Combined levels of carotenoids and vitamin E in diets for gilthead seabream broodstocks (*Sparus aurata* L.): effect on the quality of egg spawning and composition

Grupo de Investigación en Acuicultura

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Introduction

Watanabe *et al*. (1991 a,b) state that, vitamin E and carotenoids perform an essential role on the quality of egg spawning. Vitamin E is one of the main nutrients for the reproduction of fish (Izquierdo *et al*., 2001), and it has been proved that its inclusion in diets for broodstocks favors the quality of egg spawning in several species of fish (Watanabe and Takashima,1977; Takeuchi *et al*., 1981; Watanabe *et al*., 1985, 1991 a,b; Sutjaritvongsanon, 1987; Watanabe, 1990; Schimittou, 1993; Mushiake *et al*., 1993; Dube, 1996; Shiranee and Natarajan, 1996; Izquierdo *et al*., 2001; Morehead *et al*., 2001; Fernández-Palacios *et al*., 2005). On the other hand, the carotenoids which also perform an antioxidizing function (including the protection of lipids from oxidation), have been involved in the reproductive processes of marine organisms: crustaceans (Liñan-Cabello *et al*., 2002), marine fish (Watanabe y Kiron, 1995; Verakunpiriya *et al*., 1997 a,b; Vassallo-Agius *et al*., 2001 a,b,c, 2002; Watanabe and Vassallo-Agius 2003) and fresh water fish (Ahmadi *et al*., 2006).

Materials and methods

Broodstocks: Thirty six gilthead seabream broodstocks (2-4 years old) (*Sparus aurata*), were randomly distributed in twelve circular fiber glass tanks with a capacity of 1000 l.

Experimental diets: Four isolipidic and isoproteic diets were formulated with squid flour and fish oil as sources of protein and lipids correspondingly, with a content of two levels of vitamin E (100 and 250 mg/kg) combined with or without the inclusion of carotenoids (CRT´s) (0 and 60 mg/kg) utilizing paprika oleoresin as the source of these.

Table 1 Composition and proximal analysis of experimental diets.

***Ingredients* (g 100 g diet $^{-1}$)**	0/100	60/100	0/250	60/250
Squid meal [a]	59	59	59	59
Fish oil	6	6	6	6
Mixture of Vitamins [b] Vitamin E	2 0.010	2 0.010	2 0.025	2 0.025
Mixture of Minerals [b]	2	2	2	2
Starch [c]	25.26	25.26	25.26	25.26
Paprika oleoresin [d]	0	0.14	0	0.14
Oleic Acid	0.74	0.74	0.74	0.74
α – Celulose	5	5	5	5
***Analytical Composition* (% base dry matter)**				
Protein (%)	49.44	48.60	48.61	48.21
Lipids (%)	12.54	10.98	11.10	11.54
Ash (%)	4.43	4.25	4.28	4.24
Moisture (%)	9.22	9.39	9.71	8.35
Carbohydrates[1]	33.59	36.17	36.01	36.01
n-3 HUFA (% dry weight)	3.51	3.45	3.25	3.56
Total carotenoids (μg/g sample)	11.01	52.19	10.76	54.09
Vitamin E (mg/kg) Vitamin E / n-3 HUFA Total carotenoids / n-3 HUFA	89 25.36 3.14	87 25.22 15.13	134 41.23 3.31	135 37.92 15.19
Total carotenoids / Vitamin E	0.12	0.60	0.08	0.40

[a] Rieber and Son Ltd., Bergen,Norway
[b] Fernández-Palacios et al. (1998)
[c] Merigel 100 Amylum Group
[d] Paprika oleoresin, José Martinez y Cía. S.A. (Murcia, Spain)
[1] Calculated per difference Carbohydrates = 100 – (%Protein +% Lipids + % Ash) %

Table 2 Composition in principal fatty acids of the experimental diets (% Total fatty acids).

	Diet			
Fatty Acids	**0 / 100**	**60 / 100**	**0 / 250**	**60 / 250**
14:0	5.70	5.31	5.60	5.41
16:0	24.72	22.33	24.59	22.21
16:1n7	0.17	0.16	0.17	0.17
18:0	3.70	3.39	3.70	3.44
18:1n9	11.62	11.24	10.95	11.35
18:2n6	4.13	4.67	4.14	4.67
18:3n3	0.78	0.87	0.77	0.87
18:4n3	1.10	1.18	1.11	1.16
22:4n6	0.57	0.61	0.58	0.60
20:5n3 (EPA)	10.47	11.56	10.83	11.39
22:6n3 (DHA)	15.98	18.17	16.81	17.82
Σ Saturated	36.01	32.69	35.66	32.78
Σ Monoenoic	24.24	23.30	23.27	23.77
Σ *n*-3	29.94	33.54	31.27	33.01
Σ *n*-6	6.35	6.94	6.36	6.94
Σ *n*-9	17.97	17.34	19.60	20.09
Σ *n*-3 HUFA	27.99	31.42	29.29	30.87
AA / EPA	0.08	0.08	0.08	0.08
EPA / DHA	0.65	0.63	0.64	0.63
EPA / ARA	11.15	11.64	11.43	11.66
Oil / DHA	0.72	0.61	0.65	0.63
Oil / Σ *n*-3HUFA	0.41	0.35	0.37	0.36
***n*-3 / *n*-6**	4.71	4.82	4.91	4.75

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).
The extraction of carotenoids and vitamin E was performed by following the method from Barua *et al*. (1993) and carotenoids quantified in spectrophotometer. Vitamin E 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. Tthe differences between averages were shown by means of the test of multiple comparison of the averages of Duncan (as general criterion one took 5 % level of significance) (Sokal y Rohlf. 1979).

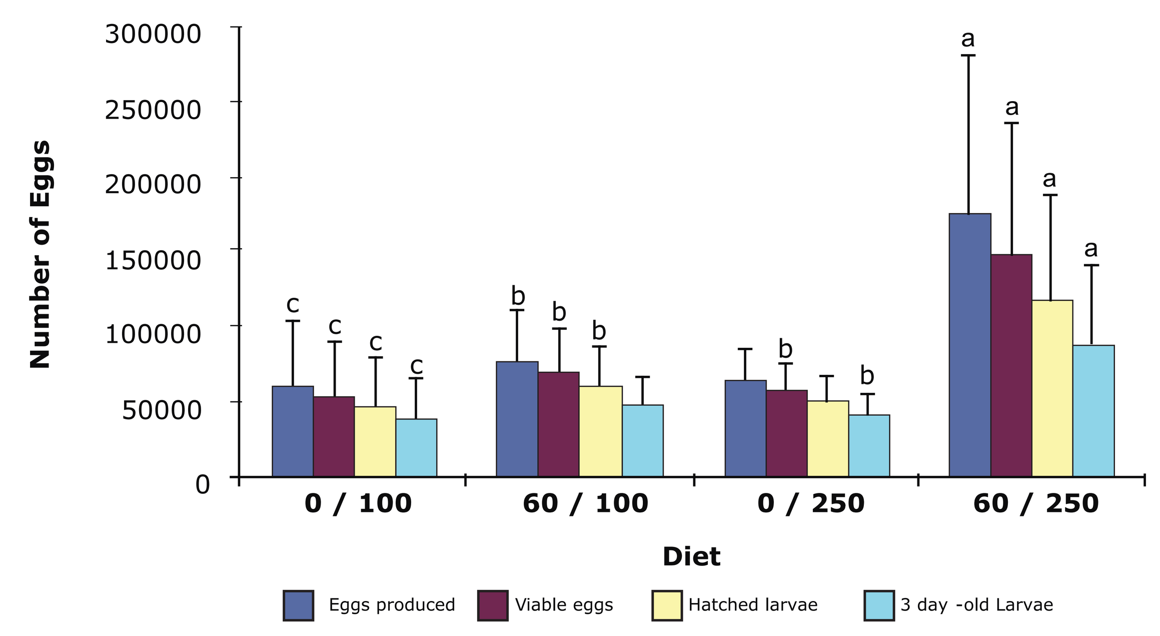
Results

Table 3 Fecundity, dimensions of eggs and larvae and egg spawning rates by broodstocks fed with the different diets (mean values ± SE).

Diets	**0/100**	**60/100**	**0/250**	**60/250**
Parameters	**n=36**	**n=38**	**n=33**	**n=23**
Fecund. (Total/kg/spawning)	60992±41536^{c}	77635±31315^{b}	64785±20057^{b}	175286±104418^{a}
Egg Diam (mm)	0.959±0.031^{a}	0.957±0.032^{a}	0.942±0.034^{b}	0.938±0.027^{b}
Lipidic drop Diam (mm)	0.231±0.013^{c}	0.239±0.014^{b}	0.231±0.013^{c}	0.248±0.018^{a}
% Egg Viability	87.35±8.75^{ab}	90.61±6.43^{a}	88.94±6.14^{ab}	83.7±8.24^{b}
% Non-viable	11.73±8.48^{ab}	6.19±4.51^{a}	7.64±5.06^{ab}	10.57±7.81^{b}
% Non-fertilized eggs	1.5±3.13^{b}	3.19±4.26^{ab}	3.40±3.98^{a}	5.71±6.13^{a}
% hatching	88.88±10.22^{a}	86.72±11.25^{ab}	88.77±7.58^{ab}	79.73±16.27^{b}
% Larval survival	82.13±10.16	78.11±12.62	80.28±8.08	75.79±15.79
3 day larva length (mm)	2.65±0.34^{c}	2.86±0.41^{ab}	2.92±0.34^{a}	2.75±0.33^{b}

Values with different superscript in the same row indicate significant variation P< 0.05

Fig.1 Relative production (per weight in kg of each female and per eggs spawn) of the gilthead seabream broodstocks fed with the various diets during the experimental period.



Bars of the same color, with or without the same letter do not represent significant differences. Bars, of the same color with different letters represent significant differences (P< 0, 05).

Discussion

All the data suggest that the quality of the eggs laid by the gilthead seabream improved when the broodstocks were fed with the diet which had a higher content of carotenoids and vitamin E (diet 60/250) observing significant differences in the productions related to eggs laid by broodstocks fed with the other diets. Followed by broodstocks fed with diets with the addition of carotenoids and lower level of vitamin E (diet 60/100) and from broodstocks fed without the supplement of carotenoids but with a greater level of vitamin E (diet 0/250).

Elevation of dietary n-3 HUFA implies the inclusion of higher levels of vitamin E (Watanabe *et al*., 1991a) and it has been suggested that the presence in the diet of antioxidants such as vitamin E is essential in order to maintain the structural integrity of the phospholipids in salmon species fed with diets rich in n-3 HUFA (Cowey *et al*., 1983). Recently, Koprücü and Seker (2003) discovered that the addition of vitamin E in diets for guppy broodstocks (*Poecilia reticulate*) or swordfish (*Xiphophorus helleri*) increases fertility of both species. On the other hand, carotenoids are also powerful antioxidants, protecting the cellular membrane from peroxidative degeneration caused by the free radicals (Miki *et al*., 1994). In addition to other functions such as predecessors of vitamin A, the regulating of chimotaxis in spermatozoids Izquierdo *et al*. (2005), thus being very important in the reproduction of fish both for embrionary development as well as that of larvae. The antioxidizing requirements are increased during the reproduction season (Izquierdo & Fernández-Palacios 1997; Fernández-Palacios *et al*. 1998), which can be related with the formation of radicals during the biosynthesis of the steroid hormone in larger vertebrate species (Rapoport *et al*. 1998).

In the current experiment, the increase of vitamin E o α-tocoferol from 100 to 250 mg/kg in addition to the supplement of carotenoids from paprika oleoresin could prevent the possible negative effect caused by the high dietary content of n-3 HUFA as was stated by (Fernández-Palacios *et al*. 1995). These requirements are more stringent than those stated by other authors for Salmon species (Furuita *et al*., 2000, 2002; Izquierdo *et al*., 2001). However, Vassallo-Agius *et al*. (2001a) discovered similar results utilizing diets which contained in addition squid flour and astaxantine, thus improving the quality of eggs laid by the striped jack *Pseudocaranx dentex*.

Conclusion

The results of this study suggest that the recommended levels of n-3 HUFA in diets for gilthead sea bream broodstocks could be increased up to 3,5 % when supplemented jointly with carotenoids from paprika oleoresin and vitamin E, thus favoring the quality of spawning.