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Larval rearing of an African catfish *Heterobranchus longifilis* (Teleostei, Clariidae): effect of dietary lipids on growth, survival and fatty acid composition of fry

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Abstract

With the aim of improving dry diets for first feeding of *Heterobranchus longifilis* larvae, the effect of dietary lipid sources on growth rate, survival rate and fatty acid composition of fry from 2 days up to 17 days of age was evaluated. Six feeding regimes were tested: *Artemia* nauplii which served as a reference, and 5 experimental dry diets differing only by the lipid source. The different oils used for the different experimental diets were the following: cod liver oil, palm oil, copra oil, peanut oil and cotton seed oil. Each diet was tested on duplicate groups of 400 larvae placed in the 40 l tanks of a recirculating system (27-29°C) and fed to excess six times per day every 4 hours. Separation and identification of the fatty acids of diets, eggs and fry were carried out by gas-liquid chromatography. After 15 days of feeding, survival rates were high for all treatments (71-87%) and did not differ significantly. By contrast, growth rates were largely influenced by the feeding regime. Fry fed with *Artemia* were significantly bigger (289 mg) than those fed artificial dry diets (79-115 mg). However, it was found that the specific growth rate of fry fed *Artemia* was superior to that of fry receiving dry diets only for fish of less than 50 mg body weight, indicating that *Artemia* presents a nutritional advantage only for fry at their youngest stages of development (first 6 days of feeding). Among the artificial dry diets, the best results were obtained with diets containing palm or copra oil, the lowest growth rate being observed with the cod liver oil diet. Peanut and cotton seed oil diets led to intermediate results.

The fatty acid composition of the whole fry reflected that of the experimental diets. All together, the results indicated the existence of an optimal ratio between *n*-3 and *n*-6 fatty acids for covering essential fatty acids requirement of the fry. Growth rates tended to be reduced by an excess of *n*-3 fatty acids (cod liver oil) or by an excess in *n*-6 fatty acids (cotton seed oil) as well. Evidence of the occurrence of HUFA (20:4*n*-6 and 22:6*n*-3) biosynthesis are given.

Keywords: *Heterobranchus longifilis*, *Artemia*, compound diet, fatty acids, larval nutrition.

Élevage larvaire d'un silure africain Heterobranchus longifilis *(Teleostei, Clariidae): effet des lipides alimentaires sur la croissance, la survie et la composition en acides gras des alevins.*

Résumé

Dans le but d'améliorer la composition des aliments secs pour l'alimentation des larves de *Heterobranchus longifilis*, l'effet de différentes sources de lipides alimentaires sur la croissance, la survie et la composition corporelle en acides gras, a été évalué depuis l'entrée en phase trophique jusqu'à l'âge de 17 jours.

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Six régimes expérimentaux ont été testés: les nauplii d'*Artemia* vivantes utilisées comme aliment de référence et 5 aliments secs ne variant que par la nature de l'huile incorporée. La composition de ces derniers était la suivante: foie de boeuf (30 %), levure (50 %), mélange vitaminique (7,5 %), huile (7,5 %) et minéraux (5 %). Les huiles utilisées sont respectivement les huiles de foie de morue, de palme, de coprah, d'arachide et de coton. Chaque aliment a été testé sur deux groupes de 400 larves élevées en bacs de 40 l en eau recyclée et nourries en excès, six fois par jour toutes les 4 h. La composition en acides gras des aliments, des ovules et des larves a été déterminée sur les lipides totaux par chromatographie en phase gazeuse.

Après 15 jours d'alimentation, les taux de survie sont élevés pour tous les traitements (71-87 %) et ne diffèrent pas significativement. En revanche, la croissance est fortement influencée par la nature de l'aliment utilisé. Le poids moyen final des alevins nourris avec *Artemia* est significativement plus élevé (289 mg) que celui des alevins recevant les aliments secs (79-115 mg). Le taux de croissance spécifique des alevins nourris avec *Artemia* est supérieur à celui des alevins recevant les aliments secs uniquement pour les individus de poids inférieur à 50 mg, ainsi *Artemia* présenterait un avantage nutritionnel lors des 6 premiers jours d'alimentation. Parmi les aliments secs, les régimes contenant de l'huile de palme ou de coprah permettent la meilleure croissance, la plus faible étant observée avec l'huile de foie de morue. Les huiles d'arachide et de coton conduisent à des résultats intermédiaires.

La composition en acides gras (AG) des alevins reflète celle des aliments. Dans leur ensemble, les résultats suggèrent l'existence d'un rapport optimal entre les AG *n*-3 et les AG *n*-6 pour la couverture des besoins en AG essentiels. La croissance des larves apparaît diminuée par un excès d'AG *n*-3 (huile de foie de morue) comme par un excès d'AG *n*-6 (huile de coton) dans les aliments. Les résultats indiquent en outre la capacité des larves de *H. longifilis* à biosynthétiser des AG hautement insaturés (20 : 4*n*-6 et 22 : 6*n*-3).

Mots-clés : *Heterobranchus longifilis*, *Artemia*, aliments composés, acides gras, nutrition larvaire.

INTRODUCTION

The potential of *Heterobranchus longifilis* Valenciennes, 1840, in aquaculture was demonstrated in the Côte-d'Ivoire (Ivory Coast). The several qualities which confer to this species a status of ideal candidate for fish culture have been extensively discussed in previous papers (Legendre, 1992; Legendre *et al.*, 1992).

Under hatchery conditions, *Artemia* nauplii proved to be highly suitable as a first feed for *H. longifilis* larvae and generally led to high growth and survival rates of fry (Legendre *et al.*, 1991). However, *Artemia* may not be the most appropriate feed for large-scale rearing of larvae in many African countries due to practical and economical reasons and other diets should be developed as substitutes (Kerdchuen and Legendre, 1994).

In comparison to live food organisms, the use of artificial diets present different advantages. They can be quality controlled during fabrication, manufactured on a large scale and distributed easily to ensure regular supplies (Uys and Hecht, 1985). Recently, it has been shown that the use of a dry diet based on yeast and beef liver, efficient in the whitefish and the common carp (Bergot *et al.*, 1986; Charlon *et al.*, 1986), leads in *H. longifilis* to survival rate as high as that obtained with *Artemia* nauplii, but with a slower growth rate during the first two weeks of rearing (Kerdchuen and Legendre, 1994). It was assumed that this slow growth rate was due, at least in part, to an inadequate covering of the nutritional requirements of the larvae.

Dietary lipids are commonly utilized in fish as a major energy source. They also provide essential fatty acids (EFA) needed for proper functioning of many physiological processes and maintenance of membrane fluidity and permeability (Cho *et al.*, 1985; Stickney and Hardy, 1989). Several works have demonstrated that EFA requirements vary considerably from species to species (Castell, 1979; Watanabe, 1982; Kanazawa, 1985). Among tropical fish, tilapias require mostly *n*-6 fatty acids (Kanazawa *et al.*, 1980; Teshima *et al.*, 1982; Takeuchi *et al.*, 1983). By contrast, cold water fish such as rainbow trout and whitefish, require *n*-3 rather than *n*-6 fatty acids (Watanabe *et al.*, 1974; Watanabe *et al.*, 1989). In other fish species, both *n*-3 (linolenic acid) and *n*-6 (linoleic acid) fatty acids have been identified as EFA, e.g. in common carp, Japanese eel and chum salmon (Kanazawa, 1985). Concerning siluriformes, the channel catfish seems to have requirements for both *n*-6 and *n*-3 fatty acids, though the ratio of the two appears of great importance (Stickney and Hardy, 1989). It has also been reported that the *n*-3 highly unsaturated fatty acids may be essential in this species (Satoh *et al.*, 1989).

In order to improve the nutritional efficiency of dry diets for larval rearing of *H. longifilis*, the present study was conducted to evaluate the effect of dietary lipids on growth rate, survival and fatty acid composition of fry during their first two weeks of life. Fatty acid compositions of ova and unfed larvae were also determined. The results were compared to those obtained for fry fed *Artemia* which served as a reference.

MATERIALS AND METHODS

The feeding experiment was carried out at the Centre de Recherches Océanologiques in Abidjan

(Côte-d'Ivoire) and was monitored for 15 days from the onset of exogenous feeding of larvae.

Experimental diets

Six diets were tested: live *Artemia* nauplii (ART) (Bio-marine, California) used as a control diet and 5 experimental dry diets differing only by the lipid source (*tabl.* 1). In the latter, the dietary lipids were modified using different oils, from animal or vegetable origins, added to a compound diet of standard composition (48.3% crude protein, 14.7% crude lipid and 21.3 $kJ.g^{-1}$ energy; Bergot *et al.*, 1986) which gave promising results for feeding *H. longifilis* larvae (Kerdchuen and Legendre, 1994). The oils used were respectively palm oil (PAL), copra oil (COP), peanut oil (PEA), cotton seed oil (COT) and cod liver oil (COL).

Table 1. – Composition (%) of the experimental diets.

Ingredient	Palm oil diet	Copra oil diet	Peanut oil diet	Cotton seed oil diet	Cod liver oil diet
Beef liver	30.0	30.0	30.0	30.0	30.0
Yeast powder (1)	50.0	50.0	50.0	50.0	50.0
Vitamin mix (2)	7.5	7.5	7.5	7.5	7.5
Mineral mix (3)	5.0	5.0	5.0	5.0	5.0
Palm oil	7.5	–	–	–	–
Copra oil	–	7.5	–	–	–
Peanut oil	–	–	7.5	–	–
Cotton seed oil	–	–	–	7.5	–
Cod liver oil	–	–	–	–	7.5

(1) Yeast powder "Protibel", Bel, France.

(2) EIFAC (1971).

(3) Luquet (1971).

Fish and experimental conditions

The larvae were obtained from *H. longifilis* breeders held at the Layo Aquaculture Research Station (Côte-d'Ivoire), using the procedure of artificial reproduction established by Legendre (1986). These brooders were fed a 35% protein pelleted feed supplemented with 0.3% cod liver oil, distributed at a daily rate of 1-2% of fish biomass. Artificial fertilization was performed using a pool of gametes from two females and two males. On the day after hatching, the larvae were individually counted and randomly transferred to the rearing facilities. Feeding started on the evening of the second day, when the yolk sac was nearly completely absorbed. At this stage, the larvae were approximately 7 mm in total length and 2 mg mean body weight.

Each diet was tested in duplicate on groups of 400 larvae placed in 40 l tanks of a recirculating system. The larvae were held in darkness and fed to excess six times per day at 02:00, 06:00, 10:00, 14:00, 18:00 and 22:00. For the dry diets, fish were fed sieved food particle size of 100-200 μm diameter from day 2 to day 6, 200-400 μm diameter from day 6 to day 11 and 400-600 μm from day 11 to day 17 (time corresponding to age of larvae). During the experiment, water temperature was maintained between 27 and 29°C and dissolved oxygen ranged from 6 to 7 $mg.l^{-1}$. Each tank was cleaned twice daily by siphoning off the feces and uneaten food.

Every three days from starting of experiment, 10 fish from each tank were randomly sampled and individually weighed to the nearest 0.1 mg according to the procedure of Kerdchuen and Legendre (1994). On the last day of the rearing period, 50 fish (17-day-old) per tank were sampled and individually weighed. Survival rates were determined by counting all the remaining fish in each tank.

Fatty acids analysis

In addition to each test diet, the following biological materials were used for fatty acid (FA) analysis: Ova collected from two ovulated females and pooled in equal proportions, unfed 2-day-old larvae after yolk sac absorption, starved larvae sampled when mass mortality begin to occur (6 days of age) and 17-day-old fry taken from each feeding treatment. The latter were fasted for 24 h prior to sampling in order to empty the digestive tract of its content. In all cases, fry were preserved by direct immersion in liquid nitrogen, and then freeze-dried.

Separation and identification of fatty acids in diets, ova and fry were carried out at INRA (Saint-Pée-sur-Nivelle, France). Three measurements were carried out for each sample. Total lipids were extracted from 1g of pooled freeze dried eggs or fry according to Folch *et al.* (1957). Fatty acid methyl esters were prepared by acid-catalyzed transmethylation of total lipids (Morrison and Smith, 1964). They were analyzed in a Varian 3400 gas chromatograph equipped with a DB Wax fused silica capillary column (30 m × 0.25 mm i.d., film thickness: 0.25 μm, J. and W. Scientific, Folsom, California, USA) using helium as carrier gas (1.4 $ml.min^{-1}$) and a thermal gradient from 180°C to 240°C at $4°C.min^{-1}$. Injector and flame ionization detector temperatures were 260 and 250°C respectively. Fatty acid methyl esters were identified by comparison with known standard mixtures (Sigma) and quantified using a Spectra Physics 4270 integrator.

Data analysis

Final weights and survival rates obtained with the different experimental feeds were subjected to one way ANOVA and Duncan's multiple range test to determine significant differences among means at $p<0.05$. The correspondence analysis was used in order to compare patterns of fatty acid composition in the diets (active observations) as a function of their content in major FA families (active variables). The influence of FA composition of diets on

FA compositions of fish was assessed using the supplementary observations procedure.

RESULTS

Fatty acid composition of diets, ova and larvae

The fatty acid compositions of *Artemia* nauplii and experimental diets are given in *table* 2. *Artemia* nauplii displayed high content in *n*-3 fatty acids (FA), mainly 18:3, and low content in *n*-6 FA. An absence of long-chain unsaturated FA of both *n*-3 (20:4, 22:5, 22:6) and *n*-6 (20:3, 20:4, 22:4) series was also noticed. Among the experimental diets, the COL diet was characterized by a high content in *n*-3 FA, mostly 20:5 and 22:6. By contrast, PEA and COT diets were rich in *n*-6 FA (18:2 mainly). The PAL diet had a fatty acid profile intermediate between these two latter diets and the COL diet: PAL, PEA and COT diets were similar considering their *n*-3 FA contents but differed highly in their *n*-6 FA contents, while PAL and COL diets displayed comparable *n*-6 FA contents but different *n*-3 FA contents. The COP diet was characterized by its richness in saturated FA.

Fatty acid composition of ova and 2-day-old larvae after yolk sac absorption are given in *table* 3. They were characterized by high contents of 18:2 and 20:4*n*-6 FA and of *n*-3 HUFA (22:6 particularly), and by low contents of 18:3*n*-3. After 4 days of starvation, the total lipid content of 6-day-old larvae (122 $mg.g^{-1}$ dry matter) were reduced approximately by half in comparison with 2-day-old larvae (224 $mg.g^{-1}$ dry matter; *tabl.* 3). This drop concerned mainly monoenoic FA. The content of 22:6*n*-3 remained roughly unchanged (around 10 $mg.g^{-1}$ dry matter). The complete metabolism of some FA was noticed for both *n*-6 (16:2 and 22:4) and *n*-3 (22:5) series.

Fatty acid composition of 17-day-old fry fed with the different experimental diets are given in *table* 4. The correspondence analysis allows a synthetic view of the FA composition of the diets and their influence on the FA composition of fry (*fig.* 1). The latter were strongly dependent upon dietary fatty acids. However, the variations in the FA composition of diets were attenuated in the FA compositions of fry, which were all situated in a more central position on the plane defined by axes 1 and 2. It was also found that fry fed PAL diet presented a FA composition very close to that of PAL diet. The composition of fry fed with other diets was systematically intermediate between the composition of fry fed PAL diet and the composition of their own diets.

In fish groups fed *Artemia* nauplii, total *n*-3 FA contents of fry were lower than that of nauplii while total *n*-6 FA contents remained similar. The body composition of fry was richer in *n*-3 than in *n*-6 FA. A striking point was the presence of long-chain PUFA of both the *n*-3 (20:4, 22:5 and 22:6) and *n*-6 (20:4) families despite their complete absence in the diet. The total content of docosahexaenoic acid was around 2 μg in a single 2 mg larvae after yolk sac absorption whereas it reached around 30 μg in a whole individual fry at the end of experiment after 15-day of feeding period with *Artemia*.

Table 2. – Fatty acid composition of the experimental diets (weight % of total dietary lipids).

Fatty acid	*Artemia* nauplii	Palm oil diet	Copra oil diet	Peanut oil diet	Cotton seed oil diet	Cod liver oil diet
8:0	–	–	6.1	–	–	–
10:0	–	0.2	6.2	0.2	0.2	0.2
12:0	–	0.3	37.6	0.1	0.3	0.2
14:0	1.7	0.9	12.0	0.4	0.9	4.3
15:0	0.2	0.2	0.2	0.2	0.2	0.4
Iso16	–	0.3	0.2	0.2	0.1	0.2
16:0	35.6	29.7	9.0	13.1	23.3	13.9
17:0	1.4	0.4	0.3	0.4	0.4	0.7
Iso18	–	–	tr	–	0.1	0.4
18:0	3.3	9.5	6.1	8.3	7.7	8.1
20:0	–	0.2	–	0.6	tr	0.1
22:0	–	–	–	0.9	–	–
Total Saturates	42.2	41.7	77.7	24.4	33.2	28.5
14:1	1.3	0.2	0.2	0.3	0.2	0.8
15:1	0.4	–	–	–	–	–
16:1	3.8	2.3	1.6	2.5	2.6	9.8
17:1	0.7	0	–	tr	tr	0.1
18:1	21.8	36.4	10.8	47.1	19.9	25.4
20:1	0.2	0.1	tr	0.5	0.1	5.5
22:1	–	–	–	0.1	0.3	2.8
Total Monoenes	28.2	39.0	12.6	50.5	23.1	44.4
16:2*n*-6	0.3	0.1	0.1	0.1	0.2	0.3
16PUFA*n*-6	1.3	0.5	0.4	0.5	0.5	1.2
18:2*n*-6	4.2	12.7	4.9	18.4	36.8	6.7
18:3*n*-6	–	0.1	0.1	0.1	0.1	0.2
20:2*n*-6	–	0.1	tr	0.1	tr	0.2
20:3*n*-6	–	1.1	0.9	1.0	1.0	1.2
20:4*n*-6	–	1.3	1.0	1.2	1.1	1.6
22:4*n*-6	–	0.2	0.2	0.2	0.2	0.3
Total PUFA *n*-6	5.8	16.1	7.6	21.6	39.9	11.7
18:3*n*-3	19.1	1.0	0.6	0.8	1.0	1.4
18:4*n*-3	2.6	–	–	–	tr	1.4
20:3*n*-3	–	–	–	–	–	tr
20:4*n*-3	–	0.2	0.1	0.2	0.2	0.5
20:5*n*-3	1.8	0.3	0.3	0.4	0.4	4.6
22:5*n*-3	–	1.1	0.8	1.1	1.1	2.0
22:6*n*-3	–	0.3	0.2	0.3	0.3	4.0
Total PUFA *n*-3	23.5	2.9	2.0	2.8	3.0	13.9
Sat/PUFA	1.4	2.2	8.1	1.0	0.8	1.1
n-3/*n*-6	4.1	0.2	0.3	0.1	0.1	1.2
Total lipids ($mg.g^{-1}$ dry matter)	198.5	163.7	168.9	167.6	168.7	162.1

tr: trace; –: not detected.

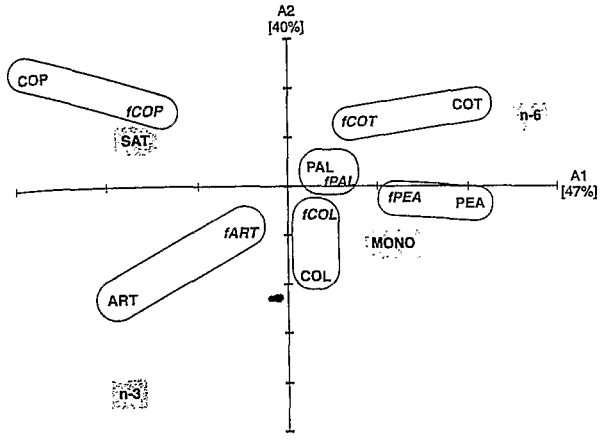

Figure 1. – Correspondence analysis applied to fatty acid composition of diets and 17 days old *H. longifilis*. Projection on the plane (axes 1 and 2) explains for 87% of total variance.

Active variables: fatty acid (FA) families. SAT: saturated FA; MONO: monoenoic FA; N3: *n*-3 FA; N6: *n*-6 FA.

Active observations: diets. ART: *Artemia* nauplii; PAL: Palm oil diet; COP: Copra oil diet; PEA: Peanut oil diet; COT: Cotton seed oil diet; COL: Cod liver oil diet.

Supplementary observations: *f* ART *fry* fed with artemia, *f* PAL *fry* fed with palm oil diet.

In fish groups receiving the dry diets, both *n*-3 and *n*-6 FA total contents were lower in fry than in diets, with the exception of the COP diet for which content of *n*-6 FA was higher in fry than in the diet. In all cases, the body composition of fry displayed higher contents in *n*-6 than in *n*-3 FA. Examination of fatty acid profiles (*tabl.* 2 and 4) indicated higher content of 20:4*n*-6 in fry than in feed for PAL and COP diets, while an inverse situation was observed for PEA, COT and COL diets. For all diets containing oil from vegetable origin, docosahexaenoic acid was the only *n*-3 FA to be found in higher proportion in fry than in diet. In contrast, for COL diet, 22:6*n*-3 content was lower in fry than in diet.

Survival and growth of fry

At the end of the 15-day feeding period, survival rates were high for all treatments and ranged from 71 to 87% (*tabl.* 5). Although survival rates tended to be lower for fish groups receiving the COL diet, no significant difference was found between treatments.

The growth response of *H. longifilis* fry to the different diets tested are shown in *figure* 2. Larvae fed *Artemia* nauplii displayed a higher growth than larvae fed dry diets, the difference in mean body weight being significant ($p<0.05$) after three days of feeding. The growth rate of larvae fed dry diets were significantly influenced by the lipid composition of diets (*table* 5). The best results were observed for PAL and COP diets, whereas the slower growth was obtained with the COL diet. Feeds supplemented with peanut (PEA) or cotton seed (COT) oils led to intermediate final weights.

Table 3. – Fatty acid composition (weight % of total lipids) of ova, 2 days old unfed larvae and 6 days old unfed larvae of *H. longifilis*.

Fatty acid	Ova	Larvae 2 days	Larvae 6 days
10:0	0.3	0.2	0.1
12:0	0.2	0.3	–
14:0	1.2	2.0	1.4
15:0	0.3	0.3	0.5
Iso16	0.1	0.3	–
16:0	35.1	33.4	39.9
17:0	0.3	0.3	0.4
18:0	15.7	13.7	19.2
20:0	–	0.1	–
22:0	–	–	0.3
Total Saturates	53.2	50.5	61.8
14:1	0.2	0.2	0.3
16:1	3.2	9.0	2.4
17:1	0.1	0.1	–
18:1	24.5	23.9	18.3
20:1	0.9	1.0	0.8
Total Monoenes	28.9	34.2	21.8
16:2*n*-6	0.3	0.3	–
16PUFA*n*-6	0.5	0.6	1.0
18:2*n*-6	4.1	3.8	2.0
18:3*n*-6	–	tr	–
20:2*n*-6	0.9	0.8	1.8
20:3*n*-6	1.0	0.9	1.0
20:4*n*-6	1.7	1.6	1.4
22:4*n*-6	0.1	0.1	–
Total PUFA *n*-6	8.6	8.1	7.2
18:3*n*-3	0.1	0.1	0.3
20:3*n*-3	–	–	0.3
20:5*n*-3	0.7	0.6	0.3
22:4*n*-3	0.3	0.3	0.4
22:5*n*-3	0.3	0.2	–
22:6*n*-3	6.6	4.8	7.6
Total PUFA *n*-3	8.0	6.0	8.9
Sat/PUFA	3.2	3.6	3.8
n-3/*n*-6	0.9	0.7	1.2
Total lipids (mg.g^{-1} dry matter)	207.9	223.8	121.7

tr: trace; –: not detected.

When the growth data were examined in terms of specific growth rates [SGR = 100(Ln W2 – Ln W1)/*t*], it appeared clearly that the growth differential between fry fed *Artemia* and dry diets concerned only small sized larvae (*fig.* 3). In fish groups fed *Artemia*, SGR decreased from 67 to 42% when larval body weight grew from 2 to 15 mg. By contrast, for a similar range of fish weight, SGRs never exceeded 35%.d^{-1} in groups receiving the dry diets. Nevertheless, when the fry reached a mean body weight of around 50 mg (after a 6-day feeding period with *Artemia*), SGRs were of similar values (around 23%.d^{-1}) irrespective of the type of feed used.

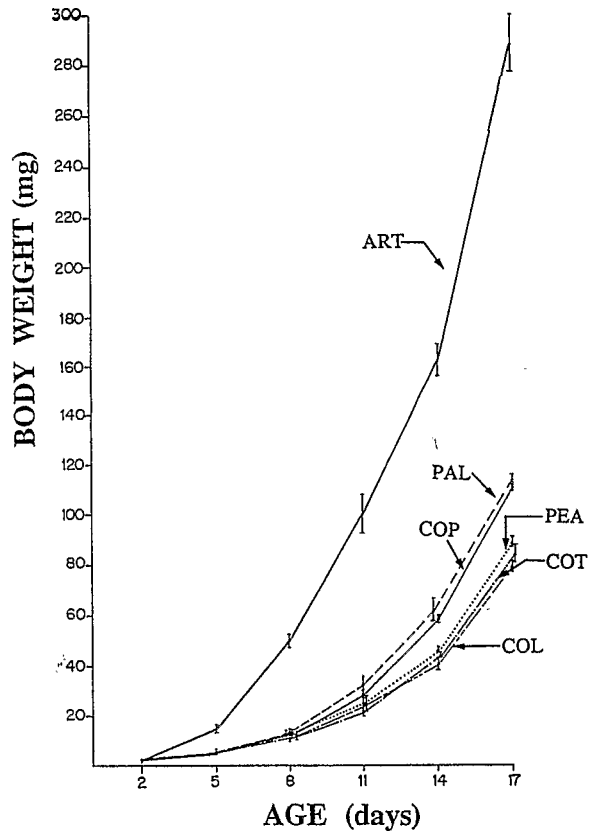

Figure 2. – Growth of *H. longifilis* from first feeding up to 17 days of age as a function of different experimental diets. Vertical bars indicate difference between replicates. (ART: *Artemia* nauplii; PAL: Palm oil diet; COP: Copra oil diet; PEA: Peanut oil diet; COT: Cotton seed oil diet; COL: Cod liver oil diet).

DISCUSSION

The present study shows that survival rates of *H. longifilis* larvae fed dry diets based on yeast and beef liver were equivalent to those obtained with *Artemia* nauplii after 15-day of feeding from the onset of exogenous feeding. This suggests that fatty acids, essential for larvae of this species, were sufficiently available in all feed types.

Growth rate of fry fed *Artemia* was high, indicating favorable rearing conditions. Mean SGR calculated over the first 12 days of feeding with *Artemia* (37%.d^{-1}; data not shown) confirmed previous results of Kerdchuen and Legendre (1994), who reported a SGR of 40%.d^{-1} for *H. longifilis* larvae grown in the same conditions. These values were similar to maximum average SGRs (40.7-41.9%.d^{-1}) obtained in *Clarias gariepinus* after a 10-day feeding period with decapsulated cysts of *Artemia* (Verreth and Den Bieman, 1987).

Although the final body weight of fry fed dry diets remained lower than that obtained with *Artemia* at the end of experiment, SGRs evolution as a function of fish weight shown that the nutritional advantage of the latter to the former concerned only larvae of less than 50 mg mean weight. It corresponded to the final weight attained after a 6-day feeding period with *Artemia*. In *C. gariepinus*, Verreth and Van Tongeren (1989) reported that the earliest weaning time to maximize growth rate of larvae was after

Table 4. – Fatty acid composition (weight % of total lipids) of 17 days old *H. longifilis* fry fed the different experimental diets.

Fatty acid	*Artemia* nauplii	Palm oil diet	Copra oil diet	Peanut oil diet	Cotton seed oil diet	Cod liver oil diet
10:0	–	0.1	0.3	tr	0.1	0.1
12:0	0.1	0.1	18.2	0.2	0.1	0.1
14:0	2.3	1.0	14.7	0.6	1.3	4.4
15:0	0.4	0.6	0.7	0.7	0.8	1.1
Iso16	0.2	0.3	0.2	0.2	0.3	0.2
16:0	29.3	27.1	18.3	18.5	29.1	20.6
17:0	1.2	0.7	0.7	0.6	0.7	0.9
18:0	7.2	9.3	10.8	10.2	9.4	10.6
20:0	0.1	0.1	0.1	0.3	0.1	0.1
22:0	0.1	–	–	0.2	–	–
Total Saturates	40.9	39.3	64.0	31.5	41.9	38.1
14:1	1.4	0.2	0.4	0.2	0.3	0.9
16:1	7.2	4.3	4.0	3.2	3.6	10.5
17:1	0.2	0.1	0.1	0.1	0.1	0.1
18:1	33.0	37.4	19.6	44.2	22.5	28.0
20:1	1.2	0.6	0.4	1.0	0.4	4.6
22:1	0.2	0.3	0.2	0.5	1.1	1.5
Total Monoenes	43.2	42.9	24.7	49.2	28.0	45.6
16:2*n*-6	0.4	0.2	0.2	0.3	0.3	0.4
16PUFA*n*-6	1.5	1.0	1.2	0.9	0.9	1.5
18:2*n*-6	2.7	9.8	5.2	12.1	21.8	5.8
18:3*n*-6	0.1	0.3	0.2	0.4	0.5	0.2
20:2*n*-6	0.1	0.2	0.2	0.2	0.3	0.2
20:3*n*-6	–	1.2	1.0	1.0	0.9	0.9
20:4*n*-6	0.1	1.3	1.1	1.1	0.8	1.2
22:4*n*-6	–	0.2	0.1	0.2	0.1	0.2
Total PUFA *n*-6	4.8	14.2	9.2	16.2	25.6	10.4
18:3*n*–3	6.5	0.6	0.4	0.4	0.5	0.9
18:4*n*-3	0.3	0.1	–	0.1	tr	0.3
20:3*n*-3	0.2	–	–	–	–	–
20:4*n*-3	0.2	0.2	0.1	0.2	0.5	0.3
20:5*n*-3	0.6	0.3	0.2	0.3	0.6	1.3
22:4*n*-3	–	0.1	–	tr	–	–
22:5*n*-3	0.2	0.6	0.3	0.5	0.4	0.9
22:6*n*-3	0.3	0.8	0.6	0.7	0.4	1.8
Total PUFA *n*-3	8.3	2.7	1.6	2.2	2.4	5.5
Sat/PUFA	3.1	2.3	5.9	1.7	1.5	2.4
n-3/*n*-6	1.7	0.2	0.2	0.1	0.1	0.5
Total lipids (mg.g^{-1} dry matter)	308.4	230.2	218.1	246.1	243.2	251.6

tr: trace; –: not detected.

Table 5. – Mean body weight, specific growth rate (SGR) and survival rate for *H. longifilis* after 15 days of feeding with the various experimental diets.

Feed	Body weight (mg)	SGR (%.d^{-1})	Survival (%)
Artemia nauplii	289.2 ^{a}	33.2	85 ^{a}
Palm oil diet	114.5 ^{b}	27.0	83 ^{a}
Copra oil diet	110.8 ^{b}	26.8	86 ^{a}
Peanut oil diet	89.2 ^{c}	25.3	87 ^{a}
Cotton seed oil diet	84.2 ^{cd}	24.9	78 ^{a}
Cod liver oil diet	79.3 ^{d}	24.5	71 ^{a}

Figures with same superscripts in the same column are not significantly different ($p < 0.05$).

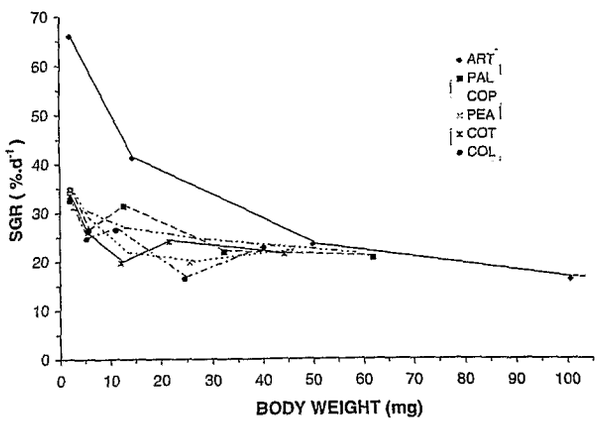

Figure 3. – Specific growth rate (SGR) of *H. longifilis* as a function of initial body weight of fish at each three days sampling periods for the different experimental diets.

(ART: *Artemia* nauplii; PAL: Palm oil diet; COP: Copra oil diet; PEA: Peanut oil diet; COT: Cotton seed oil diet; COL: Cod liver oil diet).

4 days of feeding with *Artemia*, corresponding to fish weight of around 18 mg. However, in the latter study weaning occurred gradually by decreasing the proportion of *Artemia* and increasing the proportion of a commercial trout feed, which means that fish received *Artemia* in their ration for a total period of 7 days.

In the present investigation, a distinct improvement of growth performance was demonstrated when replacing oil from animal origin (cod liver oil), commonly used for temperate fish species (Bergot *et al.*, 1986; Charlon *et al.*, 1986), by oil from vegetable origin (palm or copra oil) in the dry diet composition. The PAL diet led to a 46% increase in the final mean weight of fry in comparison to the COL diet (115 against 79 mg). This was clearly a consequence of differences in the fatty acid composition of the diets.

The strong influence of dietary lipids on body fatty acid profiles has been well documented for both marine and freshwater fish (Castell, 1979; Yingst and Stickney, 1979; Watanabe, 1982; Soivo *et al.*, 1989; Radünz-Neto *et al.*, 1994). In *H. longifilis*, the fatty acid composition of the fry also reflected their respective dietary fatty acids. This was particularly the case with the PAL diet for which striking similarities between FA composition of the fry and the diet itself were observed. The PAL diet, which gave the best growth response among artificial diet tested, appears to provide the best fatty acid composition. As a consequence the *n*-3 FA requirement seems to be low in *H. longifilis* larvae. In the PAL diet it corresponded approximately to 0.5% of the dietary dry matter. Low *n*-3 EFA requirements were also found in first feeding carp larvae (Radünz-Neto *et al.*, in press).

When comparing the growth of fry fed PAL, PEA or COT diets, the results indicated that, for a similar content of dietary *n*-3 FA, increased content of *n*-6 FA (18:2, mainly) led to a reduction in growth performance. Similarly, when comparing PAL and COL diets which had comparable *n*-6 contents, it was found that the higher *n*-3 FA content in COL diet was associated with a reduction of growth. These results indicate the existence of an optimum ratio between *n*-3 and *n*-6 FA for good growth of *H. longifilis* larvae. In the channel catfish, *Ictalurus punctatus*, requirements for fatty acids from both the *n*-3 and *n*-6 families were identified, though the proper ratio of the two was not precisely determined (Stickney and Hardy, 1989). As the PAL diet led to the best growth response among the dry diets tested, the *n*-3/*n*-6 ratio of 0.2 in the PAL diet may be considered as convenient for *H. longifilis* larvae in comparison with ratio observed in the other dry diets (0.1 in PEA and COT diets, 1.2 in COL diet).

Artemia were characterized by very high content of 18:3*n*-3 and no *n*-3 HUFA. At the start of exogenous feeding, 2-day-old larvae displayed very low content in linolenic acid and particularly high content of docosahexaenoic acid, suggesting that 18:3*n*-3 was not necessary for larvae at an early stage of development. It seems difficult to attribute the high growth rate obtained with *Artemia* to its high content in 18:3*n*-3. On the other hand, it is unlikely that the excess in this particular fatty acid in the nauplii could have a negative effect on growth of *H. longifilis* larvae. Verreth *et al.* (1994) found no growth depletion in *C. gariepinus* larvae fed high *n*-3 HUFA-enriched *Artemia* in comparison with larvae fed without *Artemia* *n*-3 HUFA-enrichment. More detailed research remains necessary to elucidate the fatty acid requirement of *H. longifilis* larvae. Nevertheless, it can be assumed that the nutritional advantage of *Artemia* over dry diets, observed during the first 6 days of feeding, was not imputable solely to its fatty acid composition.

It is of relevance to note that the (22:5+22:6)*n*-3/total *n*-6 ratios in the 17-day-old fry were identical (ratio value of 0.10, calculated from *table* 4) for fish fed *Artemia*, PAL and COP diets which led to the highest growth rates. In slow growing fry receiving

the other diets, this ratio was either lower (0.03 and 0.07 with COT and PEA diets respectively) or higher (0.26 with COL diet). A remarkable point was the presence of HUFA, particularly 22:6*n*-3 and 20:4*n*-6, in 17-day-old fry fed *Artemia* despite their complete absence in the diet. As the total content of these fatty acids in the fry were increased (by 15 times in the case of 22:6) between starting of exogenous feeding and the end of experiment, their presence in 17-day-old fry could not originate only from reserve stocks found in the eggs. This suggests that the observed increase in C20 and C22 HUFA contents resulted from biosynthesis by the larvae. In other catfish, the capacity to synthesize HUFA from C18 precursor fatty acids was reported in fingerlings of *Ictalurus punctatus* (Satoh *et al.*, 1989) and larvae of *C. gariepinus* (Verreth *et al.*, 1994). The selective retention of docosahexaenoic acid in the 6-day-old starved larvae underlines the particular importance of this fatty acid for *H. longifilis* larvae.

In conclusion, the replacement of cod liver oil by palm oil as oil supplementation in dry diet based on yeast and beef liver permitted a clear improvement of growth in *H. longifilis* larvae. This, and the high survival rates obtained, indicate that the PAL diet can already be used for fry production when *Artemia* shortage is experienced. The *n*-3/*n*-6 ratio in the dry diet should be in favor of the *n*-6 fatty acids, an excess of either *n*-3 or *n*-6 FA in the dry diet composition led to reduced growth performance. The data indicated that *H. longifilis* larvae were capable of HUFA biosynthesis, as noticeable contents of 20:4*n*-6 and 22:6*n*-3 were found in the fry body composition although these fatty acids were completely lacking in the *Artemia* FA profile. The results also suggested that the dietary requirement for *n*-3 HUFA is low and could be met by low levels of 18:3*n*-3. In comparison to the dry diets, the nutritional advantage of *Artemia* nauplii concerned only larvae of less than 50 mg body weight, a weight reached after 6 days of feeding with the nauplii. Further investigations on the nutritional requirements of *H. longifilis* larvae should focus particularly on their first week of life.

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