Arachidonic acid, arachidonic/eicosapentaenoic acid ratio, stearidonic acid and eicosanoids are involved in dietary-induced albinism in Senegal sole (*Solea senegalensis)*

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Keywords: Arachidonic acid; ARA/EPA ratio; Prostaglandins; Pigmentation; *Solea senegalensis*; *Artemia* enrichment

Abstract

Senegal sole larvae were fed live prey enriched with different amounts of arachidonic acid (ARA, 20:4n-6) and eicosapentaenoic acid (EPA, 20:5n-3) to re-evaluate the effect of these two fatty acids on flatfish pigmentation. Echium oil, a plant derived oil rich in gamma-linolenic acid (GLA, 18:3n-6) and stearidonic acid (SDA, 18:4n-3) was also used as a component of one of the enrichment emulsions. Although ARA content did not have any effect on growth there was a clear influence on pigmentation that correlated clearly with prostaglandin production. Inclusion of Echium oil, on the contrary, exerted a positive effect on pigmentation rate even though dietary ARA levels were as high as in the other emulsions. The relationships between dietary ARA levels and dietary ARA/EPA ratio, prostaglandin production and pigmentation are discussed.

Introduction

Arachidonic acid (20:4n-6, ARA) and eicosapentaenoic acid (20:5n-3, EPA) are considered essential for marine fish because both are precursors of eicosanoids (Sargent et al., 1999a) a group of extremely diverse and complex array of physiologically highly active, hormone-like compounds, which are involved in a great variety of regulatory processes in the body (Smith, 1989). The eicosanoids are mainly derived from the n-6 and n-3 C20 precursor fatty acids dihomo-gamma-linolenic acid (20:3n-6, DGLA), ARA, eicosatetraenoic acid (20:4n-3) and EPA. However, ARA-derived eicosanoids are generally the most abundant and bioactive whereas those produced from EPA are of lower efficacy (Tocher, 2003). The relative abundance of the two fatty acids ARA and EPA, or more specifically the amounts of the free acids, released from membrane phospholipids, available for enzymatic conversion, determines eicosanoid potency and mode of action. In fishes, ARA has been found to improve resistance to handling stress in gilthead seabream and striped bass (Koven et al., 2001) and maximizes stress resistance to hypersaline challenge in larval summer flounder (Willey et al., 2003). On the other hand, ARA has also been associated with malpigmentation problems in flatfish (summarized by Bell et al., 2003; Villalta et al., 2005) via a biochemical induced stress and over production of eicosanoids (Sargent et al., 1999b) although this association between eicosanoid production and pigmentation has not been clearly substantiated. In mammals, eicosanoids of the n-3 series have an anti-inflammatory effect, while most of those from the n-6 family are pro-inflammatory.

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Fears about declining fish stocks, coupled with the putative risks of pollutants from oily fish, have encouraged researchers and industry to investigate the extraction of omega-3 fatty acids from alternative sources, including plants and microorganisms that might be used to alleviate the problem (Miller et al., 2005). Different types of vegetable

oils have been used for marine fish rearing with good results, in terms of survival and growth, although they do not provide the n-3 HUFA that provide the cardiovascular protection and other health beneficial properties associated with fish consumption (Izquierdo et al., 2003; SACN/COT, 2004). In the case of larval rearing, the use of vegetable oils in the formulation of live prey enrichment emulsions has been neglected because of the usually high requirement of most marine fish larvae for n-3 HUFA. Echium oil is a complex triglyceride obtained by extracting the oil from the seeds of *Echium plantagineum*, a naturally occurring plant. The occurrence of SDA, a rare PUFA of the n-3 series that can be elongated and desaturated to EPA more effectively than alpha-linolenic acid (18:3n-3, ALA), exceed those of the n-6 equivalent, gamma-linolenic acid (18:3n-6, GLA), and has been extensively used in humans due to its hypotriglyceridemic properties (Surette et al.. 2004) and therapeutic activity on chronic diseases and inflammation (Coupland and Hebard. 2002).

Thus, the aim of this study was to further understand the importance of ARA and the ARA/EPA ratio on pigmentation and eicosanoid production in Senegal sole larvae. Oils used in emulsions for *Artemia* were specifically chosen to produce high or low ARA concentrations, a high ARA concentration but also with high EPA content (Croda), and a high ARA content but with high stearidonic acid (18:4n-3, SDA) content (Echium). These combinations would allow assessment of fatty acids with potent antagonistic activity towards ARA, thus alleviating previously reported problems on pigmentation in ARA-rich diets.

2. Material and methods

2.1. Experimental emulsions

Four experimental emulsions were prepared mixing commercially available DHA, ARA and EPA-rich oils and echium oil obtained respectively from the heterotrophically grown algae, *Crypthecodinium cohnii* (Neuromins®, Martek Bioscience, USA), fungus, *Mortierella alpina* (Vevodar®, DSM Food Specialties, Netherlands), purified fish oil (Croda Oleochemicals EPA500TG®) and the Boraginaceae, *Echium plantagineum* (Technology Crops Ltd., Braintree, Essex, England) with olive and vegetable oils, soy lecithin and alpha-tocopherol. The components used in the formulation of each emulsion and the major fatty acid composition in the emulsions and in the *Artemia* metanauplii after enrichment are shown in Tables 1 and 2. Oil mixtures were emulsified with equal amounts of distilled water by homogenising with an Ultra-turrax T25 at high speed for 60 seconds. The emulsion was then transferred to plastic syringes, the air removed after which the syringes were cooled on ice and kept in a refrigerator (4ºC), in an upright position.

2.2. Live food enrichment

Rotifers were enriched in 10 l containers at a density of 500 rotifers ml^{-1} for 12 h at 20ºC using 0.1 g l^{-1} of all emulsions. After 12 h, the rotifers were gently washed with UV filtered seawater, rinsed for a further 1 min with freshwater to reduce bacterial loads, and subsequently fed to the larvae.

Six hour-old *Artemia* nauplii (EG strain, INVE) were enriched in 10 l containers at 100 naupli ml^{-1} for 18 h at 26ºC with 0.6 g l^{-1} of the emulsions. Enriched metanauplii were thoroughly washed with UV filtered seawater and freshwater (15 min) before feeding to the larvae.

Rotifers and *Artemia* were sampled three times during the experimental period for lipid analysis.

2.3. Sole larviculture

Senegal sole (*Solea senegalensis*) larvae were generously provided by the Spanish Institute of Oceanography at Santander and transported by road to the Institut de Recerca i Tecnologia Agroalimentàries (IRTA) at San Carlos de la Rápita. The larvae were distributed (50 larvae l^{-1}) into twelve 35 l, 150 μm mesh baskets distributed amongst four, 1500 l holding tanks. The tanks were connected to a recirculation unit under the conditions described elsewhere (Villalta et al., 2005) assuring the same water quality and lighting to all the tanks. Larvae were fed rotifers from 3 to 9 dph at a density of 10 rotifers ml^{-1} and *Artemia* nauplii from 5 until 40 dph. Triplicate tanks of larvae were fed each of the experimentally enriched live feeds. The *Artemia* ration was adjusted as in Villalta et al. (in press) to avoid unenriched leftovers (70% body weight (BW) day^{-1} from 5 to 15 dph, 20% BW day^{-1} from 16 to 25 dph, 15% BW/day^{-1} from 26 to 30 dph and 7.5 % BW day^{-1} from 31 to 35 dph). Live preys were distributed twice per day (9.00 and 16.00 h). Water changes (200%) in the holding tanks were performed daily.

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When the larvae started to settle down in the bottom of the tanks, freshly enriched *Artemia* metanauplii were substituted by frozen enriched *Artemia* following the method of Morais et al. (2005)

Standard length and dry weight were measured at 1, 4, 6, 10, 12, 15, 20, 30 and 40 dph. Twenty larvae were sampled at each time point, placed in beakers and euthanased using a lethal concentration of 3-amino benzoate methane sulphonate (1000 mg/l, MS 222). Length was measured using a dissecting microscope and an image

analyser (AnalySIS, SIS Gmbh, Germany). Dry weight (DW) determination was carried out by rinsing larvae with distilled water to remove salt and then oven-dried at 60ºC for 24h. Eye migration during sole metamorphosis was assessed according to the description of Cañavate and Fernandez-Diaz (1999) and the percentage of larvae at each stage of development at the same age was calculated as well as the eye migration index (I_{EM}, Solbakken et al., 1999).

For an assessment of final biochemical composition, 20 larvae basket^{-1} were sampled at 40 dph and stored in chloroform/methanol (2:1, v:v) under nitrogen at –20ºC until lipid analysis. A further 10 pigmented and albino larvae (if available) were taken from each basket for eicosanoid analysis, put into vials containing Hank's balanced salt solution (HBSS, Sigma) with 15% (v/v) ethanol and 5% (v/v) formic acid (2 M) to reduce pH and prevent further production or metabolism of prostaglandins (Powell, 1982) and frozen until analysis. Analysis of prostaglandins (PG) was carried out by enzyme immunoassay (EIA) using commercial kits for prostaglandin E_2 (PGE_2, Cayman Chemical, MI, USA, catalog No 514010) and F_2 (PGF_2, Cayman Chemical, MI, USA, catalog No 516011) after extraction and purification following Bell et al (1994) and manufacturer's instructions. Values are expressed as nanograms (ng) per gram dry matter (DM) of the fish as a whole group and according to the pigmentation (analysing separately albino and pigmented fish).

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Survival and final pigmentation pattern were assessed at the end of the experiment (40dph).

2.4. Lipid analysis

Total lipids from enriched live food and larval tissues were extracted in chloroform/methanol (2:1, v:v) using the method of Folch et al. (1957), and quantified

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gravimetrically after evaporation of the solvent under a stream of nitrogen followed by vacuum desiccation overnight. Total lipids were stored in chloroform/methanol (2:1, 20 mg ml^{-1}) containing 0.01% butylated hydroxytoluene (BHT) at –20ºC until final analysis.

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Acid catalysed transmethylation was carried out using the method of Christie (1982). Methyl esters were extracted twice using isohexane/diethyl ether (1:1, v:v), purified on TLC plates (Silica gel 60, VWR, Lutterworth, UK) and analysed by gas-liquid chromatography on a Thermo Electron Trace GC (Winsford, UK) instrument fitted with a ZB-Wax capillary column (30 m x 0.32 mm id; Phenomenex, Macclesfield, UK), using a two-stage thermal gradient from 50ºC (injection temperature) to 150ºC at 40ºC min^{-1} and holding for 5 min at 225ºC after increasing at 2ºC min^{-1}. Hydrogen was used (2.0 ml min^{-1}constant flow rate) as the carrier gas and on-column injection and flame ionisation detection at 250^{o}C was used. Individual fatty acids were identified by comparison with known standards (Supelco Inc., Madrid) and a well characterised fish oil, and quantified by means of the response factor to the internal standard, 17:0 fatty acid, added prior to transmethylation, using a Chrompack for Windows program (Thermo Electron, Winsford, UK).

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2.5. Statistics

The variance of the data is given as standard deviation (SD) of the mean of three replicates with differences tested for statistical significance ($P \leq 0.05$) by one-way ANOVA followed by a pair-wise multiple comparison of means using Tukey´s test. Prior to statistical analysis percentage data were arcsin transformed (Sokal and Rohlf, 1995). A Statgraphics package (Microsoft, Inc.) was used for all statistical analysis.

including regression and correlation analysis between FA composition, PG production and pigmentation.

3. Results

3.1. Emulsion and Artemia fatty acid profile

The fatty acid composition of the emulsions was as expected. ARA-L was rich in oleic (18:1n-9), linoleic (18:2n-6, LA) and DHA fatty acids and no ARA could be detected whereas ARA-H, Echium and Croda emulsions contained the same amounts of ARA and DHA and only varied in the contents of SDA in the case of the Echium emulsion and EPA in the case of the Croda emulsion. However, once given to the nauplii significant differences were only observed in terms of total monounsaturated fatty acids higher in ARA-L diet, GLA, 20:3n-6 and ARA, lower in ARA-L enriched *Artemia*, ALA and SDA that were higher in Echium oil enriched *Artemia* and EPA higher in Croda oil enriched *Artemia* although all the metanauplii showed high amounts of this fatty acid, and DHA content that was lower in ARA-L enriched prey (Table 2). Consequently, only ARA-L enriched live prey showed a significantly lower ARA content and ARA/EPA ratio and ARA being the same in the other three diets.

No significant differences were observed between the fatty acid composition of fresh and frozen enriched metanuplii (data not shown).

3.2. Larval performance

Final growth in length and dry weight, survival rate, and percentage pigmentation of Senegal sole larvae after the 40 day feeding trial are shown in Table 3. Significantly higher growth in length and weight was obtained if *Artemia* nauplii were enriched with Echium or Croda emulsions. No differences in survival rate could be

detected but a clear effect of the diet on pigmentation rate was observed, being the lowest using ARA-H and C diets and intermediate when *Artemia* nauplii were enriched with Echium oil while ARA-L fed fish were normally pigmented and only one non pigmented individual (used for eicosanoid analysis) (there might be a problem with only using one fish for the analysis but leave it in for now) could be sampled.

Eye migration and settlement were similar for all the groups during development (data not shown).

3.3. Larval biochemistry

Whole larval body fatty acid composition reflected the composition of the diet given during the feeding trial (Table 4). ARA-L fed larvae showed significantly lower ARA, total n-6 PUFA and DHA content whereas LA, 20:4n-3, EPA, DPA and total n-3 PUFA were significantly higher than in the rest of the treatments. ARA-H, Echium and Croda fed fish showed similar amounts of ARA and DHA and significant differences were found in ALA, GLA, 20:3n-6 and SDA content, that were higher for Echium fed fish, and EPA and DPA that were present in significantly higher amounts in Croda fed fish. Total n-6 and n-3 PUFA were also significantly higher if fish were fed Echium and Croda oil enriched nauplii. ARA/EPA ratio was the lowest for ARA-L fed fish and the highest for Echium oil fed fish with intermediate levels observed in the ARA-H and Croda groups.

PG production (PGE and PGF) was measured in pigmented and albino individuals although in some cases, especially those of ARA-L and Echium groups low quantities of non pigmented individuals were observed and used for the analysis (only 1 individual for ARA-L). In both PGs, higher production was detected in non pigmented individuals compared to pigmented larvae in all the dietary groups except for ARA-H

fed fish which showed higher PG production in pigmented individuals. No significant differences were obtained for PGF production between albino and pigmented fish (Table 5, Fig 1) due to the high variability in the results. When all the fish, pigmented and non pigmented together, were considered, the influence of the diet on PG production was evident. ARA-L and Echium fed fish showed lower PGE and PGF production compared to that obtained in the fish from ARA-H and Croda groups (Table 5). There was also a clear relationship between PG production and dietary ARA content (Fig. 1), in such a way that the higher dietary ARA content resulted in higher PG production and increased abnormal pigmentation of fish.

Finally, a significant linear relationship between dietary ARA content (%TFA), dietary ARA/EPA ratio and final results in pigmentation rate was recorded (Fig. 2) when results from this study together with those obtained from previous experiments (Villalta et al., 2005 and 2007 in press) were plotted.

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4. Discussion

Larval growth and survival obtained in this experiment were lower than previously recorded (Villalta et al., 2005 and submitted) probably due to the different origin (Santander, NW Spain versus Cádiz, SW Spain) and initial quality of the larvae. In both cases natural spawnings obtained from wild broodstock were used. In previous experiments fish were fed freshly enriched rotifer and *Artemia* metanauplii from the beginning of larval rearing until the final sampling time. In the present study frozen, enriched *Artemia* metanauplii were given from 20 to 40 dph. Fish showed active feeding activity consistent with previous trials, however the use of frozen prey might have contributed to the lower growth rate obtained. The quality of frozen enriched

metanauplii, in terms of fatty acid composition, was not affected (data not shown). Feeding frozen Artemia was undertaken as a time management strategy, and as larvae are generally bottom feeding having metamorphosed and settled. Frozen Artemia will also retain fatty acid profiles for longer compared to living nauplii who continue to metabolise the lipids (Navarro et al., 1999).

Fatty acid profile of metanauplii differed to that of the emulsions. Dependent on the emulsion, greater variation particularly in SDA and EPA was anticipated in the *Artemia*. However, as is the usual trend in *Artemia* enrichment, EPA content increased in all the treatments to final content of about 2.5-3% TFA even when this fatty acid was not provided in the food, probably derived from chain shortening of DHA (Navarro et al., 1999). As a consequence, ARA/EPA ratio that was expected to vary among the treatments, showed similar results in ARA-H, Echium and Croda treatments, and only ARA-L enriched metanauplii, in which no ARA was provided in the food, showed the expected low ARA/EPA ratio.

The main dietary effect on Senegal sole larvae was obtained in pigmentation and growth rates. Fish fed ARA-L enriched *Artemia* showed lower growth rate and higher proportion of normally pigmented individuals contrasting with the effects of ARA-H and Croda enrichment which produced fish with higher growth but lower pigmentation rates. Similar results were obtained by Villalta et al. (2005) and other authors in different flatfish species (Estévez et al., 1999; Copeman et al., 2002) in terms of pigmentation rate. ARA given in excess to flatfish larvae produces dietary-induced albinism. Differences in growth are not believed to be directly attributed to ARA. ARA-L and ARA-H fed fish had similar final TL and DW. Echium and Croda fed larvae were larger than their ARA-L and ARA-H counterparts. The reason for this not clear.

The fatty acid composition of the fish reflected that of the feed. Although in humans, SDA has been cited to be four times more effective than ALA in increasing the levels of EPA in plasma phospholipids (James et al., 2003), and in salmon cell cultures elongation to 20:4n-3 has been observed (Ghioni et al., 2002), in the case of Senegal sole no differences were detected in the levels of EPA of fish fed Echium oil enriched *Artemia* when compared with the rest of the diets. While there were some significant differences in EPA and SDA in the various feeds, these were relatively minor, which probably explains this lack of change otherwise observed in humans and salmon. On the other hand, docosapentaenoic acid (22:5n-3, DPA) increased significantly in all the groups of fish considering the low dietary levels provided, as has been noted previously suggesting significant elongation of EPA or retroconversion of DHA (Villalta et al., 2005).

A specific effect of Echium oil on PG production was observed (see table 5). Although fish fed Echium oil enriched metanauplii received the same dietary level of ARA, PGE_2 and PGF_2 production was significantly lower and comparable to that of ARA-L fed fish. A similar effect of SDA on prostanoid production has been cited by Horia and Watkins (2005) in mammals and Bell et al. (2006) in cod. Horia and Watkins (2005) related the lower production of PGs with an inhibition exerted by SDA on the expression of cyclooxygenase-2 (COX-2), the inducible enzyme that catalyzes prostaglandin production, and on NFκB and PPARγ transcription. However, Bell et al. (2006) considered the reduction in prostaglandin production a consequence of decreased tissue ARA rather than increasing tissue levels of 20:3n-6 or 20:4n-3. Other studies have suggested that SDA may reduce formation of ARA derived eicosanoids in vitro (Guichardant et al., 1993). In this experiment all the fish, except those receiving ARA-L diet, received the same amount of dietary ARA that was consequently accumulated

similarly in fish tissue. Considering this result in tissue ARA accumulation, it might be the case that Senegal sole larvae receiving Echium oil had reduced PG production as a consequence of COX-2 inhibition. In contrast, dietary EPA supplementation (Croda fed group), that in mammals (Hansen Petrik et al., 2000) was associated with low PG levels, resulted in PG levels similar to those of ARA-H fed fish, probably derived from the high amounts of ARA (almost 2 fold EPA content) observed in Croda fed fish. It is also possible that the reduced eicosanoid production seen in fish fed Echium oil is not solely due to SDA but might be a combined effect of elevated 18:3n-3, SDA, GLA and 20:4n-3, that are all effectively anti-ARA antagonists acting at different points of the eicosanoid sysnthesis pathway.

In the present study, lower PG production was correlated with better pigmentation ($\log_{10}$(% pigmentation) = 1.927–0.00783 PGE, r^2=0.545, P=0.006, regression line not shown). However, Bransden et al. (2005) couldn't find any relationship between PG production with pigmentation on head or body of Atlantic cod cultured in green and grey tanks, although most of the fish (50 to 80% for the head and 35 to 50 % for the body) were heavily pigmented. These authors didn't find any relationship between dietary ARA content and PG production while in our study there is a clear relationship, except for Echium fish, as discussed previously.

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To conclude, ARA and ARA/EPA dietary composition and consequently, fish tissue composition, has a clear influence on the pigmentation rate of Senegal sole, as already described in previous studies (Villalta et al., 2005 and in press). Furthermore, increasing dietary ARA increased whole body PG concentrations and subsequently a direct correlation was found between PG concentration and pigmentation. The inclusion of SDA-rich Echium oil in the enrichment emulsion contributed to a reduction in PG production and resulted in better pigmentation and growth rates although ARA levels in

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the diet and fish tissue were similar to the other treatments. SDA, or a combination of anti-ARA fatty acids, had a clear effect in reducing PG production probably through the inhibition of COX-2 production as already observed in mammals (Horia and Watkins, 2005). Clearly, the apparent efficacy of SDA, other C18 PUFA and their metabolites should be investigated for their anti-ARA activity in future experiments. If SDA has a more powerful effect on suppressing ARA-induced pathologies than EPA, the implications of using SDA as an anti-inflammatory agent in place of, or in addition to EPA-rich oils warrants further investigation.

Acknowledgement

Funding was provided to AE by the Spanish Ministries of Agriculture, Fisheries and Food (Jacumar) and Education (INIA project ACU-02-006). MV acknowledges the scholarship provided by Generalitat de Catalunya (DURSI scholarship). We would like to acknowledge the technical expertise and assistance of Fiona Strachan in the analysis of tissue eicosanoids as well as Magda Monllaó, Noelia Gras and Olga Bellot in the rearing of the fish and live food and sampling. MPB was funded by the Australian Aquafin CRC.

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Table 1

Formulation of the experimental emulsions and fatty acid composition (%TFA) of the emulsions used for *Artemia* enrichment

	ARA-L	**ARA-H**	**Echium**	**Croda**
Formulation (mg g^{-1})				
Vevodar® oil[a]	0.0	300.0	300.0	300.0
Neuromins® oil[b]	400.0	400.0	400.0	400.0
Olive oil[c]	392.6	123.7	24.2	84.2
Vegetable oil[d]	97.4	66.3	27.6	71.4
Croda EPA500 oil[e]				34.3
Echium oil[f]			138.2	
Soy lecithin	70.0	70.0	70.0	70.0
Alpha tocopherol	40.0	40.0	40.0	40.0
Total Fatty acids (mg g^{-1})	407.8	417.8	415.4	402.3
Fatty acids				
14:0	5.4	5.8	6.0	6.1
16:0	11.4	12.7	12.4	12.6
18:0	2.4	5.0	5.1	4.8
Total saturated	19.9	24.8	24.6	24.6
16:1n-7	0.1	0.9	0.9	0.9
18:1n-9	47.7	27.4	18.6	23.5
18:1n-7	1.9	1.2	0.7	1.0
20:1n-9	0.2	0.4	0.4	0.4
20:1n-7	0.0	0.0	0.0	0.0
Total monounsaturated	51.1	30.1	21.0	26.0
18:2n-6 (LA)	10.7	9.5	10.7	10.9
18:3n-6 (GLA)	0.0	1.1	2.7	1.1
20:2n-6	0.0	0.2	0.2	0.3
20:3n-6 (DGLA)	0.0	1.4	1.4	1.4
20:4n-6 (ARA)	0.0	13.1	13.0	13.1
Total n-6 PUFA	10.7	25.3	28.1	26.8
18:3n-3 (ALA)	0.7	0.4	5.1	0.5
18:4n-3 (SDA)	0.0	0.0	1.9	0.3
20:4n3	0.0	0.0	0.0	0.0
20:5n-3 (EPA)	0.0	0.0	0.0	2.0
22:5n-3 (DPA)	0.1	0.1	0.1	0.3
22:6n-3 (DHA)	17.5	19.4	19.3	19.5
Total n-3 PUFA	18.4	19.9	26.3	22.5
Total PUFA	29.1	45.2	54.4	49.3

[a] DSM Food Specialties, The Netherlands
[b] Martek Biosciences, USA
[c] Cornicabra type olive oil, D.O. Montes de Toledo, Spain
[d] Commercial corn oil, spain
[e] Croda Oleochemicals, UK
[f] Technology Crops Ltd., UK

Table 2
Fatty acid composition (%TFA) of *Artemia* metanauplii enriched for 18 h with the experimental emulsions (mean±SD, n= 3). Superscripts indicate significant differences (P<0.05, ANOVA and Tukey's test)

	ARA-L	**ARA-H**	**Echium**	**Croda**
Total lipids (mg g^{-1} DW)	136.9±14.9	160.9±22.9	170.0±30.1	150.8±38.3
Total Fatty acids (mg g^{-1} DW)	54.9±6.9	73.0±11.3	72.7±19.7	73.0±14.0
Fatty acids				
14:0	1.3±0.2	1.4±0.2	1.4±0.1	1.4±02
16:0	10.4±0.3	9.9±0.3	10.1±0.6	9.9±0.3
18:0	5.0±0.1	4.9±0.1	5.0±0.2	4.9±0.1
Total saturated	17.5±0.3	16.9±0.5	17.3±0.9	17.1±0.6
16:1n-7	$2.3±0.1^{b}$	$2.2±0.0^{ab}$	$2.2±0.1^{a}$	$2.1±0.1^{a}$
18:1n-9	$27.3±3.6^{b}$	$24.0±0.6^{b}$	$22.7±3.7^{a}$	$22.8±0.5^{a}$
18:1n-7	$6.9±0.3^{b}$	$6.7±0.2^{ab}$	$6.5±0.4^{a}$	$6.4±0.4^{a}$
20:1n-9	$0.6±0.0^{ab}$	$0.5±0.0^{ab}$	$0.6±0.0^{b}$	$0.6±0.0^{ab}$
20:1n-7	$0.1±0.0^{b}$	$0.1±0.0^{ab}$	$0.1±0.0^{ab}$	$0.1±0.0^{a}$
Total monounsaturated	$37.9±3.8^{b}$	$34.2±0.6^{ab}$	$32.7±4.2^{a}$	$32.6±0.4^{a}$
18:2n-6 (LA)	8.6±0.5	8.7±0.3	8.9±0.2	9.5±0.5
18:3n-6 (GLA)	$0.6±0.4^{a}$	$0.7±0.0^{a}$	$1.1±0.4^{b}$	$0.7±0.0^{ab}$
20:2n-6	0.2±0.0	0.3±0.0	0.3±0.0	0.3±0.0
20:3n-6 (DGLA)	$0.2±0.3^{a}$	$0.6±0.0^{b}$	$0.5±0.3^{b}$	$0.6±0.1^{b}$
20:4n-6 (ARA)	$1.8±2.4^{a}$	$6.0±0.2^{b}$	$5.2±2.6^{b}$	$6.4±0.3^{b}$
Total n-6 PUFA	$11.4±3.4^{a}$	$16.3±0.4^{b}$	$16.1±3.3^{b}$	$17.5±0.8^{b}$
18:3n-3 (ALA)	$21.6±1.4^{b}$	$20.3±1.1^{ab}$	$21.4±0.8^{b}$	$19.5±1.6^{a}$
18:4n-3 (SDA)	$2.9±0.3^{bc}$	$2.7±0.2^{ab}$	$3.1±0.2^{c}$	$2.6±0.3^{a}$
20:4n3	0.5±0.1	0.5±0.1	0.5±0.0	0.5±0.1
20:5n-3 (EPA)	$2.9±0.1^{b}$	$2.7±0.1^{ab}$	$2.7±0.1^{a}$	$3.5±0.1^{c}$
22:5n-3 (DPA)	$0.04±0.0^{a}$	$0.1±0.0^{b}$	$0.1±0.0^{b}$	$0.1±0.0^{b}$
22:6n-3 (DHA)	$4.0±0.5^{a}$	$5.6±0.3^{b}$	$5.6±1.1^{b}$	$6.1±0.5^{b}$
Total n-3 PUFA	33.2±1.5	32.5±1.2	34.0±1.9	32.9±1.6
Total PUFA	$44.6±4.0^{a}$	$48.9±0.9^{ab}$	$50.1±5.1^{b}$	$50.4±0.9^{b}$
ARA/EPA	$0.64 ±0.92^{a}$	$2.21±0.10^{b}$	$1.95±0.96^{b}$	$1.81±0.11^{b}$

Table 3

Growth, survival and pigmentation of Senegal sole larvae after 40 days of feeding *Artemia* metanauplii enriched on experimental emulsions (mean±SD, n=3). Superscript letters indicate significant differences (P<0.05, ANOVA and Tukey's test)

	ARA-L	**ARA-H**	**Echium**	**Croda**
Final length (mm)	9.53±0.81[b]	10.13±0.92[b]	11.62±1.48[a]	10.92±1.65[a]
Final weight (mg)	2.29±0.27[c]	2.61±0.27[c]	4.05±0.45[a]	3.43±0.45[b]
Survival (%)	26.1±21.0	30.3±25.8	19.2±9.6	47.5±1.6
Pigmentation (%)	100±0.0[a]	26.8±9.1[c]	64.4±6.7[b]	26.7±1.6[c]

Table 4

Fatty acid composition (%TFA) of whole body of Senegal sole larvae after 40 days of being fed *Artemia* enriched on experimental emulsions (mean±SD, n=3). Superscripts letters as in table 2 and 3.

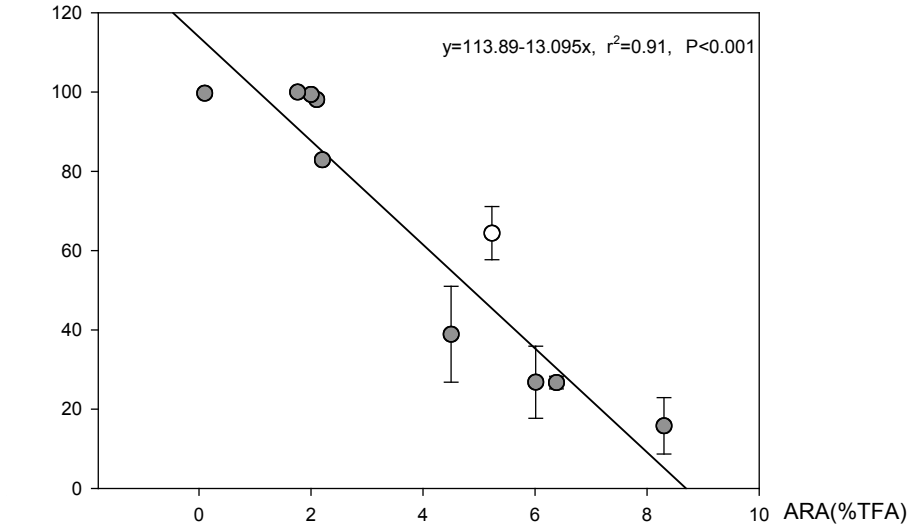
	ARA-L	**ARA-H**	**Echium**	**Croda**
Total lipids (mg g^{-1} DW)	*137.2±1.7^{b}*	*110.3±12.3^{a}*	*134.4±3.2^{ab}*	*138.9±16.2^{b}*
Total FA (mg g^{-1} DW)	*34.6±0.3*	*40.0±4.4*	*36.2±1.0*	*40.2±4.1*
14:0	0.9±0.0	0.9±0.0	1.0±0.0	0.9±0.0
16:0	12.0±0.2^{b}	11.7±0.1^{ab}	11.3±0.3^{a}	11.4±0.3^{a}
18:0	7.2±0.2	7.1±0.1	6.8±0.4	6.8±0.2
Total saturated	20.9±0.5	20.7±0.2	20.0±0.9	20.1±0.6
16:1n-7	1.8±0.1^{b}	1.7±0.1^{a}	1.7±0.1^{ab}	1.7±0.0^{a}
18:1n-9	23.8±0.1^{d}	20.5±0.3^{c}	18.4±0.4^{a}	19.5±0.3^{b}
18:1n-7	6.9±0.0^{b}	6.4±0.1^{a}	6.4±0.1^{a}	6.3±0.1^{a}
20:1n-9	0.8±0.0^{b}	0.7±0.0^{a}	0.7±0.0^{a}	0.7±0.0^{a}
20:1n-7	0.4±0.0^{c}	0.3±0.0^{a}	0.3±0.0^{ab}	0.3±0.0^{b}
Total monounsaturated	35.2±0.0^{d}	30.7±0.4^{c}	28.7±0.5^{a}	29.6±0.4^{b}
18:2n-6 (LA)	8.5±0.0^{c}	7.3±0.1^{a}	7.6±0.1^{b}	7.7±0.1^{b}
18:3n-6 (GLA)	0.4±0.0^{a}	0.6±0.0^{b}	0.9±0.0^{c}	0.6±0.0^{b}
20:2n-6	0.4±0.0^{b}	0.4±0.0^{a}	0.4±0.0^{b}	0.4±0.0^{b}
20:3n-6 (DGLA)	0.4±0.0^{a}	0.7±0.0^{b}	0.8±0.0^{d}	0.7±0.0^{c}
20:4n-6 (ARA)	1.8±0.0^{a}	9.1±0.2^{b}	9.0±0.3^{b}	9.1±0.1^{b}
Total n-6 PUFA	11.6±0.1^{a}	19.1±0.2^{b}	20.0±0.1^{c}	19.8±0.2^{c}
18:3n-3 (ALA)	13.3±0.2^{b}	11.9±0.3^{a}	13.0±0.2^{b}	11.8±0.1^{a}
18:4n-3 (SDA)	1.4±0.0^{b}	1.3±0.1^{a}	1.5±0.0^{c}	1.3±0.0^{a}
20:4n3	0.8±0.0^{b}	0.6±0.0^{a}	0.6±0.0^{a}	0.6±0.0^{a}
20:5n-3 (EPA)	2.4±0.1^{d}	1.4±0.0^{b}	1.3±0.0^{a}	1.7±0.0^{c}
22:5n-3 (DPA)	2.2±0.0^{c}	1.8±0.1^{a}	1.7±0.0^{a}	2.0±0.1^{b}
22:6n-3	10.9±0.2^{a}	11.3±0.2^{b}	11.6±0.2^{b}	12.0±0.2^{c}
Total n-3 PUFA	32.3±0.4^{d}	29.5±0.1^{a}	31.3±0.6^{c}	30.5±0.2^{b}
Total PUFA	43.9±0.5^{a}	48.6±0.2^{b}	51.3±0.4^{d}	50.3±0.4^{c}
ARA/EPA	0.8±0.0^{a}	6.6±0.2^{c}	6.9±0.2^{c}	5.3±0.1^{b}

Table 5

Prostaglandin production (in ng g^{-1} dry matter, DM) of albino and pigmented fish fed the experimental diets. Superscript letters indicate significant differences among the groups ARA-L, ARA-H, Echium and Croda (ANOVA and Tukey's test, $P< 0.05$, n= 3 samples per group and 1-10 fish per sample) when the same type of fish, that is albino, pigmented and overall, were compared.

Experimental	**PGE2 (ng g^{-1} DM)**			**PGF2 (ng g^{-1} DM)**		
groups	Albino	Pigmented	Overall	Albino	Pigmented	Overall
ARA-L	20.99^{a}	5.12 ± 1.25^{a}	10.41 ± 9.21^{a}	23.08^{a}	6.36 ± 1.06^{a}	11.94 ± 9.68^{a}
ARA-H	49.36 ± 18.47^{ab}	62.09 ± 4.31^{b}	55.73 ± 13.19^{b}	93.72 ± 19.96^{a}	109.95 ± 97.42^{a}	101.83 ± 58.17^{bc}
Echium	14.22^{a}	8.20 ± 5.40^{a}	10.21 ± 5.16^{a}	38.67^{a}	23.06 ± 8.12^{a}	28.26 ± 10.69^{ab}
Croda	84.04 ± 9.95^{b}	55.23 ± 19.10^{b}	69.64 ± 20.85^{b}	185.07 ± 63.03^{a}	125.29 ± 57.56^{a}	162.57 ± 63.14^{c}

Fig. 2 Regression lines and correlation coefficients (r^2) obtained for the relationship between dietary ARA and dietary ARA/EPA ratio and pigmentation rate. Data from this study and previous results (Villalta et al, 2005; Villalta et al, unpublished results). White symbol denotes Echium oil fed fish.



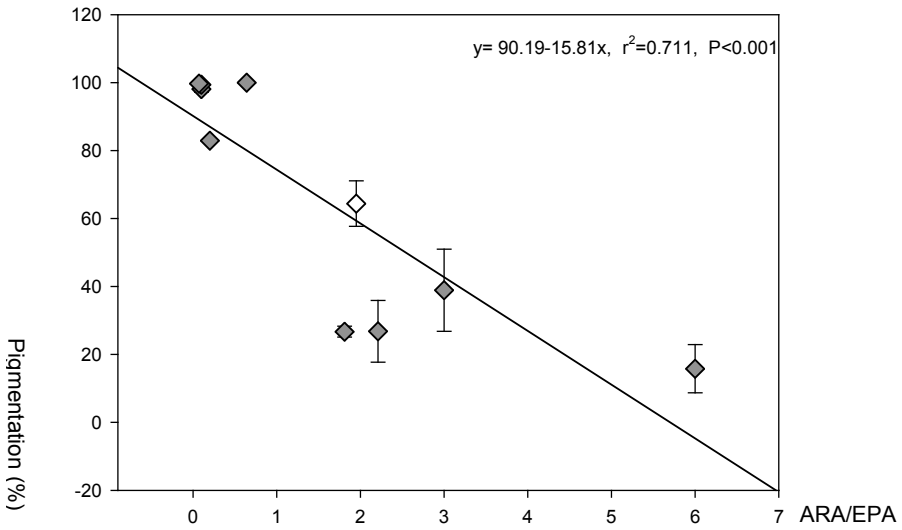


Fig. 1 Prostaglandin production of albino (white symbols) and pigmented (black symbols) fish related to ARA dietary content. Letters indicate significant differences among the groups and type of fish. Numeric data represent pigmentation rates obtained after the feeding trial. Echium oil fish results appear inside a circle.

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