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EFFECT OF THE DIETARY AA/EPA RATIO ON THE n-3 HUFA NEEDS OF GILTHEAD SEA BREAM (*SPARUS AURATA*) LARVAE FED MICRODIETS

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Introduction

Recent studies have demonstrated the importance of arachidonic acid (20:4n-6, AA) in fish larvae, as well as the importance of simultaneously considering the requirement of AA, eicosapentaenoic (20:5n-3, EPA), and docosahexaenoic (22:6n-3, DHA) acids (Bessonart, 1997; Sargent et al., 1999). Thus, besides the well documented importance of DHA for survival, growth, and development of several marine fish larvae, the elevation of dietary AA in gilthead sea bream larvae has been found to improve growth, survival (Bessonart et al., 1999), and resistance to handling stress (Koven et al., 2001).

In 15-day-old gilthead sea bream (*Sparus aurata*) larvae, n-3 highly unsaturated fatty acid (n-3 HUFA) requirement has been found to be variable, depending on factors such as the dietary DHA/EPA ratio (Bessonart, 1997) or the polar lipid content and n-3 HUFA content of polar lipids (Salhi et al., 1999).

The present work was conducted in order to study the effect of the AA/EPA ratio on the n-3 HUFA requirement of 16-day-old gilthead sea bream larvae fed microdiets.

Materials and methods

Nineteen-day-old gilthead sea bream larvae (5.63mm in total length) were fed for 20 days on two experimental microdiets differing in the total n-3 HUFA, EPA, and AA content, but containing the same amount of DHA (Table I) .

Microdiets were based on freeze-dried squid powder (Rieber and Son, Bergen, Norway) and lipid-extracted (73.5g·100g^{-1}diet), and contained 5.3g·100g^{-1}diet of vitamin mixture, 4.5g·100g^{-1} diet of mineral mixture, 3.0g·100g^{-1}diet of attractant, 3.0g·100g^{-1}diet of soybean lecithin, and free arachidonic acid (Sigma, St. Louis, USA), oleic acid (Merk, Darmstadt, Germany), and fish oils EPA28 and DHA27 (Nippai Co., Ltd, Tokyo, Japan). Dietary n-3 HUFA content was lower in Diet II due to a lower EPA level. This EPA reduction was compensated by increasing the amount of AA in Diet II (Table I).

Table I. Protein, lipid, and essential fatty acid content (% dry weight) of the experimental microdiets.

	Diet I	Diet II
Crude protein	79.3	79.7
Total lipids	15.1	12.8
Σ n-3 HUFA	1.91	1.49
DHA	1.08	1.00
EPA	0.82	0.46
AA	0.36	0.60
DHA / EPA	1.32	2.17
AA / EPA	0.44	1.30

Larvae were divided into groups of 2000 fish and distributed into six 100-l tanks (3 tanks per diet), where they were fed 1.0-2.0g·tank^{-1}·day^{-1} of the experimental microdiets using automatic feeders. Larvae were also fed rotifers (3.5×10^5 per tank twice a day) cultured on baker's yeast, which contained only trace amounts of n-3 HUFA. No microalgae were added to the rearing tanks. During the trial, temperature ranged from 19.5-20.0°C, and a photoperiod of 12h artificial light was maintained.

Larval growth and survival were assessed by measuring the total length of 30 fish per tank and individual counting of live fish at the end of the trial. The methodology used for lipid and fatty acid analyses is described in Salhi et al. (1999). A Student test was used for statistical comparison of means.

Results and discussion

After 20 days of feeding the experimental diets, larval growth (total length) and survival were not significantly affected ($P<0.05$) by the reduction of dietary n-3 HUFA from 1.91 % (Diet I) to 1.49% (Diet II) based on a reduction of the EPA content from 0.82 to 0.46% (Table II). In a previous study (Salhi, 1997), an experiment conducted under similar conditions – feeding 10-day-old gilthead sea

bream larvae with the same kind of microdiets – showed that a dietary n-3 HUFA reduction from 2.1 to 1.6%, based on a reduction of both EPA (from 0.73 to 0.57%) and DHA (from 1.27 to 0.94%) and maintaining a DHA/EPA ratio of ~1.7, resulted in a lower larval growth (larval total length and dry body weight).

Table II. Growth (mm total length), survival (%), and total, neutral, and polar lipid content (% dry weight) of the larvae.

	Initial	Diet I	Diet II
Total length	5.63 ± 0.61	8.64[a] ± 1.09	8.52[a] ± 0.66
Survival	–	28.8[a] ± 6.5	23.6[a] ± 7.6
Total lipids	17.37	15.79[a] ± 1.19	16.04[a] ± 0.31
Neutral lipids	7.72	6.45[a] ± 0.84	6.58[a] ± 1.56
Polar lipids	9.45	9.34[a] ± 1.34	9.38[a] ± 1.53

Values having the same letter within a row were not significantly different ($P<0.05$)

In the present study, dietary DHA was similar in both diets and the reduction of EPA was accompanied by an increase of AA in Diet II. Thus, it seems that a combination of increasing dietary AA and decreasing dietary EPA could result in an improved larval performance, reflected in this study in a lower need of n-3 HUFA in microdiets. In this regard, Bessonart et al. (1999) found that feeding 17-day-old gilthead sea bream larvae on microdiets containing ~2% n-3 HUFA and 0.7-0.8% EPA, the dietary inclusion of 1% AA resulted in an improved larval growth, while survival was improved by including 1.8% AA in the diet. Besides the importance of dietary AA as an essential fatty acid for these fish, an importance of the dietary AA/EPA ratio comes from the competitive interactions between these fatty acids – AA and EPA compete for the cyclo-oxygenase and lipoxygenases that produce eicosanoids from these fatty acids; the eicosanoids produced from AA are generally more biologically active than those produced from EPA, and the respective eicosanoids compete for the same cell membrane receptors (Sargent el al., 1999). Moreover, a competition between these 20C HUFA in phospholipid biosynthesis has also been observed in gilthead sea bream larvae fed microdiets with different AA/EPA ratios (Bessonart, 1997).

Total, polar, and neutral lipid content of the larvae (% dry basis) was not affected by the different diets. However, compared to the initial larvae, a slight reduction of larval total lipids mainly due to a reduction in neutral lipid content was observed, reflecting the low lipid content of the experimental microdiets (Table I).

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