



# Arachidonic acid enriched live prey induce albinism in Senegal sole (*Solea senegalensis*)

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Senegal sole is a warm-water species being cultured in the Iberian Peninsula. In recent years research has focused on the larviculture of this species, however there have been no studies addressing optimal lipid and fatty acid requirements. This study was designed to investigate the role of arachidonic acid (ARA, 20:4n-6) on growth, survival, and pigmentation success of this species.

## Materials and Methods

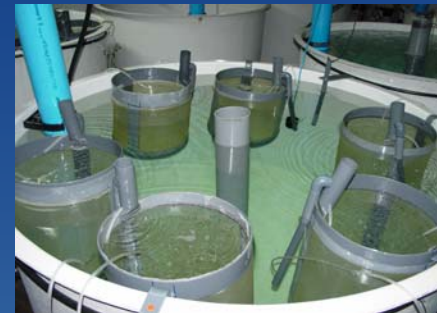
Three emulsions were formulated by mixing specialty oils and other ingredients (Table 1). Emulsions were characterised by low (0.14 mg/g, ARA L), medium (63.9 mg/g, ARA M) or high (127.7 mg/g, ARA H) concentrations of ARA (Table 2). Emulsions were used to enrich rotifers and *Artemia nauplii* that were subsequently fed to Senegal sole larvae. A commercial emulsion, DHA-Protein Selco (INVE), was used as a reference (2.25 mg/g ARA).

Table 2. Fatty acid profile (%TFA) of emulsions.

	Selco	ARA L	ARA M	ARA H
<b>Total lipids (mg/g)</b>	<b>250.0</b>	<b>318.0</b>	<b>381.9</b>	<b>325.9</b>
14:0	2.0	7.8	8.3	8.5
16:0	30.8	14.6	15.4	15.7
18:0	6.0	1.8	2.9	4.1
<b>Saturates</b>	<b>43.7</b>	<b>24.7</b>	<b>27.5</b>	<b>28.9</b>
18:1n-7	9.4	1.6	1.3	1.2
18:1n-9	13.1	46.4	35.9	24.7
20:1n-9	0.0	0.2	0.2	0.2
<b>Monounsats</b>	<b>24.3</b>	<b>49.3</b>	<b>38.6</b>	<b>28.7</b>
18:2n-6	8.8	4.4	4.0	3.9
20:2n-6	0.9	0.2	7.8	14.8
<b>n-6 PUFA</b>	<b>10.3</b>	<b>4.6</b>	<b>15.4</b>	<b>20.1</b>
18:3n-3	1.6	0.5	0.3	0.1
20:3n-3	4.6	0.2	0.4	0.7
22:5n-3	14.7	20.0	18.9	19.2
<b>n-3 PUFA</b>	<b>21.7</b>	<b>20.7</b>	<b>19.8</b>	<b>20.9</b>
n-3:n-6	2.1	4.5	1.3	1.0
<b>EPA/ARA</b>	<b>5.0</b>	<b>1.0</b>	<b>0.1</b>	<b>0.0</b>
<b>ARA:DHA</b>	<b>0.1</b>	<b>0.0</b>	<b>0.4</b>	<b>0.8</b>

Table 1. Formulation of experimental emulsions.

Ingredient (g/kg)	ARA L	ARA M	ARA H
Vegetable oil	300	150	0
18:2n-6 oil	0	18	35
Olive oil	90	222	355
Neomuns oil	500	500	500
Soy lecithin	70	70	70
Vitamin E	40	40	40



Larvae were distributed (50 larvae/L) into triplicate, 35 L mesh tanks placed in two 1500 L holding tanks (see picture; Carbó et al., 2002). Live prey were fed at 35% larval body weight during pre-metamorphosis and 20% larval body weight after metamorphosis. Prey density in the rearing tanks was adjusted to avoid unenriched prey remaining. At 37 days after hatch (dah), larvae were assessed for length, dry weight, pigmentation and survival, and samples taken for lipid analysis.

## Results

At 37 dah, no significant differences were recorded between treatments in weight or survival (Table 3). Larvae were significantly longer when fed Selco-enriched live feeds compared to larvae fed ARA L or ARA M enriched live feeds during most stages of the experiment (Figure 1). Significant differences were detected in the proportion of larvae with albinism. Most notably a higher degree of albinism was recorded as more dietary arachidonic acid was included.

Table 3. Performance of larvae fed experimental diets.

	Final	Final		
Group	Length (mm)	Weight (mg)	Survival (%)	Albinism (%)
Selco	12.76±1.24 b	5.11±0.74 a	48.95±4.78 a	0.16±0.14 a
ARA L	11.82±1.53a	4.49±1.30 a	44.42±18.4 a	0.28±0.40 a
ARA M	11.89±1.93 a	5.12±1.55 a	58.86±16.69 a	61.04±12.06 b
ARA H	12.16±1.52 a	4.75±1.33 a	54.93±5.70 a	84.18±7.07 c

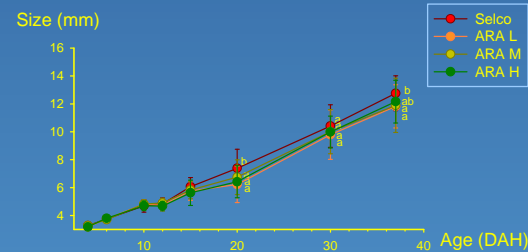


Figure 1. Temporal changes in larval length.

Total lipid content in the brain and eyes (Figure 2) was not significantly different among the 4 treatments, however significant differences were recorded in lipid accumulation in the gut and liver (Figure 3) and carcass (Figure 4). Fatty acid analysis of heads (including brain and eyes), gut (including the liver), and the carcass revealed similar compositions to the emulsions. Significant correlations were found between % albinism and ARA content in the tissues ( $r^2 = 0.85$ ; % Albinism = 19.55 \* % ARA - 50.94) as well as with the tissue EPA/ARA ratio ( $r^2 = 0.92$ ; % Albinism = 99.19 \* EPA/ARA - 100.04).

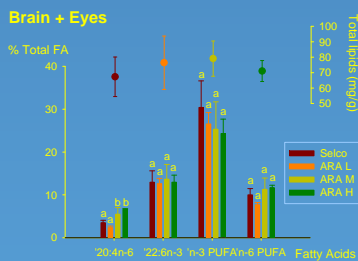


Figure 2. Lipid and fatty acid composition of brain and eyes.

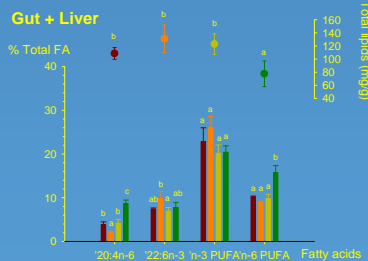


Figure 3. Lipid and fatty acid composition of gut and liver.

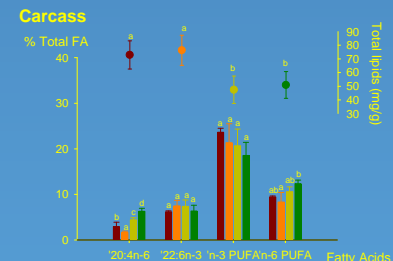


Figure 4. Lipid and fatty acid composition of carcass.

## Discussion

Arachidonic acid had no effect on growth or survival but had a negative effect on pigmentation of Senegal sole larvae. Dietary EPA/ARA ratios of 0.5:1 or lower have been found to induce malpigmentation in other flatfish species such as halibut, turbot, Japanese flounder and yellowtail flounder (McEvoy et al., 1998; Estévez et al., 1999, 2001; Copeman, et al., 2002). Similarly in our experiment, emulsion EPA/ARA ratios > 0.5:1 were found not to cause albinism while ratios < 0.5:1 resulted in malpigmentation. An imbalance in the dietary EPA to ARA ratio would cause irregular tissue eicosanoid production, and could in turn result in malpigmentation, as previously hypothesised (Sargent et al., 1999). Further, experiments carried out with melanoma cells *in vitro* showed that linoleic acid accelerates spontaneous degradation of tyrosinase, the enzyme responsible for melanin synthesis (Ando et al., 1999). Like linoleic acid, ARA acid is believed to have similar but more pronounced effects on tyrosinase degradation (Ando, personal communication, 2004), which may also explain albinism recorded in flatfish species in relation to dietary ARA. Further research is required to support this theory.

**References** Ando et al. (1999) J. Lipid Res., 40: 1312-1316; Carbó et al. (2002) EAS spec. Publ. 32: 171-172; Copeman et al. (2002) Aquaculture, 210: 285-304; Estévez et al. (1999) Aquaculture, 180: 321-343; Estévez et al. (2001) Aquaculture, 192: 309-319; McEvoy et al. (1998) Bull. Aquacul. Assoc. Canada 88-4: 17-20; Sargent et al. (1999) Aquaculture, 179: 217-229

**Acknowledgements** This experiment was funded by the Spanish Ministry of Agriculture, Fisheries and Food (Jacumar) and Ministry of Science and Technology (INIA project ACU-02-006). M. Villalta thanks Generalitat de Catalunya (DURS) pre-doctoral fellowship) for financial support for this research. DSM Food Specialties (The Netherlands) generously provided the Vedovar oil and some financial support.