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Effects of different dietary protein and lipid levels on growth, feed utilization and body composition of red porgy (*Pagrus pagrus*) fingerlings

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Abstract

Two feeding trials were conducted to determine the minimum dietary protein level producing maximum growth, and the optimum protein to energy ratio in diets for red porgy (*Pagrus pagrus*) fingerlings, respectively. In the first trial, six isoenergetic diets were formulated with protein levels ranging from 400 to 650 g kg^{-1} in increments of 50 g kg^{-1}, and fed for 11 weeks to 2.8 g average initial weight fish. Weight gain, specific growth rate and feed efficiency were significantly higher with diets containing higher protein levels, when compared with dietary levels below 500 g kg^{-1}. The highest protein efficiency ratios were obtained in fish fed 500 g kg^{-1} dietary protein. The minimum dietary protein level producing maximum fish growth was found to be 500 g kg^{-1}. In the second trial, 15 g average initial weight fish were fed for 12 weeks, six diets containing three different lipid levels (100, 150 and 200 g kg^{-1}) combined with two protein levels (450 and 500 g kg^{-1}). Weight gain values increased when dietary lipids increased from 100 to 150 g kg^{-1}, with a further decrease for 200 g kg^{-1} lipids in diets; the lowest fish growth being supported by 200 g kg^{-1} dietary lipids. Fish growth was significantly higher when dietary protein increased from 450 to 500 g kg^{-1}. There was no evidence of a protein-sparing effect of dietary lipids. Liver protein and lipid contents were low when compared with other fish species. All diet assayed produced high liver glycogen accumulation. The recommended protein and lipid levels in diets for red porgy fingerlings were 500 and 150 g kg^{-1}, respectively.

KEY WORDS: lipids, nutritional requirements, protein, red porgy

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Introduction

Species diversification is considered a major approach for the sustainable development of Mediterranean aquaculture (Stephanis & Divanach 1993; Papandroulakis *et al.* 2005). The red porgy has particular characteristics making it an appropriate candidate: high growth rate (Kentouri *et al.* 1994), ease to adapt to existing farming techniques for other widely cultured sparids such as gilthead seabream (Basurco & Abellan 1999), and constantly high market prices all year around throughout the Mediterranean region (FEAP 2005). In addition, its life cycle has been closed under captive conditions (Hernández-Cruz *et al.* 1990, 1997, 1999; Kentouri *et al.* 1994; Conides *et al.* 2000; Conides & Glamuzina 2001; Mihelakakis *et al.* 2001; Pavlidis *et al.* 2003; Mylonas *et al.* 2004).

Studies on juveniles culture fed with commercial diets for gilthead seabeam showed high feed conversion rates (3.0–3.5) (Divanach *et al.* 1993), and in 1994 around 130 tonnes were commercially produced in Cyprus and Greece, using also commercial gilthead seabream diets and resulting in poor growth rates and an abnormal dark external colour or the fish (Stephanou *et al.* 1995; Klios *et al.* 1997). There have been also some preliminary attempts to formulate specific diets for this species (Kalinowski *et al.* 2005). However, no published data up to date are available on the basic nutritional requirements of the red porgy.

The optimal dietary protein levels found for different sparid species have been reported to be around 500 g kg^{-1} (Yone *et al.* 1974; Takeuchi *et al.* 1991; Vergara *et al.* 1996a). In addition, the current trend in fish feed production is to increase the lipid content in diets to spare proteins, to improve feed

Table 1 Composition (fed basis) and proximate analysis of the diets fed (g kg^{-1}) to *P. pagrus* fingerlings in trial 1

Diet protein/lipid (g kg^{-1})	400/150	450/150	500/150	550/150	600/150	650/150
Ingredients						
Fish meal[1]	518	592	666	739	813	887
Fish oil	83	75	65	58	50	42
Cornstarch	354	289	223	157	92	32
Vitamin premix[2]	20	20	20	20	20	20
Mineral premix[3]	20	20	20	20	20	20
C.M.cellulose[4]	5	5	5	5	5	5
Nutrient content (dry matter)						
Crude protein (g kg^{-1})	391	439	493	553	605	652
Total lipids (g kg^{-1})	147	150	146	152	149	147
Ash (g kg^{-1})	78	86	95	104	128	133
Moisture (g kg^{-1})	99	139	83	111	116	79
NFE[5] (g kg^{-1})	384	325	265	191	117	67
GE[6] (MJ kg^{-1})	19.93	19.98	20.03	20.08	20.13	20.17
P:E[7] (g MJ^{-1})	19.64	22.00	24.63	27.52	30.06	32.34

[1] Proximate analysis (g kg^{-1} dry wt): crude protein: 743, total lipids: 121, ash: 123.
[2] Vitamin premix contains (mg kg^{-1} or IU kg^{-1} of dry diet): thiamine 40 mg, riboflavin 50 mg, pyridoxine 40 mg, calcium pantothenate 117 mg, nicotinic acid 200 mg, biotin 1 mg, folic acid 10 mg, cyanocobalamin 0.5 mg, choline chloride 2700 mg, inositol 600 mg, ascorbic acid 1000 mg, alpha tocopherol 250 mg, menadione 20 mg, cholecalciferol 2000 IU, ethoxyquin 100 mg, retinol acetate 5000 IU.
[3] Mineral premix contains (g kg^{-1} of dry diet): calcium orthophosphate 1.60 g, calcium carbonate 4 g, ferrous sulphate 1.5 g, magnesium sulphate 1.6 g, potassium phosphate 2.8 g, sodium phosphate 1 g, aluminium sulphate 0.02 g, zinc sulphate 0.24 g, copper sulphate 0.20 g, manganese sulphate 0.08 g, potassium iodate 0.02 g. Both premixes were prepared with α-cellulose to include at 2% of the experimental diets.
[4] Carboxy methyl cellulose (sodium salt).
[5] Nitrogen free extract, calculated as 1000 – (protein + lipid + ash) g kg^{-1}.
[6] Calculated gross energy content (Brafield, 1985).
[7] Protein to energy ratio in g MJ^{-1}.

conversion and to decrease the amount of nitrogen waste produced by the fish. Most of the research in this area has been done with salmonid species (Hillestad & Johnsen 1994; Weatherup *et al*. 1997; Helland & Grisdale-Helland 1998; Hemre & Sandnes 1999; Lee & Kim 2001; Gélineau *et al*. 2002; Nordgarden *et al*. 2002), whereas less information exists on warmwater marine species such as sparids (Takeuchi *et al*. 1991; Ballestrazzi & Lanari 1996; Tibaldi *et al*. 1996; Vergara *et al*. 1996b, 1999; Pérez *et al*. 1997; Company *et al*. 1999; Peres & Oliva Teles 1999; Lupatsch *et al*. 2001; Boujard *et al*. 2004; Skalli *et al*. 2004). The present work aimed to investigate protein and lipids levels required for an optimal growth of fingerlings from this species.

Materials and methods

Fish and feeding

Trial 1 Six experimental diets were prepared containing a range of dietary protein from 400 to 650 g kg^{-1} by replacing fish meal with cornstarch in order to produce isoenergetic diets. Dietary lipid level was fixed at 150 g kg^{-1} in all diets. Dietary protein and lipids sources were Norwegian fish meal (LT 95) and capelin oil (Norsalmoil; Norsildmel, Bergen, Norway), respectively. Data for ingredients and chemical compositions are shown in Table 1.

Red porgy fingerlings of 2.8 g initial average body weights were anaesthetized (chlorobutanol 200 mg L^{-1}; at 70 mL/100 L sampling tanks), weighed and randomly stocked in 100-L fibreglass tanks in triplicate groups of 17 fish. All tanks were supplied with continuous flow of natural seawater. Average water temperature and dissolved oxygen concentration along the experimental period (11 weeks) were 21.8 °C ± 0.35 and 6.4 mg L^{-1} ± 0.62, respectively. A controlled photoperiod of 14 h light/10 h dark was maintained during the trial. Fish were hand fed until apparent satiation, five times a day, 6 days a week.

Trial 2 Six experimental diets were prepared in order to obtain three different dietary lipid levels (100, 150 and 200 g kg^{-1}) combined with two dietary protein levels (450 and 500 g kg^{-1}). Dietary protein and lipid sources were Norwegian fish meal (LT 95) and capeling oil, respectively. Diet ingredient contents and chemical composition are shown in Table 2.

Table 2 Composition (fed basis) and proximate analysis of the diets fed (g kg^{-1}) to *P. pagrus* fingerlings in trial 2

Diet (protein/lipids) (g kg^{-1})	450/100	450/150	450/200	500/100	500/150	500/200
Ingredients						
Fish meal[1]	646	646	646	729	729	729
Fish oil	32	82	132	23	73	123
Cornstarch	277	227	177	203	153	103
Vitamin premix[2]	20	20	20	20	20	20
Mineral premix[3]	20	20	20	20	20	20
C.M.cellulose[4]	5	5	5	5	5	5
Nutrient content (DM)						
Crude protein (g kg^{-1})	482	477	471	534	529	523
Total lipids (g kg^{-1})	111	147	195	96	147	200
Ash (g kg^{-1})	81	78	76	75	83	85
Moisture (g kg^{-1})	83	78	75	87	91	89
NFE[5] (g kg^{-1})	326	298	258	295	240	192
GE[6] (MJ kg^{-1})	21.29	22.13	23.23	21.39	22.38	23.51
P:E[7] (g MJ^{-1})	22.64	21.55	20.27	24.96	23.64	22.24

[1] Proximate analysis (g kg^{-1} dry wt): crude protein: 743, total lipids: 121, ash: 123.
[2] Vitamin premix contains (mg kg^{-1} or IU kg^{-1} of dry diet): thiamine 40 mg, riboflavin 50 mg, pyridoxine 40 mg, calcium pantothenate 117 mg, nicotinic acid 200 mg, biotin 1 mg, folic acid 10 mg, cyanocobalamin 0.5 mg, choline chloride 2700 mg, inositol 600 mg, ascorbic acid 1000 mg, alpha tocopherol 250 mg, menadione 20 mg, cholecalciferol 2000 IU, ethoxyquin 100 mg, retinol acetate 5000 IU.
[3] Mineral premix contains (g kg^{-1} of dry diet): calcium orthophosphate 1.60 g, calcium carbonate 4 g, ferrous sulphate 1.5 g, magnesium sulphate 1.6 g, potassium phosphate 2.8 g, sodium phosphate 1 g, aluminium sulphate 0.02 g, zinc sulphate 0.24 g, copper sulphate 0.20 g, manganese sulphate 0.08 g, potassium iodate 0.02 g. Both premixes were prepared with α-cellulose to include at 2% of the experimental diets.
[4] Carboxy methyl cellulose (sodium salt).
[5] Nitrogen free extract, calculated as 1000 – (protein + lipid + ash) g kg^{-1}.
[6] Calculated gross energy content (Brafield, 1985).
[7] Protein to energy ratio in g MJ^{-1}.

Red porgy fingerlings of initial average body weight 15 g were anaesthetized (chlorobutanol 200 mg L^{-1}; at 70 mL/100 L sampling tanks), weighed and randomly stocked in 500-L fibreglass tanks in triplicate groups of 50 fish. All tanks were supplied with continuous flow of natural seawater of 6 L min^{-1}. Average water temperature and dissolved oxygen concentration along the experimental period (12 weeks) were 22.4 ± 0.05 °C and 6.2 ± 0.07 mg L^{-1}, respectively. A controlled photoperiod with artificial light of 13 h light/11 h dark was maintained during the trial. Fish were hand fed until apparent satiation, three times a day, 6 days a week, due to the bigger size of fish.

Samples and analysis

Twelve fish at the start of each experiment and three per tank at the end were killed by an overdose of chlorobutanol and stored at −20 °C for subsequent chemical analysis. Besides, fish were individually weighed at the beginning, after 5 weeks and at the end of the experiment.

Dry matter and ash in feeds and fish samples were determined gravimetrically after drying for 24 h until constant weight, at 105 °C and after combustion for 24 h at 450 °C, respectively. Crude protein (N × 6.25) and lipid contents were determined by Kjeldahl (AOAC 1995) and Folch (Folch *et al.* 1957) methods, respectively.

Red porgy livers from three fish per tank at the end of the experiment were fixed in 10% buffered formaldehyde for histological evaluations. Livers were then dehydrated in ethanol series, embedded in paraffin, serially sectioned at 4–5 m, and stained with haematoxylin and eosin (H&E), and periodic acid Schiff-reactive haematoxylin (PAS-Hx) (Garcia Del Moral 1993). The PAS-Hx was used to selectively stain glycogen.

One- and two-way analysis (independent variable protein and lipid) of variance (ANOVA), and 5% level probability (Tukey 95%) were used to compare data ($P < 0.05$), which are presented as mean ± SD of three replicate groups (Sokal & Rolf 1995). The broken line test was also used to estimate minimum dietary protein level supporting optimal growth (Zeitoun *et al.* 1976).

Table 3 Mean growth performance and feed conversion of *P. pagrus* fingerlings fed the experimental diets in trial 1

Diet (protein/lipid) (g kg^{-1})	400/150	450/150	500/150	550/150	600/150	650/150
Average initial weight (g)	2.62 ± 1.07	2.66 ± 0.98	2.77 ± 1.17	2.70 ± 0.97	2.74 ± 1.1	2.66 ± 0.99
Average final weight (g)	15^{a} ± 4	18^{b} ± 4	23^{c} ± 6	22^{bc} ± 7	23^{c} ± 6	23^{c} ± 7
Weight gain (% of initial weight)	451^{a} ± 60	581^{b} ± 41	714^{c} ± 34	724^{c} ± 76	712^{c} ± 50	771^{c} ± 70
SGR[1]	2.28^{a} ± 0.14	2.56^{ab} ± 0.08	2.83^{bc} ± 0.01	2.81^{bc} ± 0.16	2.81^{bc} ± 0.10	2.88^{c} ± 0.11
FCR[2]	1.67^{b} ± 0.12	1.59^{b} ± 0.17	1.20^{a} ± 0.1	1.20^{a} ± 0.09	1.12^{a} ± 0.02	1.05^{a} ± 0.09
PER[3]	1.54 ± 0.12	1.43 ± 0.17	1.66 ± 0.22	1.51 ± 0.11	1.46 ± 0.05	1.45 ± 0.11

Values in the same row with no superscripts are not significantly different ($P < 0.05$).
[1] SGR = (($\log_e$ average final weight − $\log_e$ average initial weight)/no days) × 100.
[2] FCR = feed consumption (g)/weight gain (g).
[3] PER = weight gain (g)/protein intake (g).

Results and discussion

Trial 1

Growth response of red porgy fingerlings fed with different experimental diets is shown in Table 3. Final body weights were significantly higher ($P < 0.05$) in fish fed diets with protein levels 500, 550, 600 and 650 g kg^{-1} than in those fed protein levels of 400 and 450 g kg^{-1}. Similarly, weight gain and food conversion ratios were significantly poorer ($P < 0.05$) with diets containing protein levels below 500 g kg^{-1}. Dietary protein levels below 500 g kg^{-1} also produced lower specific growth rates (SGR), the values of fish fed protein level 400 g kg^{-1} was significantly lower ($P < 0.05$) than fish fed with protein levels of 500, 550, 600 and 650 g kg^{-1}. Protein efficiency ratios (PER) values were highest in fish fed 500 g kg^{-1} protein, but not significantly different from other fish treatments (Table 3).

Among fish fed the lower protein contents, the linear increase in weight gain, together with the decrease in food conversion ratios suggested a possible limitation of certain amino acid contents in those diets to completely promote growth. The broken line test applied to weight gain with different dietary protein levels showed a value of minimum dietary protein level supporting optimal growth of 508 g kg^{-1} (Fig. 1), which is within the range of levels found for different sparid species, such as common dentex (*Dentex dentex*) (Cardenete *et al.* 1999), Japanese seabream (Takeuchi *et al.* 1991) and gilthead seabream (Vergara *et al.* 1996a). Dietary protein levels above 500 g kg^{-1} did not show an additional effect on either weight gain or feed conversion, this might be explained as dietary energy needed to deaminate and excrete excess absorbed amino acids (Cowey *et al.* 1972; Teng *et al.* 1978; Shi *et al.* 1988).

The average muscle protein content showed a similar trend to that of PER, with the highest values corresponding to fish fed diets with 450, 500 and 550 g kg^{-1} protein, being significantly different from ($P < 0.05$) fish fed diets with 400, 600 and 650 g kg^{-1} protein (Table 4), suggesting again that fish treatment with protein level 400 g kg^{-1}, with the lowest protein level, was unable to meet the requirement for the optimal protein synthesis, whereas dietary protein levels above 550 g kg^{-1} lead to a reduced muscle protein synthesis. In general, fish fed protein levels between 400 and 500 g kg^{-1} tended to show higher muscle lipid contents than those fed either higher or lower protein levels. This could be due to enhanced lipogenesis from either high protein or carbohydrate levels in these diets. Liver protein and lipid contents were low when compared with other fish species (red porgy average P/L 370/210 g kg^{-1} and for other species 500/380 g kg^{-1}) (Tibaldi *et al.* 1996; Robaina *et al.* 1997; Dias *et al.* 1998; Company *et al.* 1999; Santinha *et al.* 1999; Boujard *et al.* 2004), and liver protein content showed an increment as dietary protein increased (Table 4). In general, fish diets with high levels of protein tend to promote fatty acids or glycogen synthesis derived from proteins (Wilson 1989), while diets with high levels of carbohydrate tend to reduce protein digestibility and increase liver glycogen concentration and liver size (NRC, 1993; Krogdahl *et al.* 2005). However, these effects could not be observed in this trial, all different treatments leading to a high liver glycogen accumulation.

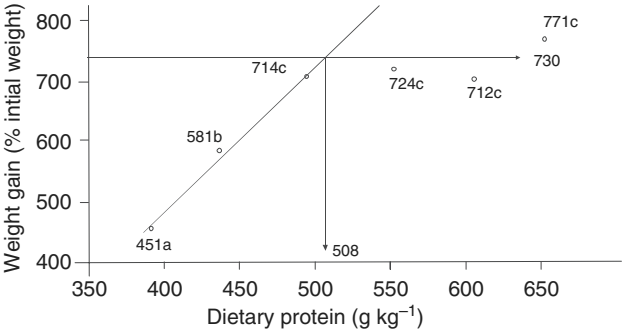

Figure 1 Broken line test applied to weight gain (% of initial weight) with different dietary protein levels in trial 1.

Table 4 Liver and muscle composition of *P. pagrus* fingerlings fed the experimental diets in trial 1 (g kg^{-1} dry weight basis)

		Diet (protein/lipid) (g kg^{-1})					
	Initial	400/150	450/150	500/150	550/150	600/150	650/150
Liver composition							
Moisture	653	613	603	592	608	613	601
Protein	358	226^{a}	248^{a}	271^{ab}	293^{ab}	312^{bc}	342^{c}
Lipids	201	235^{ab}	209^{a}	290^{b}	236^{ab}	203^{a}	246^{ab}
Muscle composition							
Moisture	749	722^{a}	743^{b}	733^{ab}	749^{c}	734^{ab}	736^{ab}
Protein	825	745^{b}	819^{d}	811^{d}	821^{d}	723^{a}	779^{c}
Lipids	82	92^{b}	90^{b}	94^{b}	75^{a}	91^{b}	78^{a}

Values in the same row with no superscripts are not significantly different ($P < 0.05$).

A moderate vacuolization of hepatocyte was shown in the fish livers both at the start of the experiment (fed with commercial gilthead seabream diets: 450 g kg^{-1} protein, 210 g kg^{-1} lipids) (Fig. 2a), and in fish fed diets with P/L 400/150 and 450/150 g kg^{-1} (Fig. 2c). The hepatocyte size of livers from fish fed diets with protein levels of 600 and 650 g kg^{-1} was smaller than other experimental groups, and the hepatocyte cytoplasm showed high acidophilia (Fig. 2e). A clear lipidic degeneration of hepatocytes in the fish liver at the start of the experiment was found (Fig. 2b). In general, and independently of diets, all fish livers showed high glycogen accumulation in hepatocytes cytoplasm, revealed as the eosinophile material found with the PAS technique (Fig. 2d,f). This elevated glycogen accumulation in hepatocytes, to a higher extent than that observed for gilthead seabream (Robaina *et al*. 1997), could partially explain the low liver protein and lipid contents found in the proximate analysis. Similar high liver glycogen accumulation has been observed in Japanese seabream (*Pagrus major*), with values up to 360 g kg^{-1} liver glycogen (Jeong *et al*. 1991).

The results from this trial suggest an optimum protein level in diets for *P. pagrus* fingerlings of 500 g kg^{-1} when hand fed to satiation, and for a fixed dietary lipid level of 150 g kg^{-1}. Similar results have been found for other sparids (Millikin 1983; Alliot & Pastoureaud 1984; Takeuchi *et al*. 1991; Tibaldi *et al*. 1996; Vergara *et al*. 1996a; Cardenete *et al*. 1999).

Trial 2

Red porgy fingerlings fed different protein and lipid levels showed the best response in the final body weight ($P < 0.05$) and SGR ($P < 0.05$) when fed diet with P/L 500/150 g kg^{-1} (Table 5). No significant differences were found in total ingested feed or PER between different treatments, although for each protein level ingested feed tended to decrease with increase in dietary lipids. PER was improved for each dietary protein level when dietary lipids increased from 100 to 150 g kg^{-1}, followed by a decrease for diets containing 200 g kg^{-1} lipids (Table 5). Both dietary protein and lipid levels, as well as their interactions significantly affected final body weight ($P < 0.05$) and SGR ($P < 0.05$). Thus, diets containing 500 g kg^{-1} protein produced higher final body weights and SGR than diets with 450 g kg^{-1} protein, regardless of the lipid levels, and diets containing 100 and 150 g kg^{-1} lipids produced highest final body weights and SGR, irrespective of the protein levels (Table 6). The fact that diets with 200 g kg^{-1} lipid produced the lowest values could be due to a poorer capability of this species to digest dietary lipids, when compared with other sparid species (Arantzamendi 2002). Average muscle and liver protein and lipid contents were not significantly affected by different treatments (Table 7), although there was a trend towards a decrease in muscle lipid when dietary protein content increased from 450 to 500 g kg^{-1} in all dietary lipid levels. As in the previous trial, liver protein and lipid contents were low when compared with other fish species (Takeuchi *et al*. 1991; Tibaldi *et al*. 1996; Robaina *et al*. 1997; Peres & Oliva Teles 1999; Lupatsch *et al*. 2001; Skalli *et al*. 2004), and for dietary lipid levels of 100 and 150 g kg^{-1}, both liver protein and lipid contents tended to increase as dietary protein increased from 450 to 500 g kg^{-1}.

Liver histology showed areas of esteatosis, migration of hepatocyte nucleus, together with a high amount of lipidic vacuoles in fish livers both at the start of the experiment (fed with commercial gilthead seabream diets: 400 g kg^{-1} protein, 210 g kg^{-1} lipids), and in fish fed with P/L 450/150 g kg^{-1}. Eosinophile material was present in hepatocytes cytoplasm in all treatments, with a higher intensity in livers of fish fed diets with P/L 450/200 and 500/150 g kg^{-1}. High glycogen accumulation was apparent in hepatocytes cytoplasm of all fish livers, without differences between different treatments. This effect, also observed in trial 1, suggests that red porgy has a higher capability to store glycogen in liver than other sparid species such as gilthead seabream (Robaina *et al*. 1997).

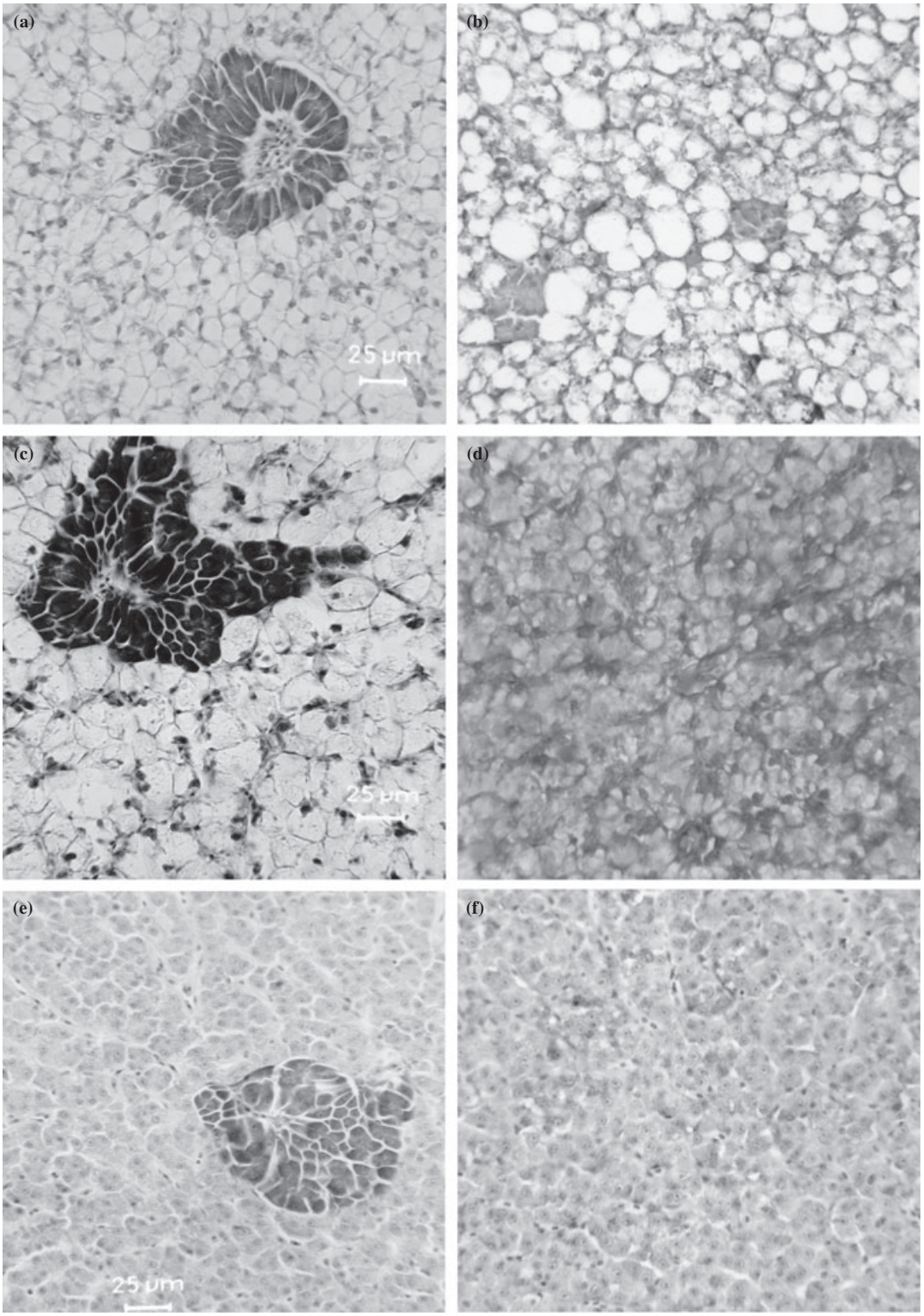

Figure 2 Livers of red porgy fed different experimental diets (trial 1). (a) Initial livers. Hepatocytes with migration of nuclei (H&E). (b) Initial livers. Increased cytoplasm vacuolation and severe lipid vacuoles accumulation (PAS-Hx). (c) Diet 2. Hepatocytes with migration of the nuclei (H&E). (d) Diet 2. Hepatocytes with significant glycogen accumulation in the cytoplasm (PAS-Hx). (e) Diet 5. Hepatocytes with large centrally located nuclei and acidophile cytoplasma (H&E). (f) Diet 5. Hepatocytes with glycogen deposition in the cytoplasm.

Table 5 Mean growth performance and food conversion of *P. pagrus* fingerlings fed the experimental diets in trial 2

Diet (protein/lipid) (g kg^{-1})	450/100	450/150	450/200	500/100	500/150	500/200
Average initial weight (g)	15.23 ± 0.49	15.88 ± 0.20	15.31 ± 0.68	15.40 ± 0.99	15.47 ± 0.19	15.69 ± 0.76
Average final weight (g)	60.2^{ab} ± 13.4	61.5^{ab} ± 11.8	56.6^{a} ± 10.9	64.9^{c} ± 15.3	73.9^{d} ± 3.6	58.8^{a} ± 15.0
Weight gain (% of initial weight)	295^{abc} ± 11	287^{ab} ± 18	269^{a} ± 5	341^{cde} ± 29	377^{e} ± 4	315^{abcd} ± 20
Total ingested food (g)	2214.7 ± 157	1980.9 ± 108	2120.5 ± 139	2237.0 ± 142	2178.5 ± 68.3	2017.2 ± 31.4
SGR[1]	1.65^{abc} ± 0.05	1.61^{ab} ± 0.07	1.55^{a} ± 0.02	1.76^{bcd} ± 0.08	1.86^{d} ± 0.01	1.69^{abc} ± 0.07
FCR[2]	1.71^{abc} ± 0.28	1.64^{abc} ± 0.16	1.78^{bc} ± 0.08	1.40^{abc} ± 0.03	1.25^{ab} ± 0.14	1.95^{c} ± 0.13
PER[3]	1.92 ± 0.78	2.23 ± 0.18	2.07 ± 0.10	2.38 ± 0.06	2.71 ± 0.28	1.76 ± 0.09

Values in the same row with no superscripts are not significantly different ($P < 0.05$).
[1] SGR = (($\log_e$ average final weight − $\log_e$ average initial weight)/days) × 100.
[2] FCR = feed consumption (g)/weight gain (g).
[3] PER = weight gain (g)/protein intake (g).

Table 6 Factorial analysis of variance (two-way) of the effect of dietary protein and lipid levels (g kg^{-1}) on final body weight (FBW) and specific growth rate (SGR) in trial 2

	Mean FBW
Dietary protein level[1] ($P = 0.0000$)	
450	59.42^{a}
500	69.27^{b}
Dietary lipid level[1] ($P = 0.0000$)	
100	64.97^{ab}
150	67.39^{b}
200	62.95^{a}
	Mean SGR
Dietary protein level[2] ($P = 0.0000$)	
450	1.60^{a}
500	1.78^{b}
Dietary lipid level[2] ($P = 0.0058$)	
100	1.73^{b}
150	1.75^{b}
200	1.66^{a}

[1] Interactions between protein and lipid levels ($P = 0.076$).
[2] Interactions between protein and lipid levels ($P = 0.023$).

Table 7 Liver and muscle composition of *P. pagrus* fingerlings fed the experimental diets in trial 2 (g kg^{-1} dry weight basis)

	Diet (protein/lipid) (g kg^{-1})						
	Initial	400/100	400/150	400/200	500/100	500/150	500/200
Liver composition							
Moisture	645	667	647	652	651	650	650
Protein	358	348	337	413	374	360	396
Lipids	201	165	199	246	291	218	182
Muscle composition							
Moisture	759	769	758	753	759	758	758
Protein	846	889	877	837	828	833	873
Lipids	82	80	104	83	74	54	63

Values in the same row with no superscripts are not significantly different ($P < 0.05$).

The result from this trial suggests optimum protein and lipid levels in diets for red porgy fingerlings of 500 and 150 g kg^{-1}, respectively, when hand fed to satiation. No protein-sparing effect due to dietary lipids could be detected in trial 2, as fish growth values increased when dietary lipids increased from 100 to 150 g kg^{-1}, with a further decrease for 200 g kg^{-1} lipids in diets. Similar results to our experiment were found in common dentex (Cardenete *et al.* 1999). Although in various sparids the authors have obtained a protein-sparing effect due to dietary lipids, such as, the *D. dentex* (Tibaldi *et al.* 1996; Skalli *et al.* 2004), and *S. aurata* (Vergara *et al.* 1996b; Company *et al.* 1999; Santinha *et al.* 1999; Lupatsch *et al.* 2001).

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