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Live food mediated vitamin C transfer to *Dicentrarchus labrax* and *Clarias gariepinus*

By G. MERCHIE[1], P. LAVENS[1], Ph. DHERT[1], R. PECTOR[2], A.F. MAI SONI[1], H. NELIS[3], F. OLLEVIER[2], A. DE LEENHEER[4] and P. SORGELOOS[1]

[1]*Laboratory of Aquaculture & Artemia Reference Centre, University of Gent, Rozier 44, B-9000 Gent, Belgium;* [2]*Laboratory of Ecology and Aquaculture, Catholic University of Leuven, Naamsestraat 59, B-3000 Leuven, Belgium;* [3]*Laboratory of Pharmaceutical Microbiology, University of Gent, Harelbekestraat 72, B-9000 Gent, Belgium;* [4]*Laboratory of Medical Biochemistry and Clinical Analysis, University of Gent, Harelbekestraat 72, B-9000 Gent, Belgium*

Summary

Live food enrichment techniques, using formulated diets and emulsions for improving the nutritional quality of *Brachionus* and *Artemia*, were studied as a tool for transferring ascorbic acid (AA) to fish larvae.

Artemia nauplii enriched for 24 h with an experimental emulsion containing 20% HUFA and 0%, 10% and 20% ascorbyl palmitate (AP) were administered to catfish larvae in a 20-day feeding trial. Survival was not affected by the dietary AA, but from day 7 onwards a significantly positive effect of supplemental AA on growth was demonstrated. At the end of the experiment the 20% AP group weighed 30% more than the control (0% AP), i.e. 9.5 and 6.3 mg DW, respectively. Evaluation of the physiological condition was demonstrated by salinity tests. In all three treatments larval growth was relatively low, and it still has to be verified if extra vitamin C in the diet really promotes growth. Seabass larvae fed on AP-enriched rotifers (days 4–12) and *Artemia* nauplii (days 13–46) showed no significant differences in production characteristics nor in stress resistance, however, for all salinity stress tests the 20% AP group performed better. AA was well incorporated into the predator larvae from the *Brachionus* feeding onwards.

Introduction

Few nutrition studies have been devoted to larval stages, mainly because of the complexity of the rearing process, the need of large numbers of animals for evaluation, and the dependency on live food as the diet source instead of formulated feeds. The development of enrichment techniques to enhance the fatty acid profile in *Artemia* nauplii (LÉGER et al. 1987) enabled the study on essential fatty acid needs during larval development. Whereas these requirements are now well documented, more information is needed on other important larval dietary components, including vitamin C, or more specifically ascorbic acid (AA). Several biological (prevention of skeletal deformities, growth, survival) and physiological (resistance to toxicants and stress, immunoactivity) functions might be enhanced in larval aquaculture species by supplemental dietary ascorbate (DABROWSKI 1992).

High levels of essential fatty acids can be incorporated in *Artemia* nauplii (LÉGER et al. 1987). Enriched nauplii resulted in significantly better survival and growth for several aquaculture species. Similar enrichment techniques have been developed to enhance the levels of HUFA in rotifers (LAVENS et al. 1992). Vitamin C supplementation was studied as a further improvement of the nutritional quality of *Artemia* nauplii and rotifers (e.g. *Macrobrachium rosenbergii* feeding, MERCHIE et al. 1993).

In this study live food enrichment was further investigated as a tool to deliver vitamin C to fish larvae via the food chain, a method that had been verified for the African catfish

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(*Clarias gariepinus*) and for European seabass (*Dicentrarchus labrax*), using *Brachionus* and *Artemia*.

Materials and methods

The nutritional quality of *Artemia* and *Brachionus*, with respect to AA, was modified using three different enrichment emulsions, respectively, artificial diets.

Clarias gariepinus trials (Experiment 1)

The freshwater species *Clarias gariepinus* was reared in aquaria (14 l) in continuous flow-through, at 27 ± 1 °C. Larvae, obtained by artificial reproduction, were kept in an incubation tank and were fed Great Salt Lake (GSL) instar I *Artemia* nauplii three times a day at 25% of their body weight (w/w). Five days after yolk sack resorption the aquaria were stocked with 350 larvae each, and a diet of enriched live food was administered. Three treatments (four replicates each) were employed where *Artemia* nauplii were delivered, enriched through an experimental emulsion containing 20% HUFA (E20) and 0%, 10% and 20% ascorbyl palmitate (AP) as a source of vitamin C. As proven by MERCHIE et al. (1993) the latter stable derivative of AA is quickly converted by the *Artemia* nauplii into the free and bioactive AA: after 24 h of enrichment the levels of free AA in the nauplii were roughly 600, 1200 and 2400 p.p.m., respectively, while only a minor amount of unconverted AP is still present in the latter two groups. The catfish larvae were fed four times a day at the same feeding level, according to the procedure of VERRETH and DEN BIEMAN (1987). The experiment was terminated after 20 days of feeding enriched *Artemia*.

Criteria used for evaluating the effects of extra dietary ascorbate in live food on catfish fry were production characteristics (survival and dry weight), stress resistance, and incorporation of AA into body tissue. Stress resistance of the larvae was assessed via a salinity test in which the physiological response capacity of the animals to osmotic stress determines their survival with time. Four times 10 larvae per treatment were exposed to a salinity of 25 ppt. The percentage mortality was calculated after 1 h of salinity shock. At regular intervals samples of both the enriched *Artemia* nauplii and the predator larvae were taken for determination of the levels of free AA.

Dicentrarchus labrax trials (Experiment 2)

Eggs of the seabass *Dicentrarchus labrax* were obtained from the commercial hatchery SEPIA (Gravellines, France), hatched at 16 °C. Larvae were cultured in conical tanks (60 l), connected to a recirculation system (DHERT et al. 1991). The fry were reared at a density of 30 larvae/l, in 35 ppt seawater of 19 ± 1 °C and were successively fed *Brachionus* (days 4–12) and enriched *Artemia* nauplii (days 13–46). Rotifers were cultured on an composed diet (Culture Selco, Artemia Systems N.V., Belgium) supplemented with 0%, 10% and 20% AP, respectively. These enrichment levels resulted in AA-values in the rotifers of 150, 900 and 1200 p.p.m., respectively, after three days of culture (LAVENS et al. 1992). *Artemia* nauplii (GSL) were enriched for 24 h with an experimental emulsion containing 50% HUFA (E50) in which, respectively, 0%, 10% and 20% AP was included (cfr. Experiment 1). Each treatment consisted of three replicates. The fish larvae were fed for 35 days until weaning.

The diets were evaluated on the basis of production characteristics, stress resistance and ascorbic acid-incorporation. Susceptibility of the seabass fry to stress was also assessed in a salinity test. The animals were immersed for 1 h in 60 ppt seawater and their survival monitored at constant time intervals until no mortalities were recorded. The cumulative

Table 1. **Final survival [%], dry weight [DW; mgl, stress resistance [% mortality after 1 h at 25 ppt] and AA-incorporation [μg AA/g DW] fir *Clarias gariepinus* larvae (day 20) fed with diets containing 20% HUFA (=E20) and three levels of ascrobyl palmitate (AP)**

	E20/0 % AP	E20/10 % AP	E20/20 %AP
Vitamin C content in *Artemia* diet [μg AA/g DW]	555[a]	1378[a,b]	2255[b]
Survival [%]	94.9[a]	95.6[a]	95.5[a]
Dry weight [mg]	6.30[a]	7.13[a]	9.38[b]
Stress resistance [% mortality after 1 h 25 ppt]	40.0[a]	17.5[b]	2.5[c]
AA-incorporation [μg AA/g DW] (day 15)	469[a]	543[b]	547[b]

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

stress sensitivity index (CSI) reflects the resistance of the larvae. It is calculated average of replicate treatments, obtained by addition of the cumulative mortalities in consequent observation intervals (DHERT et al. 1992).

Analysis of AA in rotifers, enriched *Artemia* nauplii and in fish larvae was performed by the HPLC-technique as described by NELIS et al. (1993).

Statistical methods include means and significance tests using one way analysis of variance (Tukey).

Results and discussion

Clarias gariepinus

Table 1 and Fig. 1 summarize the results on survival, growth, stress resistance and AA incorporation for the catfish larvae at the end of the experiment.

Boosting of the *Artemia* diet with vitamin C resulted in high levels of free ascorbic acid

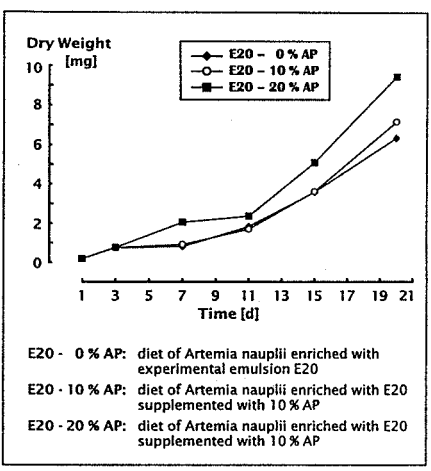

Fig. 1. Growth of *Clarias gariepinus* larvae fed three dietary levels of vitamin C

content in live food (Table 1). The content of vitamin C in the nauplii was comparable to the values obtained during standardization of the enrichment procedure and in the previous feeding experiments with the prawn larvae of *M. rosenbergii* (MERCHIE et al. 1993).

Survival was high (± 95%) and independent of the dietary vitamin C level (Table 1). However, from day 7 onwards a significantly positive effect on dry weight of the highly supplemented AA diet (20% AP) was demonstrated when compared to 0% and 10% AP feeds (Fig. 1).

At the end of the experiment, fish fry of the 20% AP group weighed 30% (9.4 mg DW) more than control specimens (0% AP, 6.3 mg DW) (Table 1). In all three treatments larval growth was relatively low (*C. gariepinus* larvae can reach 15 mg DW in 10 day feeding; VERRETH et al. 1987, 1992). There is still a need to verify if extra vitamin C in the diet does promote growth. It should be noted that previous trials with *Macrobrachium rosenbergii* showed that under optimal culture conditions high AA levels caused no effects. However, it may be expected that when conditions turn suboptimal, supplementation with high ascorbate concentrations may enhance production characteristics (MERCHIE et al. 1993).

A significant correlation was demonstrated between the dietary vitamin C level, AA-incorporation and stress resistance of the larvae. The results of the salinity stress test at day 13 (55%, 35% and 23% mortality for 0%, 10% and 20% AP in the diet) were confirmed at day 20 (Table 1). In *Macrobrachium rosenbergii*, the larval quality of the 20% AP-supplemented larvae was also remarkably higher than for the control group.

Dicentrarchus labrax

Production characteristics, stress resistance of seabass larvae in response to dietary AA content are presented in Tables 2, 3 and 4.

The data show no significant differences in the survival, dry weight and length (Table 2) as well as in stress resistance, however, for all salinity stress tests the 20% AP group performed best (Table 3).

AA concentrations obtained in the enriched *Artemia* nauplii were comparable to these mentioned in Experiment 1 (Tables 1 and 4). In continuous cultures of rotifers higher values of AA were observed than in a batch culture. After 3 days an AA content in *Brachionus* of about 900 and 1200 p.p.m. was reached in the 10% and 20% AP groups (LAVENS et al. 1992), whereas in this experiment values of about 1400 and 3600 p.p.m. were noticed.

AA was incorporated into the predatory larvae when feeding both *Brachionus* and

Table 2. **Survival [%], dry weight [mg] and length [mm] of *Dicentrarchus labrax* larvae fed live diets containing various levels of ascrobyl palmitate (AP) enrichments and a high level of HUFA (E50)**

Day 20	E50/0 % AP	E50/10 % AP	E50/20 % AP
Survival [%]	77.2[a]*	72.6[a]	75.8[a]
Dry weight [[mg]	0.54[a]	0.57[a]	0.53[a]
Length [mm]	8.63[a]	8.91[a]	8.89[a]
Day 35	**E50/0 % AP**		**E50/20 % AP**
Survival [%]	75.6[a]		3.8[a]
Dry weight [mg]	2.52[a]		2.64[a]
Length [mm]	15.61[a]		15.53[a]

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

Table 3. Cumulative stress sensitivity index (salinity challenge test) for *Dicentrarchus labrax* larvae of different ages fed live diets containing various levels of ascrobyl palmitate (AP)

	E50/0 % AP	E50/10 % AP	E50/20 % AP
Day 18	65[a]*	62[a]	52[a]
Day 21	120[a]	113[a]	105[a]
Day 27	135[a]	n.d.	89[b]
Day 35	110[a]	n.d.	84[a]

* means with common superscript are not significantly different ($P < 0.05$); n.d.: not determined

Table 4. Ascorbic acid content [μg AA/g DW] in seabass larvae fed different ascrobyl palmitate (AP) levels

	E50/0 % AP	E50/10 % AP	E50/20 % AP
Vitamin C content in *Brachionus* [μg AA/g DW]	154	1393	3319
Vitamin C content in *Artemia* [μg AA/g DW]	581	1427	3581
Day 12 (end of rotifer phase)	346[a]*	1034[b]	1244[b]
Day 21	756[a]	1603[b]	1624[b]
Day 35 (end of experiment)	610[a]	n.d.	1185[b]

* means with common superscript are not significantly different ($P < 0.05$); n.d. not determined

Artemia (10% and 20% AP groups compared to control group), although there was no difference in AA content between the 10% and the 20% treatments (Table 4). The incorporation of AA into the fish larvae proves that the transfer of different vitamin C levels via live food is attainable.

Conclusions

These experiments prove the importance of vitamin C as nutritional factor in larval fish-culture. In the case of *Clarias*, the vitamin C requirements appear to be much higher for larval fish than for juveniles and ongrowing fish, e.g. a need for 15, 15 and 50 p.p.m. AA proved sufficient for normal growth in channel catfish (MUSTIN and LOVELL 1992), seabass (BOONYARATPALIN et al. 1992) and red seabream (KOSUTARAK et al. 1992). Moreover, the effect of AA-supplementation at high levels on stress resistance (in terms of salinity challenge) are shown. Such resistance might be of importance under suboptimal rearing conditions in commercial hatcheries if it can be proven that similar effects occur when the stressors are handling, transportation or disease. Several deformities have recently been observed in Mediterranean seabass and seabream hatcheries which are not related to an EFA deficiency or non-inflation of the swimbladder, but generally claimed to be a vitamin C deficiency during the startfeeding period.

The results of this study show that the requirements for vitamin C are species specific, and might differ with culture conditions.

The enrichment procedure used to manipulate the levels of different nutrients in live food

has proven to be a valuable technique for improving larval culture techniques. However, in the case of AA, the endogeneous content in *Brachionus* and *Artemia* of ± 180 and 500 p.p.m., respectively, impairs the determination of the exact requirements when they are lower than these initial levels (e.g. in seabass). The effects of vitamin C supplement on salinity resistance of early stages shows promising results and further studies should examine if other stress effects would be similarly mitigated.

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