bioencapsulation technique improved the commercial larviculture success of seabass and bream in the Mediterranean area, or of dolphinfish where an increase in survival rate at weaning from 0.1% to more than 10% could be achieved (Bhert et al., 1993). Whereas EFA requirements are now relatively well documented, more information still needs to be obtained on other important dietary components during the larval stages. Vitamin C, more especially ascorbic acid (AA), is in this respect considered as a potentially essential nutrient during larviculture. Several biological (e.g. prevention of skeletal deformities, growth, survival) and physiological (e.g. resistance to toxicants and stress, immunocompetence) functions might be enhanced in larval aquaculture species by supplemental dietary ascorbate. In this respect a further improvement of the nutritional quality of Artemia nauplii and rotifers via enrichment was investigated with regard to vitamin C supplementation. The boosting of live food with AA was then evaluated as a tool to deliver vitamin C to fish and shrimp larvae via the food chain.

VITAMIN C ENRICHMENT OF LIVE FOOD

Using the standard procedure for HUPA-enrichment (Léger et al., 1987) and experimental self-emulsifying concentrates supplemented with 10, 20 and 30% (w/w) of ascorbyl palmitate (AP), high concentrations of free ascorbic acid (AA) could be incorporated into the brine shrimp nauplii (Merhian et al., 1993a). As vitamin C source because of its stable and lipophilic characteristics which allowed its incorporation in booster emulsions. A 20% AP addition increases AA-levels up to 2003 ppm after 18 h enrichment at 27°C and 24 h after transferring the nauplii to seawater of 1500 ppm can be detected. 10% AP supplementation only slightly enhances the AA-content occurring in freshly-hatched nauplii (600 ppm), while 30% AP increases the AA-concentration in Artemia 6-fold. In both treatments HUPA levels remain equal to normal enrichment procedures. When the total amount of enrichment product (0.6 g/l) is split over three administrations instead of two, even higher levels of AA are incorporated into the Artemia, respectively 3.4 and 2.4 mg AA/g DW (Fig. 1). These concentrations do not drop when the 24 h-enriched nauplii are stored for 24 h in seawater of 28°C, respectively 4°C. A further increase is even noticed after 12 h storage, due to a continued assimilation of AA from AE remaining in the gut. AP is very fast converted into free and bioactive AA, and after the 24h enrichment period only a small fraction of non-assimilated AP (< 300 ppm) remains.

For the boosting of the vitamin C levels in the rotifer Brachionus plicatilis two sets of experiments have been carried out (Lavens et al., 1993) (Fig. 2). Direct incorporation only allows inclusion of maximum 0.55% AP into the artificial diet. Nevertheless, the AA level in the rotifers changes in function of the concentration of AP in the diet and stabilizes at 100% increase of AA when fed the highest level of AP for three culture days. The indirect incorporation method allows inclusion of much higher levels of AP in the diet and, consequently, much higher levels up to 2000 ppm AA are assimilated in the rotifers. Applying the extra boosting method for 6h at the end of the culture period also increases the AA content but to a far lesser extent. Storage of the AA-enriched rotifers in fresh seawater (25°C) during 24h has no effect on the AA content of the rotifers.
**Fig. 1:** Ascorbic acid content during enrichment of Artemia nauplii

**Fig. 2:** Combined culture and enrichment of Brachionus plicatilis
This indicates that the rotifers maintain their nutritional value when fed to the larval fish tanks.

Elevated levels of a bio-active vitamin C source can thus be transferred through the live food chain towards the fish or shrimp larvae, in this way providing an important tool to build up stress and disease resistance during larviculture.

EVALUATION OF ELEVATED LEVELS OF DIETARY VITAMIN C IN ENRICHED LIVE FOOD

The effect of the high levels of vitamin C in the live food has been verified for the giant freshwater prawn, Macrobrachium rosenbergii, and two fish species, the African catfish, Clarias gariepinus, and the European seabass, Dicentrarchus labrax, using three different enrichment levels in the live food (0%, 10% and 20%).

For M. rosenbergii (Merchie et al., 1993b), no differences on growth or survival can be observed under standard culture conditions (Fig. 3.). However, a significantly positive effect on the physiological condition of the postlarvae, measured by means of a salinity stress test (Romdhane et al., 1993), is demonstrated when the vitamin C-boosted live food is administered. Since the extra vitamin C has been incorporated in the predator larvae as AA (>500 ppm for the 20% AP-diet), it may be assumed that a positive influence on stress resistance is caused by feeding vitamin C-enriched live food. Moreover, a significant drop in AA-concentration is detected in the postlarvae as compared to the levels found in the larvae. This may reflect a need for vitamin C during metamorphosis, a stress sensitive period. It is expected that under suboptimal conditions (e.g., stress situations), supplementation of high vitamin C levels might also enhance production characteristics.

Seabass larvae were successively fed enriched rotifers (day 4-12) and Artemia nauplii (day 13-46), supplemented with the same three vitamin C enrichment levels (Merchie et al., 1993a). No significant differences in production characteristics nor in stress resistance can be observed, however, for all salinity stress tests (Dhert et al., 1992) the 20% AP group performs best (Fig. 4). AA is well incorporated into the predator larvae from the Brachionus feeding onwards.

For larvae of the African catfish fed vit C-boosted Artemia, supplemental dietary ascorbaxe resulted in a significantly positive effect on growth: in an initial feeding trial (Merchie et al., 1993a) the 20% AP group weighed 30% more than the control (0% AP) on the final day of the experiment, respectively 9.5 and 6.3 mg DW. Evaluation of the physiological condition by means of salinity tests demonstrated a significantly higher resistance of the larvae according to the dietary vitamin C level. AA was incorporated into the fish body tissue (data not shown). As for all three treatments the larval growth was relatively low - C. gariepinus larvae can reach 15 mg DW in a 10 days feeding period (Verret et al., 1987, 1992) - it was verified if extra vitamin C in the diet really promotes growth or only slows down growth retardation under suboptimal conditions. Therefore six treatments were designed (Merchie et al., 1993b): three treatments (Artemia-series) received only enriched Artemia nauplii (0, 10 and 20% AP, respectively), the other 3 treatments (co-feeding-series) were fed the same Artemia diets but which were partially
Effect of Vit C enrichment III Artemia nauplii on the larviculture success of Macrobrachium rosenbergii (until day 28)

<table>
<thead>
<tr>
<th></th>
<th>First Experiment</th>
<th>Control</th>
<th>10% AP</th>
<th>Control</th>
<th>20% AP</th>
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<tbody>
<tr>
<td>Survival (%)</td>
<td>72.1</td>
<td>48.4</td>
<td></td>
<td>57.5</td>
<td>57.1</td>
</tr>
<tr>
<td>Ind. Length (mm)</td>
<td>9.31</td>
<td>9.34</td>
<td></td>
<td>9.68</td>
<td>9.73</td>
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<tr>
<td>Ind. Weight (µg)</td>
<td>83.1</td>
<td>88.8</td>
<td></td>
<td>115.0</td>
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<td>Metamorphosis (%)</td>
<td>12.7</td>
<td>16.2</td>
<td></td>
<td>49.6</td>
<td>53.3</td>
</tr>
<tr>
<td>Stress Resistance (SS)</td>
<td>8.7</td>
<td>32.7</td>
<td></td>
<td>80</td>
<td>62</td>
</tr>
<tr>
<td>AA in Larvae (µg/g DW)</td>
<td>365</td>
<td>552</td>
<td></td>
<td>352</td>
<td>448</td>
</tr>
<tr>
<td>AA in Postlarvae (µg/g DW)</td>
<td>388</td>
<td>328</td>
<td></td>
<td>255</td>
<td>388</td>
</tr>
</tbody>
</table>

Fig. 3: Effect of vitamin C-enriched Artemia on prawn larvae

Stress resistance of seabass larvae
Cumulative mortality at 60 ppt (day 27)

Fig. 4: Stress resistance of Dicentrarchus labrax larvae fed different vitamin C levels

AP = ascorbyl palmitate
(20:80) substituted by an artificial diet containing no vitamin C (Coutteau et al., 1993). From day 6 onwards the 20% AP group shows a significantly better growth compared to the 0% and 10% AP treatments (Artemia-satellite) [Fig. 5]. For the co-feeding series the same positive, however, not significant, influence of the extra vitamin C administration on the dry weight is found. Moreover, the animals receiving the highest vitamin C supplementation display a considerable lower stress sensitivity than the ones of the 0% and the 10% AP group (Fig. 6). These differences can already be observed on day 2, which might indicate the importance of AA for early development. Fig. 5 illustrates the incorporation into the body tissue of the extra vitamin C delivered by the live food. For the 0 and 10% AP groups in the co-feeding-series the dietary vitamin C is not sufficient to maintain the initial AA-concentration (280 μg AA/g DW). A high correlation is found between the the AA-content in the catfish larvae and their growth: r² = 0.985. Although growth numbers are comparable to other results (Verreth et al., 1987, 1992), growth is still affected by a fungal infection, coupled with suboptimal enrichment of Artemia during the first days of the experiment.

In a third experiment performed in optimal culture conditions, the same trend of increasing growth according to the dietary ascorbate level is shown, although not statistically significant. Non-significant differences in stress resistance can be noticed either; however, for all salinity stress tests the 20% AP group performed best. Higher levels of AA are incorporated into the catfish larvae compared to the previous experiment (data not shown), due to optimal enrichment and thus a higher AA-accumulation in the live food diet (Table 1). These higher AA-concentrations in the larvae (saturation of requirements for growth ?) and the optimal culture conditions might explain the smaller differences in growth.

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**Fig. 5:** Growth and AA-incorporation in the African catfish fed different vitamin C levels
### Stress resistance (% mortality 1h 15ppt) for *Clarias gariepinus* (Artemia series, day 2)

<table>
<thead>
<tr>
<th></th>
<th>0% AP</th>
<th>10% AP</th>
<th>20% AP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td><img src="image" alt="Diagram" /></td>
<td><img src="image" alt="Diagram" /></td>
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</tr>
</tbody>
</table>

**Fig. 6:** Stress resistance of *Clarias gariepinus* larvae fed different vitamin C levels

### Table 1: AA-incorporation (μg/g DW) into the live food diet and final survival (%) of *Clarias gariepinus* larvae (day 10)

<table>
<thead>
<tr>
<th></th>
<th>0co</th>
<th>10co</th>
<th>20co</th>
<th>0*</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C content in Artemia diet (μg AA/g DW) First experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival (%)</td>
<td>73a</td>
<td>69a</td>
<td>70a</td>
<td>74a</td>
<td>72a</td>
<td>75a</td>
</tr>
<tr>
<td>Vitamin C content in Artemia diet (μg AA/g DW) Second experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival (%)</td>
<td>71a</td>
<td>74a</td>
<td>72a</td>
<td>71a</td>
<td>71a</td>
<td>76a</td>
</tr>
</tbody>
</table>

* means with common superscript are not significantly different (P<0.05)

x 0, 10, 20: diet of Artemia nauplii enriched with 0%, 10% en 20% AP, respectively;
y 0co, 10co, 20co: co-feeding of Artemia nauplii enriched with 0%, 10% en 20% AP, respectively with an artificial diet in a ratio 20:80
CONCLUSIONS

1. These experiments prove that vitamin C is indeed an important nutritional factor in larviculture of fish and prawn. In the case of Clarias the vitamin C requirements appear even to be much higher for larval fish than the values reported for juveniles and ongrowing fish - e.g., a need for 15, 15 and 50 ppm AA has been proved to be sufficient for normal growth in channel catfish (Mustin and Lovell, 1992), seabass (Boonyaratpalin et al., 1992) and red seabream (Kosutarak et al., 1992), respectively -, which might be due to the higher rates of growth and metabolism in the larvae. Johnston et al. (1989) demonstrated that fish take several weeks to increase their tissue ascorbate concentrations when they are fed a diet containing more than 100 mg vitamin C/kg. Therefore, when hatchery fish are fed diets low in vitamin C for extended periods, the ascorbate levels in tissues will quickly decrease. Moreover, extra effects of AA-supplementation at high levels on stress resistance are shown, which might be of importance under suboptimal rearing-conditions in commercial hatcheries (handling, transportation, disease outbreaks).

2. Comparing the results, it appears that the requirements for vitamin C are species specific, and might even differ according to the culture conditions.

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Effect of Dietary Supplementation of Vitamin C and Seeds of Achyranthes aspera on Growth, Digestive Enzyme Activities, Immune System and Lipid Peroxidation of Snow Trout Schizothorax richardsonii. Moses Rinchui Ngasainao1, Kjell J Nilssen2 and Rina Chakrabarti3.* Incorporation of plant ingredients in diets enhanced the survival rate, growth and disease resistance in carps Catla catla, catla [21], Labeo rohita, rohu [22] and Cyprinus carpio, common carp [23]. Most of the growth performance studies in snow trout have been conducted with grow-out stage [24] [25] [26] [27] and brooders [28].