Grupo de Investigación en Acuicultura

Effects of diets, with different levels of *n*-3 HUFA, designed specifically for gilthead seabream (*Sparus auratus* L.) broodstock on spawning quality

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Introduction

The effects of dietary lipid levels in the spawning quality has been studied in several cultured fish species. Works like those of Watanabe *et al*. (1984 a); Mourente *et al*. (1989); Dhert *et al*. (1991); Bruce *et al*. (1993); Navas *et al*. (1997); Rodriguez *et al*. (1998); Lavens *et al*. (1999); Furuita *et al*. (2002, 2003 b); Mazorra *et al*. (2003); Fernandez-Palacios (2005) and Aijun *et al*. (2005) show that lipids and fatty acids are the dietetic components that have more influence in the spawning quality, specially in those species with continuous spawning which display short vitellogenesis periods and are able to incorporate these dietetic components in eggs during the spawning period.

Materials and methods

Broodstocks: Forty five gilthead sea bream (*Sparus aurata*) broodstocks (3-5 years of age) were randomly selected and distributed in fifteen fibre glass tanks of 1000 l capacity.

Diets: After a period of common feeding with a commercial diet and making sure that there were no significant differences in the spawning quality parameters, one proceeded to feed the broodstocks, with the experimental diets twice a day.

Table 1 Composition and analyzed content of the experimental diets.

	DIET				
	1	2	3	4	5
Ingredients					
Squid meal	---	60.85	64.98	64.98	64.98
Defatted Squid meal	61.52	5.82	---	---	---
Fish oil	---	---	1.11	3.43	4.80
Cow fat	13.32	8.47	7.09	4.77	---
EPA (28)	---	---	---	---	3.40
Minerals[1]	2.00	2.00	2.00	2.00	2.00
Vitamins[1]	2.00	2.00	2.00	2.00	2.00
Vitamin E	0.025	0.025	0.025	0.025	0.025
Carboximetil celulose	1.00	1.00	1.00	1.00	1.00
Alfa celulose	0.30	0.00	1.96	1.96	1.96
Starch	7.40	7.40	7.40	7.40	7.40
Dextrin	2.46	2.46	2.46	2.46	2.46
Wheat barn	10.00	10.00	10.00	10.00	10.00
Analyzed composition					
Protein	52.35	54.62	54.69	53.87	54.05
Lipids	19.45	18.12	19.18	19.23	16.50
Ash	5.37	5.74	5.46	5.48	5.15
Humidity	6.97	7.85	9.99	7.82	14.12
Vitamin E (mg/kg)	270.23	265.35	272.23	256.54	269.25
n-3 HUFA (% dry weight)	0.36	1.90	2.84	4.38	6.67

[1] Fernández-Palacios *et al* (1995).

Table 2 Main composition of principal fatty acids (% total fatty acids) of the experimental diets

Fatty acids	DIET 1	DIET 2	DIET 3	DIET 4	DIET 5
14: 0	2.69	2.52	2.90	3.87	5.32
16: 0	25.40	26.62	25.72	24.74	19.30
16: 1n-7	1.62	1.21	2.02	3.49	6.54
18: 0	27.47	22.11	18.59	13.80	2.58
18: 1n-9	31.51	24.61	22.47	18.78	7.06
18: 2n-6	3.42	3.37	3.25	3.23	3.16
18: 3n-3	0.29	0.30	0.34	0.49	0.81
18: 4n-3	0.14	0.09	0.37	1.12	3.15
20: 4n-6	0.02	0.12	0.38	0.57	0.96
20: 5n-3	0.65	3.15	4.91	9.32	19.47
22: 6n-3	1.02	7.46	8.90	12.65	17.84
Saturated	56.78	52.29	48.19	43.77	25.74
Monounsaturated	34.09	28.67	26.46	24.91	16.90
n-3	2.71	12.91	16.05	25.02	40.91
n-6	4.08	4.04	4.54	4.54	5.21
n-9	32.31	27.29	24.20	20.99	10.33
n-3 HUFA	1.87	11.11	14.84	22.78	40.45
AA/EPA	0.030	0.038	0.077	0.061	0.049

Spawning and evaluation of egg quality: Naturally produced spawns by each female were collected every day during the experimental period. The parameters used to determine spawning and egg quality were as described by Fernández-Palacios *et al.* (1995).

Analytical methods: Total Lipid Folch *et al* (1957). Fatty acids were prepared from crude lipid as described by Christie (1982). Liquid-gas chromatography operating conditions were as described by Izquierdo *et al*. (1990).
Moisture, ash and protein (AOAC. 1995).
Vitamin E (α-tocopherol) was determined by high performance liquid chromatography (HPLC) according to the method modified by Lambertsen (1983) and Lie *et al*. (1994).

Statistical analysis: The results obtained have been always expressed as average ± standard deviation of the average. The data were compared statistically using the analysis of variance ANOVA. The differences between averages are shown by means of the test of multiple comparison of the averages of Tukey (as general criterion one took 5 % level of significance) (Sokal y Rohlf. 1979).

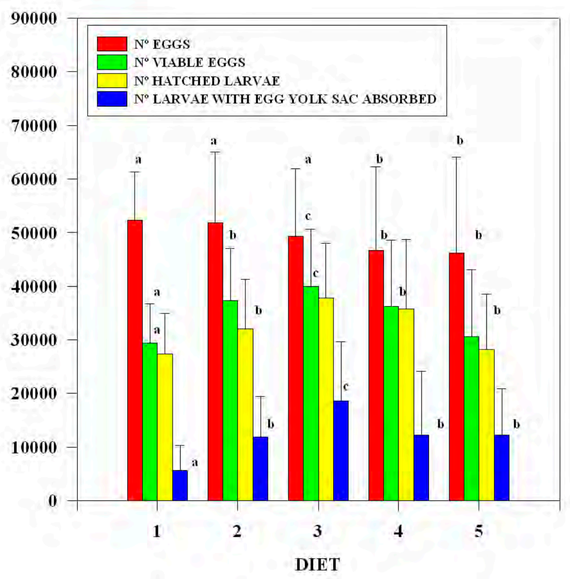
Results

Table 3 Rates of spawning from broodstocks fed with experimental diets (Average ± standard deviation).

DIET	% EGG VIABILITY	% ABNORMAL EGGS	% NON-FERTILIZED EGGS
	P < 0.01	**P < 0.01**	**P < 0.01**
1 (n = 84)	56.50±11.71[a]	5.54±3.25	15.57±9.13[a]
2 (n = 96)	72.15±7.49[b]	2.94±1.65 [b]	5.22±2.92[b]
3 (n = 97)	81.05±6.71[c]	2.12±1.04[c]	4.67±3.77[b]
4 (n=117)	77.94±7.55[d]	0.93±0.54[d]	2.77±2.83[c]
5 (n =118)	67.99±8.98[b]	0.16±0.11[b]	1.98±4.88[c]
	% HATCHING	**% ABNORMAL LARVAE**	**% LARVAL SURVIVAL**
	P < 0.01	**P = 0.25**	**P < 0.01**
1 (n = 84)	92.61±5.41[a]	1.96±1.02	22.62±14.86[a]
2 (n = 96)	88.26±5.12[b]	2.02±0.99	26.23±16.79[ab]
3 (n = 97)	98.00±2.51[c]	2.16±1.52	38.75±17.64[c]
4 (n = 107)	97.53±3.19 [cd]	1.98±1.25	30.58±18.20[b]
5 (n = 118)	94.86±5.66[d]	1.89±1.86	27.71±16.7[b]

*Rows of a same column without or with equal superscripts do not display significant differences. Different Superscripts indicate significant differences.

Fig.1 Relative productions (by kg of female and spawning) of gilthead broodstock fed with experimental diets.



*Columns. with the same plot. without or with a same letter does not display significant differences. Columns. with the same plot. with different letters displays significant differences.

Discussion

In the present study the higher percentage of live eggs was observed with the broodstock fed with Diet 3 (2.84% n-3 HUFA in dry weight) in comparison with the broodstock fed with diets containing much lower as well as much higher amounts of n-3 HUFA. Nevertheless, levels superior to 2.84% of n-3 HUFA considerably reduced the percentage of live eggs. These results suggest that excessive levels as insufficient levels of n-3 HUFA in broodstock diets have a negative effect on the spawning quality of gilthead seabream (*Sparus aurata*). Similar results are found by Furuita *et al*. (2000, 2002), which in two experiments with Pacific sole (*Paralichthys olivaceus*) broodstock fed diets containing different levels of n-3 HUFA.

As pointed out by Fernandez-Palacios *et al*. (1995, 1997) which found a clear correlation between the percentage of non-fertilized eggs and dietary levels of n-3 HUFA. Rodriguez *et al*. (1998) pointed out that the percentage of egg fertilization in spawning of gilthead seabream is smaller with a deficient diet in n-3 HUFA.

The dietary relation AA/EPA can be a critical nutritional factor in diets for broodstock and larvae. Thus, in this experiment are found positive correlations between this relation and the percentage of hatching and larval survival. Navas *et al*. (2001) observed in seabass (*Dicentrarchus labrax*), an increase in the percentage of hatching with the increase in the relation of AA/EPA.

Fernandez-Palacios *et al*. (1995) observed a significant decrease of the percentage of larval survival when the broodstock were fed diets containing superior levels of n-3 HUFA to 1.6%, nevertheless Fernandez-Palacios (2005) with a percentage of 2.5% HUFA obtained the best results as in the present experiment as obtained with 2.84%, in both experiments these diets were supplemented with 250mg/kg of vitamin E. This suggests that the results observed by Fernandez-Palacios *et al*. (1995) can be explained by an inadequate protection of the lipids due to the oxidation of these during the embryonic development.

The number of eggs by spawning and kg of female was greater in the spawns from the broodstocks fed with Diet 1 and decreased progressively with the increase of dietary n-3 HUFA, observing a negative correlation between both parameters. Similar results are reported by Furuita *et al.* (2000) for the Pacific sole (*Paralichthys olivaceus*). The broodstocks fed with Diet 3 had the spawnings with the highest percentage of live eggs, hatching and larval survival, still in spite of not putting the greater number of eggs by spawns and kg.

Conclusion

Diets for gilthead sea bream (*Sparus aurata*) broodstock with dietary levels of 2.84% n-3 HUFA, combined with levels of 250 mg/kg vitamine E rasure good spawning quality. Putting so indicative the importance for an effective utilization of essential fatty acids the use of adequate levels of antioxidants.