The effect of supplemental ascorbic acid in enriched live food for *Clarias gariepinus* larvae at startfeeding

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Abstract

The effect of three dietary ascorbic acid (AA) concentrations, each applied via two feed types, on production characteristics and physiological condition of African catfish (*Clarias gariepinus*) larvae has been assessed in two 10-day culture trials. Three treatments received only *Artemia* nauplii enriched with an experimental emulsion containing 0, 10, or 20% ascorbyl palmitate (AP) and yielding 530, 1200 and 1600 μg AA g⁻¹ DW *Artemia*, respectively; the other three treatments were fed the same *Artemia* diets which were partially substituted by an artificial diet containing no vitamin C (ratio 20:80). No differences in survival could be observed; however, from day 6 onwards the 20%-AP group showed significantly better growth compared to the 0%- and 10%-AP treatments. For the cofeeding series, the same positive, but not significant, influence of vitamin C on dry weight was found. Moreover, the animals receiving the highest vitamin C supplementation displayed a considerably lower stress sensitivity than those of the 0%- and the
10%-AP groups, for both the 100%- and the 20%-Artemia series. These differences had occurred by day 2, which might be indicative of the importance of AA in early development.

A second trial, which was a repetition of the first one, revealed the same tendencies; however, growth differences were smaller, probably due to the higher incorporation levels of AA obtained in the live diet (530, 1700 and 2300 µg AA g⁻¹ DW) and in the catfish larvae.

Growth results of both experiments were supported with data from the ultrastructural evaluation of the hepatocytes; i.e. a more organized cell compartmentation and better-structured cell organelles in the 20%-AP group of the Artemia series compared to the control are indicative of a more active metabolism. The slow growth in the cofeeding series was documented by the poor condition of the hepatocytes.

In a third experiment it was verified that the growth effect of the 20%-AP boosted Artemia diet was the result of the extra AA incorporation and not of the concomitant palmitic acid (PA), which was set free after hydrolysis of AP in the Artemia nauplii, and which could possibly be used as a supplemental energy source. The three treatments were fed Artemia nauplii enriched with 0% AP, 12% PA and 20% AP, respectively. Growth and stress resistance of the latter group were significantly higher compared to the control and the PA-supplemented fish.

To our knowledge this is the first evidence for the positive role of high dietary vitamin C levels (more than 1500 µg AA g⁻¹ DW) on larval development of an aquaculture species, and more specifically of C. gariepinus. © 1997 Elsevier Science B.V.

**Keywords:** Live food; Larvae nutrition; Ascorbic acid; Clarias gariepinus; Artemia

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**1. Introduction**

One of the major improvements in the production of fish and shrimp larvae has been the optimization of the feeding strategy to function in accordance with the feeding behaviour and nutritional requirements of the predator larvae (Sorgeoloos and Léger, 1992). Whereas in recent years several methods have been developed to determine nutritional requirements for juveniles, the study of the larval needs appeared to be far more difficult. Dependency on live food as the dietary source is a major constraint in larval nutritional research. The development of a live food enrichment technique for the manipulation of the fatty acid profile in Artemia and rotifers (Léger et al., 1987) contributed to the detection of essential fatty acid requirements during hatchery production. However, larval requirements for other dietary components are still insufficiently documented. Ascorbic acid (AA) is considered a potential essential nutrient during the larval stages (Dabrowski, 1992). In order to better assess the needs for this nutrient during larviculture, the AA content has been determined in the various live foods currently being used in aquaculture (i.e. microalgae, rotifers and Artemia; Merchie et al., 1995a). A further improvement of the nutritional quality of Brachionus (Lavens et al., 1994) and Artemia (Merchie et al., 1995a) via vitamin C enrichment was investigated. Recently, it has been demonstrated for Dicentrarchus labrax and Macrobrachium rosenbergii larvae that the delivery of high AA levels through incorporation of ascorbyl palmitate (AP) into the live food resulted in a pronounced effect on stress resistance and correlated with a high level of the vitamin in the body tissue (Merchie et al., 1995a,b). However, since the requirements for survival and growth were lower than

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**2. Materials and methods**

**2.1. Set-up**

In experiments 1 and 2 (performed at the Department of Fish Culture and Fisheries of the Wageningen Agricultural University, The Netherlands), C. gariepinus fry were cultured in aquaria (17 l) connected to a recirculation system including a sedimentation tank, a 100-l biological filter (lava rock) and an oxygenation tower. The flow rate was about 1 l min⁻¹ and the temperature was maintained at 27.5 ± 0.5°C. The larvae were obtained by artificial reproduction of laboratory broodstock (Hogendoorn and Vismans, 1980) and kept for 2 days in a stock tank. On the final day of yolk absorption, each aquarium was stocked with 600 larvae (35 larvae l⁻¹); each treatment had three replicates. Feeding started the next morning when all yolk was absorbed. In the third experiment (performed at the Laboratory for Ecology and Aquaculture, Catholic University of Leuven, Belgium), African catfish fry were reared in aquaria (14 l) in a continuous flow-through system at 27 ± 1°C. Each treatment consisted of four replicates. Other culture conditions were identical to those in the first two experiments.

**2.2. Feeding**

Daily, Artemia franciscana nauplii (origin: Great Salt Lake, UT, USA) were hatched, consequently bioencapsulated using experimental emulsions (prepared by INVE Aquaculture, Baasrode, Belgium) and cold stored between feedings according to standard...
procedures (Dhont et al., 1993). The boosting emulsions contained 20% highly unsaturated fatty acids and various levels (0, 10 or 20%) of ascorbyl palmitate (AP; Roche, Basel, Switzerland) or 12% palmitic acid (PA; Sigma, Belgium), respectively. The level of PA incorporated in the latter emulsion corresponded to the amount of palmitate present in the 20%-AP-enriched emulsion. In the cofeeding series the Artemia diet was partially (20:80) substituted by an experimental pellet, formulated using semi-purified ingredients (Couteau et al., 1995) but no vitamin C.

Feeding was done five times a day at 3-h intervals between 10:00 and 22:00 h. After the food was administered to the aquaria, the water supply was shut down for 10–15 min to allow the larvae to feed undisturbed. Every day a newly enriched batch of Artemia nauplii was administered. Feeding occurred at the same feeding level (a near satiation level, using a b-value of 0.35) determined according to the procedure of Verreth and den Bieman (1987).

2.3. Evaluation

Survival was estimated by counting the live larvae at the end of the experiment (day 10), taking into account the larvae sampled during the test. Growth results were analysed by a single regression analysis (regression coefficient b) of larval weight (y=bx) on culture period (days) (Hogendoorn, 1980). The physiological condition of the larvae was assessed by means of 15-ppt and 25-ppt salinity tests (Dhert et al., 1992) on day 2 and day 8, respectively. Additional information was obtained from the ultrastructure of the liver cells (Storch et al., 1983, 1984; Verreth et al., 1987). The observations of this electron-microscopic analysis were evaluated according to the following criteria: cell compartmentalization, size and structure of mitochondria, size and distribution of the rough endoplasmic reticulum (rER), and the quantity of glycogen and fat reserves.

A paired-ion, reversed-phase, high-performance liquid chromatography (HPLC) procedure coupled with electrochemical detection and internal standard quantitation based on isoascorbic acid was used for the determination of AA in Artemia and fish larvae. The HPLC apparatus consisted of a Varian 8500 pump (Varian Associates, Palo Alto, CA, USA), a N60 valve injector fitted with a 20-µl loop (Valco, Houston, TX, USA), and a Coulochem 5100A electrochemical detector (ESA, Bedford, MA, USA) equipped with a Model 5010 or 5011 analytical cell. The HPLC column (15 cm x 0.46 cm), packed with 5 µm Hypersil ODS (Shandon, Runcorn, UK) and equipped with a 5 cm x 0.3 cm Chromguard RP guard column (Chrompack, Middleburg, The Netherlands), was eluted with an aqueous buffer (40 mM sodium acetate, 0.54 mM Na2EDTA, 1.5 mM dodecyltrimethylammonium phosphate adjusted to pH 4.75 with glacial acetic acid). The flow rate was 0.8 ml min⁻¹. Settings of the electrochemical detector were as follows: detector 1, +0.05 V; detector 2, +0.5 V; guard cell, +0.95 V. Extraction was done following a modification of the method of Kutnik et al. (1987). AA levels are expressed per gram dry weight (DW).

One-way analysis of variance (ANOVA) combined with Tukey's test was employed to evaluate statistical differences between treatment means. Data were controlled for normality and homoscedasticity of variances.

3. Results and discussion

3.1. AA incorporation

Analytical data for the three experiments are summarized in Tables 1–3. During the first experiment, values of AA enrichment into the Artemia nauplii were lower than the levels reported earlier for a 10%- and 20%-AP addition (1300 and 2700 µg AA g⁻¹ DW, respectively; Merchie et al., 1995a), probably due to water quality problems during the first days of the experiment. In the second and third tests, AA levels were higher (1700 and 2300 µg AA g⁻¹ DW, respectively) and comparable to previously reported data.

The AA content in the fish larvae reflected the dietary AA administration for all experiments: considering both cofeeding and Artemia series in experiments 1 and 2, a linear correlation coefficient of 0.961 was found between incorporated and dietary AA. The initial AA level in the last test (460 µg AA g⁻¹ DW) was twice the value found in the fry (230–280 µg AA g⁻¹ DW) used for both first trials. In this respect, data of Lavens and Sorgeloos (1991) and Watanabe and Miki (1993) indicate that the biochemical composition of eggs and freshly hatched larvae is influenced by the quality of the food available to the maturing female broodstock and interferes with the hatchery output.

3.2. Survival

During all tests the survival was independent of dietary treatment and relatively low (average 72%: Tables 1–3). Some mortality occurred at the start of the first trial due to a fungal infection (Saprolegnia parasitica) and in the second due to the ruptured intestine syndrome (Boon et al., 1987). A survival of 95% or more has been reported for C. gariepinus larval rearing when using Artemia nauplii as the sole diet (Uys and Hecht, 1985; Verreth and van Tongeren, 1989; Verreth et al., 1987, 1992). Comparing the data to the survival percentage calculated by syphoning from the bottom and counting dead larvae during the feeding trial, a difference between the two methods of estimation varying between 6–10% and 7–12% (first and second trial, respectively) was found. The underestimation of mortality via syphoning is probably due to cannibalism which could be observed in the first days of the experiments. As the percentage of missing larvae was not different among the treatments, overfeeding of one particular treatment (in which mortality was underestimated) did not occur.

3.3. Growth

Growth results (DW and b-value) of the cofeeding series were clearly, and in most cases significantly, inferior to these of the Artemia series (Tables 1 and 2). Most literature data indicate a lower growth rate of C. gariepinus larvae fed an artificial diet compared to live Artemia nauplii (Verreth and van Tongeren, 1989; Verreth et al., 1987, 1992). Even supplementation with Artemia nauplii (20%) could not completely fulfil the nutritional requirements of the fish larvae.
Table 1

<table>
<thead>
<tr>
<th>Cofeeding series</th>
<th>Artemia series</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
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<tr>
<td>AA content in diet (μg AA g⁻¹ DW)</td>
<td>106a</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>73</td>
</tr>
<tr>
<td>Missing larvae (%)</td>
<td>6</td>
</tr>
<tr>
<td>Dry weight (mg)</td>
<td>5.7</td>
</tr>
<tr>
<td>b-value</td>
<td>0.20</td>
</tr>
<tr>
<td>Stress resistance, day 2 (%) mortality</td>
<td>93</td>
</tr>
<tr>
<td>Stress resistance, day 8 (%) mortality</td>
<td>65</td>
</tr>
<tr>
<td>AA incorporation</td>
<td>222</td>
</tr>
</tbody>
</table>

Values represent mean ± SD. Means in the same row with common superscript are not significantly different (P < 0.05).

*AA content in Clarias before first feeding: 280 μg AA g⁻¹ DW.

Table 2

<table>
<thead>
<tr>
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<th>Artemia series</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>AA content in diet (μg AA g⁻¹ DW)</td>
<td>107a</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>71</td>
</tr>
<tr>
<td>Missing larvae (%)</td>
<td>12</td>
</tr>
<tr>
<td>Dry weight (mg)</td>
<td>4.9</td>
</tr>
<tr>
<td>b-value</td>
<td>0.18</td>
</tr>
<tr>
<td>Stress resistance, day 2 (%) mortality</td>
<td>90</td>
</tr>
<tr>
<td>Stress resistance, day 8 (%) mortality</td>
<td>50</td>
</tr>
<tr>
<td>AA incorporation</td>
<td>228</td>
</tr>
</tbody>
</table>

Values represent mean ± SD. Means in the same row with common superscript are not significantly different (P < 0.05).

*AA content in Clarias before first feeding: 235 μg AA g⁻¹ DW.

The observed dry weights of larvae of the Artemia series of 11–16 mg (Tables 1 and 2) were comparable to results of 12–18 mg published by Uys and Hecht (1985), Verreth and van Tongeren (1989) and Verreth et al. (1987, 1992). However, during the first experiment, growth was still negatively influenced by the suboptimal conditions during the enrichment of Artemia and a fungal infection. Maximal growth rate of C. garietinus larvae fluctuated around b = 0.31 (Verreth and den Bieman, 1987), which was only reached by the 20%-AP group in the Artemia series. The larvae displayed a much higher growth than in a previous study (Merchie et al., 1995b) in which a maximum dry weight of 9.4 mg was reached only after 20 days of feeding the 20%-AP diet.

From day 6 onwards, the 20%-AP group showed a significantly better growth compared to the 0%- and 10%-AP treatments (Artemia series) in experiments 1 and 3. The higher AA concentrations in the enriched Artemia and the more optimal culture conditions most likely explain the smaller differences in growth observed in the second test. As the Artemia diets (0, 10 and 20% AP) differed mainly in AA concentration (mean values of 516, 1454 and 2182 μg AA g⁻¹ DW, respectively), larval growth could be related to the AA content in the Artemia nauplii administered, yielding a correlation coefficient of 0.821 over the three experiments (Fig. 1). The repeated observation of this relationship sustains the assumption of a causal connection between dietary AA and growth. Moreover, considering the three tests, the fish body weight is clearly related to the AA incorporated in the tissues (r² = 0.872: Fig. 1).

For the cofeeding series, the same positive, but not significant, influence of the extra vitamin C administration on the dry weight was found. Variation within the cofeeding treatments was too high to allow statistical conclusions to be drawn.

For the range of dietary concentrations tested (cofeeding and Artemia series), a linear correlation was found between the AA content in the catfish larvae and their dry weight on day 8: r² = 0.957 and 0.864 (first and second experiment, respectively; Fig. 2).

For the 0%- and 10%-AP groups in the cofeeding series (Table 1), the dietary vitamin
C was not sufficient to maintain the initial AA concentration in the fish larvae before first exogenous feeding (280 μg AA g⁻¹ DW). Johnston et al. (1989) demonstrated that it takes fish several weeks to increase their tissue ascorbic concentrations when they are fed a diet containing more than 100 mg vitamin C kg⁻¹. Therefore, when hatchery fish are fed diets low in vitamin C for extended periods, the ascorbic levels in their tissues will quickly decrease. Moreover, high levels of AA were measured in eggs and freshly hatched larvae of aquatic organisms (Harpe et al. and Poulet, 1990; Lavens and Sorgeloos, 1991) which may be indicative of a need for vitamin C in early larval development. In the second experiment, higher levels of AA were incorporated into the catfish larvae (Table 2), probably due to a more optimal enrichment and thus a higher AA accumulation in the live food diet.

These results indicate that, in the case of C. gariepinus, the vitamin C requirements appear to be much higher during the hatchery phase than the values reported for juveniles and ongrowing fish; for example, a need for 10–25 ppm AA has been shown to be sufficient for normal growth in channel catfish (El-Naggar and Lovell, 1991; Mustin and Lovell, 1992), seabass (Boonyaratpalin et al., 1994), red seabream (Kosutara et al., 1994) and Atlantic salmon (Sandnes et al., 1992). This higher need may be due to a higher rate of larval growth and metabolism. Comparing these results with those reported earlier for the larvae of the prawn Macrobrachium (Merchie et al., 1995c) and the European seabass (Merchie et al., 1995b), it appears that the requirement for vitamin C may be species specific, and might even differ according to the culture conditions.

Considering the superior results for DW and b-value (Table 3) in the 20%--AP fish larvae in the third experiment (18 mg and 0.34, respectively) in relation to both other sets (13 mg and 0.30, respectively), it can be concluded that the positive effect on growth was caused by feeding extra AA, and not by the extra amount of palmitate in the diet. Extra dietary energy supplied via the PA did not cause any positive effect on growth of the catfish larvae. Also the stress resistance (day 2) of the animals of the 20%--AP group was remarkably higher compared to the control and 12%--PA treatments (45 and 10% survival for the 0%-- and 12%--PA groups, respectively).

3.4. Stress resistance

The animals receiving the highest vitamin C supplementation displayed a considerably lower stress sensitivity than those of the 0%-- and the 10%--AP group (Table 1). These differences could be observed on day 2, which might be indicative of the importance of AA in early development. During the second trial no significant differences in stress resistance was noticed; however, the 20%--AP group performed best (Table 2). Similar positive effects of high vitamin C concentrations on stress resistance have been observed in analogous tests with Macrobrachium rosenbergii and Dicentrachus labrax (Merchie et al., 1995b, c). Also for juvenile fish, inclusion of high levels of vitamin C (1000–5000 μg AA g⁻¹ DW) has been demonstrated to enhance tolerance to environmental stressors such as aldrin toxicity (Agrawal et al., 1978) and intermittent hypoxic stress (Ishibashi et al., 1992), and to increase immunoresistance (Li and Lovell, 1985; Liu et al., 1989; Navarre and Halver, 1989; Hardie et al., 1991). The extra effects of AA supplementation at high levels on stress resistance might be of importance under suboptimal rearing conditions in commercial hatcheries (handling, transportation, disease outbreaks).

Since the extra dietary vitamin C also caused an increase in body AA, it may be assumed that feeding AA-enriched live food resulted in a positive effect on stress resistance. These results support the hypothesis of Dabrowski (1992), who states that stress creates increased ascorbic requirements for larval fish, and that in this respect body vitamin C concentration may reflect fish survival potential more accurately than variation in growth rate.

3.5. Hepatocyte ultrastructure

The observations on the ultrastructure of the larval fish hepatocytes for tests 1 and 2 are presented in Table 4.
Table 4

<table>
<thead>
<tr>
<th></th>
<th>Cofeeding series</th>
<th>Artemia series</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Compartmentation</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Number of organelles</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nucleus</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Irregularity of shape</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Heterochromatin content</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Occurrence of two nucleoli</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rough endoplasmic reticulum</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Content</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Circular formation</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Number</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Organization of cristae</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lipid content</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Glycogen content</td>
<td>(+)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

- , not observed; +, seldom observed; + +, frequently observed; + + +, most frequently observed.

The polygonal cells of the 0%-AP Artemia group had an average diameter of 11–13 μm. They showed a well-developed internal compartmentalization: rER stacks and other organelles were clearly separated from the glycogen fields. The number of organelles varied from one cell to another but was generally high. The cell nucleus was located in the centre and was elongated and irregularly shaped. A large nucleolus was found in the centre of the pale nucleoplasm. Up to 11–15 long parallel cisternae of rER occurred mainly in the periphery of the cell. Sometimes the cisternae were strongly folded or arranged in circles. Many mitochondria could be observed, mainly associated with the rER fields and preferably located near the cell periphery, sometimes near lipid inclusions. They were elongated and belonged to the cristae type (sometimes swollen). Some peroxisomes and lysosomes occurred in the perinuclear layer of organelles, close to the bile canaliculi. A large reserve of energy was present in the cells. Although the main storage product was glycogen, lipid droplets of varying size were also deposited.

For the 10%-AP Artemia treatment, cellular organization and number of organelles was similar to the control, although general maintenance of the cells of the 0%-AP group seemed slightly better. The cell diameter was 14–18 μm. Some Golgi fields with many vesicles were observed between the nucleus and bile canaliculi or in the perinuclear layer of organelles.

The hepatocytes of the 20%-AP treatment were even larger (19–21 μm) than in the other Artemia treatments. Cellular compartmentalization of the cells was also clearer and more organelles, especially rER and mitochondria, were found. The nucleus was more regularly shaped and sometimes contained two nucleoli. More rER was located near the nucleus, and many (often up to 23) well-structured parallel cisternae were found in the peripheral zone. Many mitochondria were observed, roundish to cylindrical in shape and closely associated with the rER and lipid inclusions. The cristae were not swollen as was the case for the 0%- and 10%-AP cells. Golgi fields were found near the nucleus and formed many vesicles. Distribution of the storage products was shifted to less lipid and more glycogen (in compact fields) compared with the 0%- and 10%-AP Artemia treatments. General maintenance of the 20%-AP cells was better than for the other two Artemia groups.

For the cofeeding group 0%-AP, there was almost a complete lack of cellular organization and only a few organelles occurred compared with the fish fed 100% Artemia nauplii (0%-AP). The nucleus was irregularly shaped and contained a lot of heterochromatin. rER was found in a few (often fragmented) stacks around the nucleus and in the cell periphery (up to 3–4 cristae). Especially in this group the cristae were partially swollen and showed electron-dense granules. Similar swellings could be found in the nuclear membrane. Mitochondria (more than rER, but less than in the Artemia series) were variable in size and located near rER, in the lipid or the glycogen. The arrangement of the cristae was sometimes aberrant. Some Golgi fields could be found near the bile canaliculi. Many peroxisomes were observed near the nucleus and quite a few secondary lysosomes contained material of organelles. The cells were extremely rich in lipids (large droplets), while only few glycogen fields occurred.

The hepatocytes of the 10%-AP group (cofeeding series) showed characteristics similar to those of the previous treatment, although swellings and granules in the rER occurred less frequently. The state of the mitochondria seemed a little better and more glycogen could be found.

The number of organelles in the 20%-AP series was slightly lower than in the 0%- and 10%-AP groups (cofeeding). The nucleoplasm contained less heterochromatin than in the 0%-AP treatment. Like the 10%-AP cells (cofeeding), mitochondria were better structured as in 0%-AP cells, more rER was present and aberrant structures (swellings and electron-dense particles) in the rER disappeared. Lipid dominated the storage reserve, but glycogen could also be found in compact fields (more than in the 0%-AP group).

The results indicate that the artificial feed used for the cofeeding series was not suitable for C. gariepinus larvae. This diet, which was developed as a standard experimental diet to study nutritional requirements for postweaning stages of marine fish (Coutteau et al., 1995), has previously proved to be inadequate for larvae of freshwater fish (carp; unpublished data). Although glycogen and lipid were deposited, showing that the larvae are able to digest and metabolize the feed, the observed structural abnormalities in mitochondria and rER and the poorly developed cellular compartmentation indicate a severe disturbance of the cell metabolism. To evaluate the nutritional quality of a diet by means of the hepatocyte ultrastructure, the structural organization of the cell and the appearance of the organelles are much stronger criteria than the storage of energy reserves (Segner and Juario, 1986). Thus, it should be concluded that the observed signs are the expression of a deficiency of essential nutrients in the artificial diet. As shown in the hepatocyte ultrastructure of the cofeeding series, the dietary vitamin C level could not fully compensate for the general deficiency caused by the diet. However, with respect to the occurrence of swellings and granule incorporation in the rER cristae, the addition of extra AA clearly showed a positive effect. These granules were probably a pathological manifestation of incomplete proteins (Ghadiali, 1982).
The enriched *Artemia* nauplii, on the other hand, proved to be an appropriate diet for the larval rearing of African catfish. The diet resulted in a considerable storage of glycogen, and the additional deposition of lipid confirms the well-fed status of the *Artemia*-fed larvae. Moreover, the clear cellular compartmentation, the well-structured mitochondria and the distribution of the rER in long parallel cisternae in the perinuclear layer and cell periphery are characteristic features of hepatocytes in adequately fed fish larvae. Within the *Artemia* series, the 20%-AP group showed a better compartmentation, a more regularly shaped nucleus with less heterochromatin and sometimes two nucleoli, more organelles (mitochondria and rER), better developed Golgi fields and a higher glycogen content compared to 0%- and 10%-AP treatments. These characteristics are indicative of a more active cellular metabolism and protein synthesis and confirm the growth results mentioned above. The higher activity of the Golgi fields of the larvae of the 20%-AP treatment, however, might also be related to ontogeny, as *C. gariepinus* larvae start to metabolize lipid in the hepatocytes from ±100 mg body weight onwards, irrespective of their nutrition (Verreth, unpublished data). Whereas no effect could be seen in the cofeeding series, hepatocyte ultrastructure adds clear evidence of extra AA on the development of the larval stages of *C. gariepinus* in the *Artemia* series.

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