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Effect of dietary arachidonic acid levels on growth and survival of gilthead sea bream (*Sparus aurata* L.) larvae

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Abstract

In order to determine the effect of different levels of arachidonic acid (AA) in microdiets on growth and survival of gilthead seabream larvae, two experiments were carried out. In the first experiment, 17-day old larvae were fed microdiets for 14 days. In this trial, we tested four diets with a similar docosahexaenoic acid (DHA)/eicosapentaenoic acid (EPA) ratio and AA in the range of 0.1%–1.8% dry weight (d.w.) and a fifth diet with 1.0% d.w. of AA and a different DHA/EPA ratio. In the second experiment, 16-day old larvae were fed for 21 days on two microdiets with levels of 0.1% and 1.0% d.w. AA. All diets in the two experiments had the same total $n-3$ HUFA level. Growth was measured by total length and dry body weight of larvae. Larval lipids were extracted and fatty acid compositions of total neutral lipid (NL) and total polar lipid (PL) fractions were determined by gas chromatography. In the first experiment, the diet containing 1.8% d.w. of AA showed the best survival ($P < 0.05$) but did not improve growth rate. In the second experiment, an increase in the AA dietary level from 0.1% to 1.0% d.w. significantly improved ($P < 0.05$) larval growth. EPA accumulation in the PLs of the larvae was negatively affected by the inclusion of dietary AA. When working with a fixed dietary $n-3$ HUFA level (2.2% d.w.), the effect of dietary AA on larval growth was masked by that of the dietary DHA/EPA ratio. However, when DHA/EPA ratio in diet was unchanged (~ 1.8), it was

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possible to improve larval growth by supplementing the diet with 1.0 d.w. of AA. The negative effect produced by the increment of dietary AA on the EPA incorporation into the larval PLs may be related to a competition interaction.

Keywords: *Sparus aurata* larvae; Arachidonic acid; Growth; DHA/EPA ratio

1. Introduction

It has been demonstrated that marine fish larvae require $n-3$ highly unsaturated fatty acids ($n-3$ HUFA), mainly eicosapentaenoic (EPA, 20:5 $n-3$) and docosahexaenoic (DHA, 22:6 $n-3$) acids, for normal development and survival (Izquierdo et al., 1989; Koven et al., 1989, 1992; Mourente et al., 1993; Rodríguez et al., 1993; Watanabe, 1993; Rodríguez, 1994; Salhi et al., 1994; Izquierdo, 1996; Furuita et al., 1996). The essentiality of this fatty acids is based on the important structural role they play as membrane phospholipid components, together with the inability of marine fish to synthesize EPA and DHA from linolenic acid (18:3 $n-3$).

Despite the same limitation in the conversion of linoleic acid (18:2 $n-6$) to $n-6$ highly unsaturated fatty acids ($n-6$ HUFA) (Sargent et al., 1994), $n-6$ fatty acids (other than 18:2 $n-6$) have received little attention in feeding studies in marine fish nutrition (Castell et al., 1994; Bell et al., 1995; Zheng et al., 1996). Among $n-6$ HUFA, several studies have pointed out the importance of arachidonic acid (AA, 20:4 $n-6$) in fish metabolism since it is known to be the main precursor fatty acid of eicosanoids in fish (Henderson and Sargent, 1985; Henderson et al., 1985; Bell et al., 1994; Sargent et al., 1994) and it is also one of the main components of phosphatidylinositol (PI) (Bell and Dick, 1990; Bell et al., 1983), where it is incorporated more specifically than into any other lipid class (Linares and Henderson, 1991).

AA is released from membrane phospholipids in response to hormone stimulation in different tissues, and can act as a second messenger in activating protein kinase C (Mc Phail et al., 1984) or can be converted to any number of compounds including prostaglandins, thromboxanes, hidroxyeicosatetraenoic acids and leukotrienes (Smith, 1989). All these molecules are very active even at low physiological concentrations and play an important role during larval development. Nevertheless, despite having important functions as components of cell membranes and being a precursor of these substances, AA is present in very limited amounts in fish tissues, making it difficult to determine quantitative requirements for this fatty acid.

The aim of the present work was to study the effects of different levels of AA in microdiets on growth, survival and biochemical composition of gilthead seabream larvae.

2. Materials and methods

Two trials were carried out feeding *Sparus aurata* larvae squid meal-based experimental microdiets (Salhi et al., 1994) differing only in their fatty acid composition. To

obtain the desired fatty acid profile, squid meal was previously defatted three times with chloroform. Fish oils, EPA 28 and DHA 27 (Nippon Chemical Feed, Tokyo, Japan) were used as sources of EPA and DHA, respectively. AA was obtained from Sigma, St. Louis, USA. Lipid and fatty acid composition of microdiets are shown in Table 1. In the first trial, 17-day old larvae were fed microdiets for 14 days. In this trial, five diets were tested, four with AA content from 0.1% to 1.8% d.w. and similar DHA and EPA contents (DHA/EPA = 1.8) and a fifth diet with 1.0% d.w. of AA and DHA/EPA ratio of 0.7 (Table 1) included to observe AA effect under different EPA contents in the diet. In the second experiment, 16-day old larvae were fed for 21 days two microdiets with levels of 0.1%–1.0% d.w. AA and DHA/EPA ratio around 1.8. In both experiments, all diets had the same total $n-3$ HUFA level.

Gilthead seabream larvae were obtained from natural spawning of the broodstock of the Instituto Canario de Ciencias Marinas. Larvae were reared in 100 l cylindrical tanks filled with seawater filtered with 50 μm net. Tanks were provided with central constant aeration and the water flow was 450 ml min^{-1} (around 6.5 water exchanges per day). Larvae were fed microdiets at a rate of 1.5–2.0 g day^{-1}. The diet was supplied by automatic feeders for 12 h day^{-1}. Rotifers (*Brachionus plicatilis*) fed baker's yeast, which had no HUFA content, were added twice a day to keep a density of 3.5 ind. ml^{-1}. Microalgae were no added to experimental tanks. A photoperiod of 12 h artificial light was maintained during the experiments. A total of 2700 and 3000 larvae per tank (individually counted) were used in the first and second experiment, respectively. Water temperature was 17°–19°C in experiment I and 19°–21°C in experiment II.

Table 1
Crude lipid and selected fatty acid content of TL of the experimental diets (% d.w.). tr.: traces

Fatty acids	Experiment I					Experiment II	
	1A	1B	1C	1D	1E	2A	2B
16:0	1.3	1.5	1.4	1.4	1.1	1.6	1.5
18:0	0.3	0.3	0.3	0.3	0.2	0.3	0.3
18:1$n-9$	4.7	5.0	4.3	3.1	4.7	5.3	4.3
18:2$n-6$	1.5	1.7	1.6	1.6	1.6	1.8	1.7
20:2$n-6$	tr.	tr.	tr.	tr.	tr.	tr.	tr.
20:4$n-6$	0.1	0.2	1.0	1.8	1.1	0.1	1.0
20:4$n-3$	tr.	tr.	tr.	tr.	tr.	tr.	tr.
20:5$n-3$	0.7	0.7	0.7	0.7	1.1	0.8	0.8
22:5$n-6$	tr.	tr.	tr.	tr.	tr.	0.1	0.1
22:5$n-3$	0.1	0.1	0.1	0.1	0.1	0.1	0.1
22:6$n-3$	1.2	1.3	1.3	1.3	0.8	1.4	1.4
Saturated	2.0	2.2	2.1	2.1	1.7	2.3	2.2
Monoenoic	5.6	6.1	5.3	4.3	5.7	6.4	5.2
$\Sigma n-3$	2.3	2.6	2.6	2.5	2.6	2.8	2.7
$\Sigma n-6$	1.7	2.0	2.7	3.5	2.7	2.0	2.7
$\Sigma n-9$	4.8	5.1	4.4	3.2	4.8	5.4	4.4
$\Sigma n-3$ HUFA	2.0	2.2	2.1	2.1	2.1	2.4	2.3
DHA/EPA	1.8	1.8	1.8	1.8	0.7	1.8	1.8
EPA/AA	7.0	3.5	0.7	0.4	1.0	8.0	0.8
TL	15.7	15.6	16.6	18.8	17.2	17.4	16.6

Treatments were tested in triplicate. Survival was calculated by individual counting of all the surveying larvae at the beginning and the end of the experiments. Larval growth was determined by measuring at least 30 larvae of each tank, at the beginning, the middle and the end of the feeding trials, by a profile projector (Nikon V-12 A).

Samples of diets, larvae and rotifers were frozen at −80°C for biochemical analysis. Total lipids (TLs) were extracted according to Folch et al. (1957), polar lipid (PL) and neutral lipid (NL) were separated by adsorption chromatography on silica cartridges (Juaneda and Rocquelin, 1985). For fatty acid analysis, heneicosanoic acid (21:0) was added to the crude lipid as an internal standard, lipids were methylated by transesterification with H_2SO_4 in methanol solution (Christie, 1982) and purified on NH_2 silica cartridges (Sep-Pak®, Van RC, Waters, Milford MA, USA) (Fox, 1990). Fatty acid methyl esters were analyzed using a Shimadzu GC-14A gas chromatograph (Shimadzu, Kyoto, Japan) with flame ionization detector and a Supelcowax-10 fused-silica capillary column (30 m × 0.32 mm I.D., Supelco, Bellefonte, PA, USA) using helium as carrier gas. Column temperature was 180°C during 10 min, increasing afterwards to 215°C at a rate of 2.5°C min^{-1} and maintained at 215°C for 15 min. Fatty acids were identified by reference to a well-characterized fish oil.

The statistical analyses were conducted using a one way analysis of variance (ANOVA) and Tukey test for multiple comparison of means (Sokal and Rohlf, 1981). Linear regression analyses were performed on several diet-larvae parameters.

3. Results

In experiment I, 31-day old larvae fed the different AA content microdiets (diet 1A to 1D) did not show significant differences in growth after 14 days of experiment. Larvae

Table 2
Growth and survival of larvae fed microdiets containing different AA levels. Values having the same superscript letter within each column and experiment are not significantly different ($P < 0.05$)

Experiment I	Diet	Larval age (days)	17	31	17	31	31
			Total length (mm)		Dry body weight (μg)		Survival (%)
	1A		5.03	5.76[a]	86	110	8.7[a]
	1B		5.03	5.71[a]	86	112	10.0[a]
	1C		5.03	5.86[a]	86	128	19.0[ab]
	1D		5.03	5.80[a]	86	124	31.6[b]
	1E		5.03	5.54[b]	86	102	11.6[a]
Experiment II		Larval age (days)	16	37	16	37	37
	2A		5.33	7.30[a]	122	302	31.2
	2B		5.33	7.48[b]	122	318	35.6

Table 3
Crude lipid and selected fatty acid content of TLs of the larvae (% of total fatty acids). (*) % d.w. tr.: traces

Fatty acid	Initial	Treatment					Initial	Treatment	
		1A	1B	1C	1D	1E		2A	2B
16:0	18.0	14.5±0.9	13.4±0.4	14.5±0.2	13.3±0.6	13.6±0.4	17.7	15.0±0.7	14.9±0.2
16:1$n-7$	3.5	9.1±1.0	10.3±0.7	9.6±1.0	10.3±0.6	9.1±0.2	3.6	12.4±0.4	12.4±0.3
18:0	10.6	10.9±0.3	10.8±0.2	11.0±0.3	10.9±0.1	11.4±0.1	9.0	9.3±0.2	9.2±0.0
18:1$n-9$	12.6	14.4±0.2	15.6±0.5	15.3±0.6	14.8±0.9	14.0±0.2	13.8	21.2±0.4	21.1±0.3
18:2$n-6$	1.8	2.7±0.2	2.4±0.0	2.4±0.1	2.2±0.1	2.4±0.3	2.5	2.5±0.1	2.3±0.1
20:1$n-9$	0.7	3.0±0.2	1.9±0.1	2.1±0.3	2.5±0.6	2.7±0.4	0.7	0.4±0.1	0.3±0.0
20:2$n-6$	1.5	1.2±0.2	1.0±0.1	0.9±0.1	1.1±0.2	1.4±0.0	2.4	1.6±0.9	0.6±0.1
20:4$n-6$	3.9	3.7±0.4	4.1±0.5	4.8±0.2	5.7±0.2	5.1±0.0	4.4	2.0±0.1	3.4±0.1
20:5$n-3$	8.8	2.7±0.1	3.2±0.2	2.9±0.2	2.6±0.1	2.9±0.2	2.7	2.6±0.0	2.4±0.1
22:1	tr.	1.9±0.4	1.0±0.1	1.2±0.2	1.6±0.5	1.8±0.4	0.3	0.5±0.1	0.3±0.0
22:4$n-9$	0.3	1.7±0.4	0.9±0.0	1.1±0.2	1.3±0.4	1.4±0.3	0.4	tr.	0.3±0.1
22:5$n-3$	5.1	1.5±0.0	1.8±0.1	1.6±0.1	1.7±0.2	1.7±0.1	10.0	1.2±0.1	1.3±0.0
22:6$n-3$	21.8	7.8±0.2	9.1±0.8	8.5±0.5	7.9±0.2	7.2±0.5	16.8	8.2±0.2	8.2±0.3
24:1	0.3	1.4±0.1	1.4±0.0	1.4±0.1	1.9±0.5	1.7±0.2	0.5	1.2±0.1	1.2±0.1
Saturated	31.1	29.6±0.9	27.0±0.2	28.1±0.6	26.9±0.6	28.8±0.4	29.1	26.3±0.9	26.0±0.3
Monoenoic	19.1	37.2±0.5	39.4±1.5	38.2±1.2	39.3±0.6	37.2±1.2	21.3	45.5±0.7	45.1±0.7
$\Sigma n-3$	39.9	12.9±0.3	14.9±1.0	13.9±0.8	13.0±0.7	12.5±0.8	34.2	12.8±0.1	12.8±0.4
$\Sigma n-6$	8.1	8.7±0.5	8.5±0.6	9.0±0.2	10.2±0.2	9.8±0.2	10.3	6.7±0.8	6.9±0.1
$\Sigma n-9$	10.7	19.6±0.4	19.0±0.6	19.3±0.4	19.2±0.2	18.5±0.8	11.8	22.9±0.1	22.8±0.2
$\Sigma n-3$	36.1	12.1±0.3	14.2±1.0	13.1±0.9	12.3±0.5	12.0±0.8	33.5	12.1±0.1	12.0±0.4
HUPA									
EPA/AA	2.5	0.7±0.1	0.8±0.1	0.6±0.0	0.5±0.0	0.6±0.0	1.8	1.3±0.1	0.7±0.0
DHA/EPA	2.3	2.9±0.2	2.8±0.1	3.0±0.1	3.1±0.1	2.5±0.0	2.3	3.1±0.1	3.4±0.1
TLs (*)		19.5	20.1	22.3	18.9	23.0		16.2	16.5
NLs (*)		11.2	9.7	11.4	10.1	13.3		7.6	6.9
PLs (*)		8.3	10.4	12.9	8.8	9.7		8.7	9.6

Table 4
Selected fatty acid content of PLs of the larvae (% total fatty acids). n.d.: non-detected. tr.: traces

Fatty acid	Treatment						
	1A	1B	1C	1D	1E	2A	2B
16:0	17.4±0.5	16.8±0.7	17.3±0.3	17.3±0.2	17.0±0.4	18.4±0.6	18.1±0.2
16:1$n-7$	8.3±0.9	8.7±0.6	8.8±0.5	9.2±0.7	8.0±0.2	9.6±0.3	9.2±0.4
18:0	11.0±0.3	10.9±0.4	10.9±0.1	10.7±0.3	11.4±0.3	9.6±0.2	9.8±0.0
18:1$n-9$	15.9±0.3	15.9±0.7	15.9±0.3	15.5±0.3	15.7±0.4	20.6±0.4	20.1±0.4
18:2$n-6$	2.9±0.3	2.5±0.1	2.5±0.2	2.1±0.1	2.2±0.1	2.7±0.1	2.5±0.1
20:1$n-9$	1.0±0.0	0.91±0.0	1.0±0.0	0.9±0.0	0.9±0.1	1.0±0.1	1.1±0.0
20:2$n-6$	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0	0.8±0.0	0.7±0.0
20:4$n-6$	2.7±0.1	3.0±0.2	4.2±0.3	5.2±0.4	4.0±0.0	2.0±0.2	3.8±0.1
20:4$n-3$	0.3±0.0	0.3±0.0	0.2±0.0	0.2±0.0	0.3±0.0	n.d.	tr.
20:5$n-3$	4.1±0.1	4.1±0.3	3.6±0.2	3.4±0.0	4.2±0.1	3.3±0.1	3.1±0.0
22:4$n-9$	0.3±0.0	0.3±0.0	0.3±0.0	0.4±0.0	0.3±0.0	n.d.	tr.
22:5$n-3$	2.3±0.1	2.2±0.1	2.1±0.0	2.1±0.0	2.4±0.1	1.7±0.1	1.8±0.0
22:6$n-3$	11.8±0.6	11.7±1.4	10.9±0.2	10.5±0.6	10.4±0.4	11.2±0.6	10.8±0.5
24:1	0.5±0.0	0.5±0.1	0.5±0.1	0.5±0.0	0.4±0.1	0.6±0.0	0.6±0.0
Saturated	31.1±0.5	30.0±0.2	30.5±0.6	30.2±0.3	30.9±0.8	29.9±0.3	29.8±0.3
Monoenoic	34.5±0.7	35.2±1.5	35.0±0.1	35.4±1.2	34.7±0.8	38.9±0.8	38.1±1.0
$\Sigma n-3$	19.7±0.6	19.6±1.9	18.2±0.2	17.5±0.6	18.4±0.4	17.4±0.6	17.0±0.6
$\Sigma n-6$	7.0±0.3	7.0±0.3	8.1±0.3	8.7±0.5	7.7±0.1	6.0±0.1	7.7±0.2
$\Sigma n-9$	17.9±0.2	17.8±0.7	17.9±0.3	17.6±0.3	17.7±0.4	22.1±0.5	21.5±0.4
$\Sigma n-3$ HUFA	18.4±0.6	18.3±1.8	16.9±0.2	16.2±0.7	17.2±0.5	16.2±0.6	15.8±0.6
EPA/AA	1.6±0.1	1.4±0.1	0.9±0.1	0.6±0.0	1.0±0.0	1.6±0.2	0.8±0.0
DHA/EPA	2.9±0.1	2.8±0.2	3.0±0.2	3.1±0.2	2.5±0.0	3.4±0.2	3.5±0.2

Table 5
Selected fatty acid content of NLs of the larvae (% total fatty acids)

Fatty acid	Treatment						
	1A	1B	1C	1D	1E	2A	2B
16:0	12.4±1.7	9.8±0.3	10.7±0.3	9.8±1.0	11.1±0.4	11.1±0.9	10.5±0.1
16:1$n-7$	9.7±1.9	12.0±0.9	10.8±1.9	11.3±1.3	9.8±0.5	15.6±1.0	16.7±0.2
18:0	10.9±0.8	10.7±0.0	11.2±0.7	11.1±0.2	11.4±0.3	8.8±0.7	8.4±0.0
18:1$n-9$	13.2±0.5	15.2±0.6	14.5±1.2	14.2±1.8	12.8±0.1	21.8±0.8	22.6±0.1
18:2$n-6$	2.6±0.1	2.2±0.0	2.3±0.1	2.3±0.2	2.5±0.4	2.2±0.1	1.9±0.1
20:1$n-9$	4.5±0.5	2.9±0.1	3.8±0.6	3.8±1.2	4.0±0.7	0.9±0.2	0.8±0.1
20:2$n-6$	1.6±0.5	1.4±0.2	1.5±0.3	1.7±0.3	2.0±0.0	2.6±1.9	0.4±0.2
20:4$n-6$	4.5±0.9	5.3±1.0	5.6±0.8	6.1±0.4	5.8±0.0	1.9±0.1	2.8±0.0
20:5$n-3$	1.7±0.3	2.2±0.0	1.9±0.4	1.9±0.2	2.0±0.2	1.8±0.1	1.6±0.1
22:1	3.3±0.8	2.1±0.2	2.8±0.5	2.9±0.9	3.1±0.7	1.1±0.2	0.8±0.1
22:4$n-9$	2.7±0.9	1.6±0.0	2.1±0.4	2.2±0.8	2.1±0.5	0.5±0.1	0.5±0.0
22:5$n-3$	0.8±0.0	1.3±0.1	0.9±0.3	1.3±0.4	1.2±0.1	0.6±0.1	0.6±0.0
22:6$n-3$	4.9±0.1	6.3±0.3	5.1±1.4	5.7±0.5	5.0±0.7	4.7±0.2	4.5±0.0
24:1	2.0±0.2	2.4±0.0	2.7±0.3	3.0±0.9	2.7±0.5	2.0±0.3	2.0±0.2
Saturated	29.0±1.4	24.2±0.5	25.6±1.0	24.5±0.8	27.5±0.1	22.7±1.5	21.6±0.4
Monoenoic	39.2±1.1	43.9±2.2	42.5±2.9	42.6±0.9	39.0±1.4	53.0±1.9	55.0±0.6
$\Sigma n-3$	7.8±0.3	9.8±0.4	8.0±1.9	9.1±1.2	8.2±1.0	7.4±0.5	6.9±0.1
$\Sigma n-6$	10.0±1.2	10.0±1.2	10.3±0.9	11.5±0.3	11.3±0.3	7.5±1.7	5.9±0.2
$\Sigma n-9$	20.9±0.8	20.3±0.8	21.1±1.0	20.5±0.1	19.1±1.1	23.8±0.5	24.7±0.0
$\Sigma n-3$ HUFA	7.4±0.4	9.8±0.4	7.8±2.0	8.9±1.0	8.2±1.0	7.3±0.5	6.8±0.0
EPA/AA	0.4±0.0	0.4±0.1	0.3±0.0	0.3±0.0	0.3±0.0	1.0±0.0	0.6±0.0
DHA/EPA	3.0±0.4	2.8±0.1	2.7±0.2	3.0±0.0	2.5±0.0	2.5±0.0	2.9±0.2

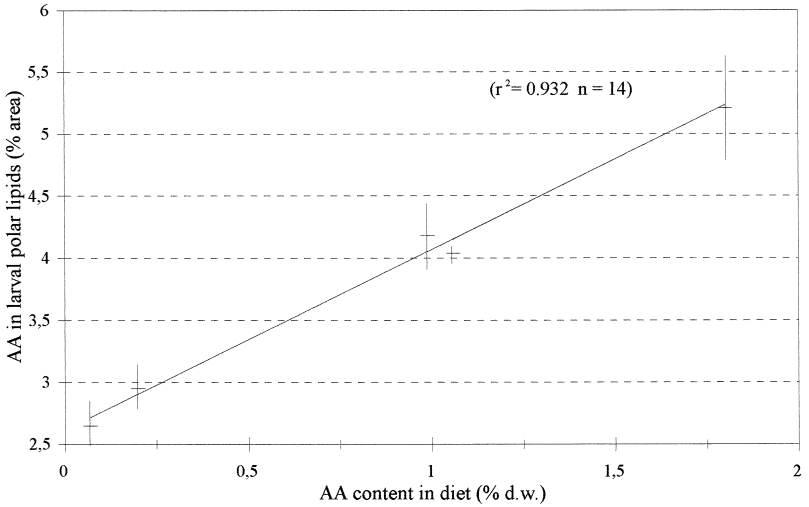

Fig. 1. Effect of different dietary AA levels on AA accumulation in larval PLs.

fed diet 1E, with 1% d.w. of AA but with a lower DHA/EPA ratio (0.7) than the other diets (1.8), presented the smallest ($P < 0.05$) size at the end of the experiment (Table 2). Larvae fed diet 1D containing 1.8% d.w. of AA showed a better survival than those fed diets 1A or 1B, with 0.1% and 0.2% d.w. of AA, respectively (Table 2).

In the second, and longer experiment, an increase in the AA level in the diets from 0.1% to 1.0% d.w. significantly improved ($P < 0.05$) larval growth after 21 days of feeding the microdiets.

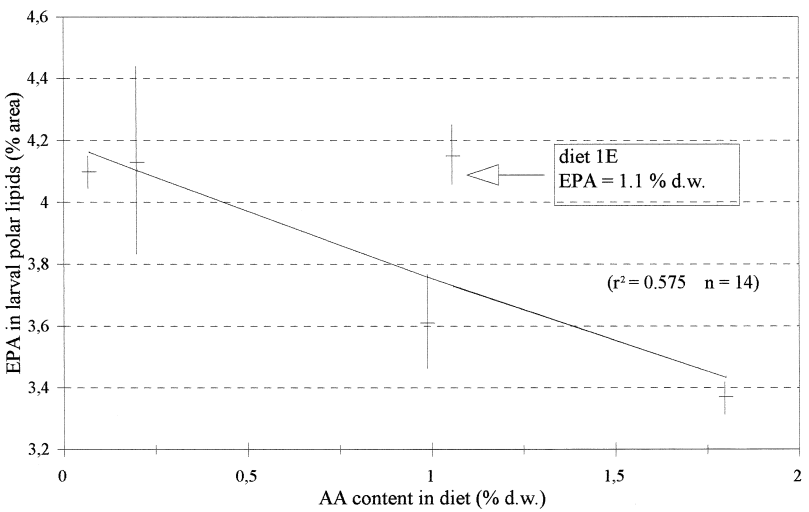

Fig. 2. Effect of different dietary AA levels on EPA accumulation in larval PLs.

Larval TL content and fatty acid composition are presented in Table 3. In the first experiment, a significant correlation ($P < 0.05$) was found between AA levels of the diet and the AA content in the larval TL. In experiment II, larvae fed diet 2B (AA = 1.0% d.w.) showed higher AA levels ($P < 0.05$) in TL than larvae fed diet 2A (AA = 0.1% d.w.).

The fatty acid composition of the PL and NL fractions are shown in Tables 4 and 5, respectively. As observed in the TL, AA contents of the NL and PL reflected those of the diets, being positively correlated ($P < 0.05$) with the dietary values (Fig. 1).

The larval EPA content was negatively affected by the AA level of the diet. In experiment I, a decrease in the EPA amount in larval PL was observed when the AA level in the diet was increased, the EPA level was negatively correlated ($P < 0.05$) with the amount of AA in the diet (Fig. 2). In experiment II, larvae fed diet 2B (1.0% d.w. AA) presented significantly lower amounts of EPA in PL than those fed diet 2A (0.1% d.w. AA). The amount of AA in larval PL did not seem to be affected when the EPA content in the diet was increased from 0.7% to 1.1% d.w.

4. Discussion

Investigations on marine fish nutrition conducted to determine the effect of AA on fish growth and survival are scarce. Zheng et al. (1996) reported that cod larvae fed enriched *Artemia* with different AA levels (3.7%–7.6% d.w.) showed poorer survival and slower growth than those larvae fed *Artemia* with less than 0.5 d.w. AA level. Castell et al. (1994) fed juvenile turbot for 11 weeks and found a rapid response to dietary supplementation with AA during the first 2 weeks. However, a compensatory growth response was observed during the rest of the feeding trial, because they did not observe differences in growth with fish fed a non-AA supplemented diet at the end of the experiment, suggesting that the fish were deficient in this fatty acid at the beginning of the experiment.

A growth promoting effect of AA was observed in the present study after 3 weeks feeding the experimental microdiets. In experiment I, the increase in AA level from 0.1% to 1.0% d.w. after 14 days feeding slightly increased growth rate but not significantly, perhaps suggesting that a longer feeding period could show an effect of AA acid supplementation on growth. Thus, a 3-week feeding trial was conducted, and the increase in the AA level from 0.1% to 1.0% d.w. significantly improved larval growth. Since all the diets had a similar $n-3$ HUFA content and the same DHA/EPA ratios (with the exception of diet 1E that showed the lowest growth), the consistently good survival of fish fed diet 1D and the bad performance of those fed diets 1A and 1B, and the better growth observed in larvae fed diet 2B (high AA) than 2A, confirms the importance of AA for gilthead seabream larvae.

Besides the importance of dietary AA, the importance of dietary DHA/EPA ratio was also marked, since larvae fed diet 1E showed the lowest growth in experiment I, in spite of the high dietary AA content (1.0% d.w.). The significance of the DHA/EPA ratio in diets for gilthead seabream larvae has already been reported (Rodríguez et al.,

1997). The results showed that, at least for larval growth and survival, the possible effect of AA could be masked by the influence of dietary DHA/EPA ratio.

AA has been described as one of the main components of certain phospholipids, specifically PI (Bell and Tocher, 1989; Bell and Dick, 1990; Bell et al., 1983). In larval gilthead seabream, the AA content in the PL fraction of the larvae was increased by the increase in dietary AA level, in agreement with the data reported by Bell et al. (1995). The EPA incorporation in the PL fraction of the larvae has also been related to the EPA content in the diet (Reitan et al., 1994). Looking for possible interaction between dietary EPA and AA values on larval composition, we observed that EPA incorporation in the PL fraction of the larvae was negatively affected by dietary AA content. Competition between EPA and AA during phospholipid esterification has been suggested to occur in fish (Tocher and Sargent, 1986; Bell et al., 1995), particularly in PI where both fatty acids are major components. The results of the present study are consistent with the competition between the two fatty acids for incorporation into PLs. Since both AA and EPA released from PI by phospholipase A_2 action are precursors of eicosanoids (Henderson et al., 1985), their respective incorporation into such PL fractions may have an influence on the synthesis of different types of eicosanoids.

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