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Dietary protein, growth, nutrient utilization and body composition of juvenile blackspot seabream, *Pagellus bogaraveo* (Brunnich)

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Abstract

When considering new marine species for Mediterranean aquaculture, blackspot seabream emerges as a potential candidate. However, there are scarce data on the nutritional requirements and optimal growth conditions of this species. A 12-week feeding trial was conducted to evaluate the effects of dietary protein on growth, nutrient utilization and body composition of blackspot seabream (23 g). Five isolipidic diets (12%) with graded levels of protein (20–60%) were distributed, twice a day, to duplicate groups of fish, until satiation. Growth increased significantly with increasing dietary protein up to 40%, but higher protein levels induced a similar daily growth index (1.4). Feed conversion ratio (FCR) decreased with increasing levels of dietary protein (4.2–1.6). No significant differences were detected in protein of whole body blackspot seabream among treatments, but fat percentage decreased with increasing dietary protein. Dry matter and energy digestibility showed a concomitant increase with the reduction in dietary wheat meal, attaining maximal values with high protein diets. These results suggested that the most favourable values for growth and FCR are obtained with diets containing 40% protein. However, the excessive lipid deposition reveals that more nutritional studies are necessary before the species can be established in aquaculture.

Keywords: sparidae, digestibility, nutritional requirements, nutrient retention, proximate composition

Introduction

In Southern Europe, marine aquaculture is currently concentrated on the production of gilthead seabream (*Sparus aurata*, Linnaeus), European seabass (*Dicentrarchus labrax*, Linnaeus) and turbot (*Psetta maximus*, Linnaeus). With the expansion of aquaculture sector in recent years there has been increased interest in other species, and blackspot seabream (*Pagellus bogaraveo*, Brunnich) is currently under consideration due to its high commercial value, scarcity in the market and biological characteristics (Peleteiro, Olmedo & Álvarez-Blásquez 2000).

The blackspot seabream is common along the continental shelf of the Southern European Atlantic and throughout Mediterranean. Studies on blackspot seabream in captivity are extremely scarce and have dealt mainly with reproduction and disease control, larvae and juveniles culture techniques (Olmedo, Peleteiro, Álvarez-Blázquez & Gómez 1998; Peleteiro *et al*. 2000; Micale, Maricchiolo & Genovese 2002, Mladineo 2003). Individuals caught in the natural environment exhibits lower growth rates (Peleteiro, Olmedo, Cal & Gomes 1994; Olmedo, Peleteiro, Linares, Álvarez-Blázquez, Gómez & Ortega 2000) compared with other farmed Sparidae, such as gilthead seabream (Aksnes, Izquierdo, Robaina, Vergara & Montero 1997; Santinha, Medale, Corraze & Gomes 1999; Izquierdo, Obach, Arantzamendi, Montero, Robaina & Rosenlund 2003), and very high lipid deposition in muscle, liver and particularly around viscera (Linares, Olmedo, Peleteiro & Arán-Echabe 2001). These results clearly suggest the need of optimizing culture conditions and improving diets for this

species for possible future consolidation of blackpot seabream farming.

Wild blackspot seabream is mainly carnivorous (Morato, Solà, Gròs & Menezes 2001), but the optimum level of dietary protein for this species in captivity was not reported yet. The aim of the present study was to determinate the effect of graded dietary protein level on growth, nutrient utilization and body composition of juvenile blackspot seabream.

Materials and methods

Diets

Five isolipidic (crude fat: 12% dry matter (DM)) diets were formulated to contain graded protein levels (20–60%) (Table 1). The dietary protein was provided from a mixture of LT fishmeal, wheat gluten, soybean meal and fish protein concentrate. All ingredients were finely ground, mixed and dry pelleted through a 2.4 mm die at 50 °C (CPM model 3000, San Francisco, CA, USA).

Experimental conditions

The feeding trial was conducted on wild-caught blackspot seabream at the Mariculture Center of Calheta, in Madeira Island. Fish were held for 6 weeks to acclimation to captive conditions. During that time, fish were fed *ad libitum* with a commercial diet (44% protein, 17% fat; Sorgal, S.A.). After acclimatization, homogeneous groups of 120 juveniles [initial body weight (IBW) = 23 g] were randomly distributed among 10 PVC tanks (500 L) in a flow-through sea-

Table 1 Formulation and proximate composition of the experimental diets

	Dietary treatments				
	D20	**D30**	**D40**	**D50**	**D60**
Ingredients (%)					
Extruded peas meal*	10.0	10.0	10.0	5.0	9.0
Wheat meal	55.7	37.5	22.4	11.2	5.0
LT fishmeal†	5.0	17.7	33.2	50.7	50.0
Wheat gluten	2.0	2.0	2.0	2.0	16.3
Defatted soybean meal (48% crude protein)	13.7	20.0	20.0	20.0	0.0
Fish protein concentrate‡	1.0	1.0	2.0	2.0	13.2
Mineral mix§	0.5	0.5	0.5	0.5	0.5
Vitamin mix¶	0.2	0.2	0.2	0.2	0.2
Binder	2.0	2.0	2.0	2.0	2.0
Fish oil	9.9	9.1	7.7	6.4	3.9
Proximate composition					
Dry matter (DM, %)	91.0	91.3	92.1	92.5	93.5
Crude protein (% DM)	24.1	35.7	45.3	54.4	63.7
Crude fat (% DM)	12.7	12.7	12.4	12.4	11.6
Ash (% DM)	5.7	7.9	10.1	12.2	11.8
NFE (% DM)‖	48.6	34.9	24.3	13.4	6.4
Gross energy (kJ g^{-1} DM)	18.8	19.3	19.5	19.7	20.4
Digestible protein (DP, % DM))	17.4	29.7	39.4	48.8	58.3
Digestible energy (DE) (kJ g^{-1} DM)	9.2	11.7	13.8	15.0	17.3
DP/DE ratio (mg kJ^{-1})	18.8	25.5	28.5	32.6	33.7

*Aquatex (20.5 Crude protein (CP); Sotexpro, Bermericourt, France).

†Norse LTay, Norse LT94, Norway.

‡Soluble fish protein hydrolizate (75.26 crude protein; Sopropêche, Boulogne sur Mer, France).

§Mineral mixture (g or mg kg^{-1} diet): calcium carbonate (40% Ca), 2.15 g; magnesium oxide (60% Mg), 1.24 g; ferric citrate, 0.2 g; potassium iodide (75% I), 0.4 mg; zinc sulphate (36% Zn), 0.4 g; copper sulphate (25% Cu), 0.3 g; manganese sulphate (33% Min), 0.3 g; dibasic calcium phosphate (20% Ca, 18% P), 5 g; cobalt sulphate, 2 mg; sodium selenite (30% Se), 3 mg; potassium chloride, 0.9 g; sodium chloride, 0.4 g.

¶Vitamin mixture (IU or mg kg^{-1} diet): DL-α tocopherol acetate, 60 IU; sodium menadione bisulphate, 5 mg; retinyl acetate, 15 000 IU; DL-cholecalciferol, 3000 IU; thiamin, 15 mg; riboflavin, 30 mg; pyridoxine, 15 mg; cyanocobalamin, 0.05 mg; nicotinic acid, 175 mg; folic acid, 5 mg; ascorbic acid, 500 mg; inositol, 1000 mg; biotin, 2.5 mg; calcium panthotenate, 50 mg; choline chloride, 2000 mg.

‖Nitrogen-free extract – estimated by difference.

water system (salinity: 38 g L^{-1}) at a temperature of 21–24 °C (min.–max.). Fish were exposed to a 12 h light/12 h dark photoperiod (dawn at 07:00 hours and dusk at 19:00 hours). The five diets were randomly allotted to the duplicates tanks, and fish were hand fed to apparent satiety, twice a day, for over 12 weeks. Every 4 weeks fish were bulk-weighed to estimate growth and register feed consumption. Before sampling, fish were deprived of food for 24 h, anaesthesized by immersion in a bath of isoeugenol (2-methoxy-4-propenylphenol) at 5–10 mL 1000 L^{-1}, weighed and measured. A pooled sample of 20 fish was taken from the initial stock, and six fish per tank at the end of the experiment. Fish were stored at − 20 °C for subsequent whole body composition analysis.

Digestibility trial

The apparent digestibility coefficient (ADC) of the dietary components of the diets was determined after incorporation of 0.5% of chromic oxide to a grounded portion of each diet. The mixtures were then dry pelleted through a 2.4 mm die at 50 °C (CPM, C-300 model). Ten homogenous groups of 10 fish (mean body weight of 60 g) were distributed by a digestibility flow-through seawater system of 55 L tanks specially constructed according to the Guelph System protocol (Cho, Slinger & Bayley 1982). The experimental fish were adapted to the new conditions for 20 days and were subjected to a 12 h light/12 h dark photoperiod regime provided by artificial illumination. The diets were randomly assigned by the tanks (two tanks per treatment) and the first 4 days were used for acclimation to the feed and no faeces were collected. Fish were fed once daily until apparent satiety and approximately 30 min after fish consumed their meal any uneaten food and faeces were removed from the system. Before the fish were fed each morning, the faeces were collected, centrifuged at 1715 *g* for 10 min and frozen at − 20 °C. The ADCs were calculated according to Maynard and Loosly (1969).

Analytical methods

Frozen whole body samples and faeces were ground and analysed for DM (105 °C for 24 h). Samples were then freeze-dried before analyses. Diets, whole body samples and faeces were analysed for DM, ash by combustion in a muffle furnace (550 °C for 12 h); crude protein (micro-Kjeldahl; N × 6.25) after acid digestion, lipids by petroleum ether extraction (at Soxhlet 40–60 °C), and gross energy, by direct combustion in an adiabatic bomb calorimeter (model PARR 1261, Parr, Moline, IL, USA). Chromic oxide in both faeces and diets was determined according to Bolin, King and Klosterman (1952).

Calculations

The growth performance, feed intake and feed utilization were described using the following parameters:

$$\text{Wet weight gain (WWG)} = \text{Final body weight (FBW)} - \text{Initial body weight (IBW) (g)}$$

$$\text{Daily growth index (DGI)} = 100 \times ((\text{FBW}^{1/3} - \text{IBW}^{1/3})/\text{days})$$

$$\text{Feed conversion ratio (FCR)} = \text{Dry feed intake (g)}/\text{WWG (g)}$$

$$\text{Voluntary feed intake (VFI)} = \text{Feed intake}/\text{Mean body weight (MBW)}/\text{days (g kg}^{-1}\text{ day}^{-1}\text{)}$$

$$\text{Protein efficiency ratio (PER)} = \text{WWG}/\text{crude protein intake}$$

$$\text{Condition factor } (K) = \text{FBW}/\text{length}^3 \times 100$$

Statistical analysis

Statistical analysis followed methods outlined by Zar (1996). All data were checked for normality and homogeneity of variance using Kolmogorov–Smirnov and the Levene tests respectively. Arcsine transformations of percentage data were preformed to achieve homogeneity of variance. Data were then submitted to a one-way analysis of variance (ANOVA), with dietary protein level as the main factor, using the STATISTICA (StatSoft) version 6. When F values showed significance, individual means were compared using the Duncan's multiple range test. Significant differences were considered when $P < 0.05$.

Table 2 Growth performance and feed utilization in juvenile blackspot seabream, *Pagellus bogaraveo*, fed the experimental diets over 12 weeks

	Dietary treatments				
	D20	**D30**	**D40**	**D50**	**D60**
Initial body weight (IBW) (g)	22.4 (0.8)	23.5 (0.1)	22.3 (0.5)	22.7 (1.6)	23.1 (0.3)
Final body weight (FBW) (g)	36.9 (2.4)^{a}	50.1 (2.1)^{b}	58.8 (1.3)^{c}	61.2 (1.1)^{c}	61.9 (2.5)^{c}
Condition factor (*K*)	2.2 (0.03)	2.3 (0.1)	2.4 (0.03)	2.4 (0.03)	2.3 (0.03)
Wet weight gain (g)	14.4 (1.6)^{a}	26.5 (2.2)^{b}	35.5 (0.8)^{c}	38.5 (2.8)^{c}	38.9 (2.2)^{c}
Daily growth index	0.7 (0.1)^{a}	1.1 (0.1)^{b}	1.3 (0.01)^{c}	1.4 (0.1)^{c}	1.4 (0.1)^{c}
Feed conversion ratio	4.2 (0.6)^{a}	2.6 (0.3)^{b}	2.1 (0.04)^{b,c}	1.8 (0.01)^{c}	1.6 (0.1)^{c}
Voluntary feed intake (g kg^{-1} BW day^{1})	26.1 (0.2)^{a}	24.2 (0.1)^{a}	22.8 (0.04)^{a,b}	20.6 (0.2)^{b,c}	19.1 (0.02)^{c}
Protein efficiency ratio	1.0 (0.1)	1.1 (0.1)	1.1 (0.02)	1.1 (0.003)	1.0 (0.03)

Values are means (SD), $n = 2$.
Within a row, means without a common superscript letter differ significantly ($P < 0.05$). Absence of superscript indicates no significant difference between treatments.

Table 3 Whole body composition (% or kJ g^{-1} of wet weight) in juvenile blackspot seabream, *Pagellus bogaraveo*, fed the experimental diets over 12 weeks

	Dietary treatments				
	D20	**D30**	**D40**	**D50**	**D60**
Final body composition*					
Moisture (%)	58.9 (0.3)^{a}	59.7 (0.3)^{a,b}	60.4 (0.2)^{b,c}	61.3 (0.3)^{c,d}	61.7 (0.8)^{d}
Ash (%)	3.5 (0.1)	3.7 (0.5)	3.5 (0.5)	4.0 (0.3)	4.0 (0.6)
Protein (%)	13.2 (1.4)	14.0 (0.6)	14.6 (0.5)	14.2 (0.3)	14.3 (0.7)
Lipids (%)	21.0 (0.6)^{a}	19.7 (0.5)^{a,b}	19.9 (0.7)^{a,b}	18.8 (0.03)^{b,c}	18.3 (0.5)^{c}
Energy (kJ g^{-1})	10.7 (0.1)^{a}	10.2 (0.3)^{b}	10.0 (0.01)^{b,c}	9.9 (0.1)^{b,c}	9.6 (0.2)^{c}

*Initial body composition (% or kJ g^{-1} of wet weight) was: moisture 63.3, protein 13.9, fat 16.3, ash 3.7 and energy 8.97.
Values are means (SD), $n = 2$.
Within a row, means without a common superscript letter differ significantly ($P < 0.05$). Absence of superscript indicates no significant difference between treatments.

Results

Growth performance

Data on growth and feed utilization are reported in Table 2. The growth of fish, expressed either as wet weight gain (WWG) or as daily growth index (DGI), was significantly improved with increasing levels of dietary protein up to 40%, but there was no further significant increase at higher dietary protein concentrations. The length–weight relationships did not vary significantly among fish in the different treatments. Voluntary feed intake decreased with increasing dietary protein level (Table 2). Feed conversion ratio (FCR) varied between 1.6 and 4.2 with no significant differences emerging when fish were fed above 40% dietary protein. Protein efficiency ratios (PERs) were not significantly different ($P > 0.05$) among treatments.

Whole body composition

Data on whole body composition are reported in Table 3. The per cent of both protein and ash were similar among treatments. There was a significant and inverse effect of dietary protein level on the per cent whole body moisture and lipids, with lowest lipid (18.3%) and highest moisture (61.7%) values in fish fed D60. These fish have also exhibited the lowest energy (9.6 kJ g^{-1}).

Digestibility

The ADC of DM increased significantly from D20 to D60 (Table 4). Protein and lipid digestibilities were highest for D60 (92 and 99% respectively), although no significant differences were observed from D50 to D60. Energy digestibility increased significantly with

Table 4 Apparent digestibility coefficients (ADC, %) of the different experimental diets

	Dietary treatments				
	D20	**D30**	**D40**	**D50**	**D60**
Dry matter	32.7 (3.0)^{a}	38.9 (4.4)^{a}	51.1 (3.0)^{b}	59.3 (0.8)^{c}	66.2 (0.3)^{d}
Protein	72.3 (0.9)^{a}	83.2 (2.2)^{b}	86.9 (1.7)^{b,c}	89.7 (0.8)^{c,d}	91.5 (1.0)^{d}
Lipids	83.9 (1.3)^{a}	93.3 (1.7)^{b}	96.3 (1.2)^{b,c}	98.1 (1.6)^{c}	99.0 (0.3)^{c}
Energy	49.1 (1.4)^{a}	60.3 (2.4)^{b}	70.9 (1.3)^{c}	76.2 (0.03)^{d}	84.6 (0.8)^{e}

Values are means (SD), $n = 2$.
Within a row, means without a common superscript letter differ significantly ($P < 0.05$). Absence of superscript indicates no significant difference between treatments.

Table 5 Intake and retention efficiency (g kg^{-1} or kJ g^{-1} of wet weight gain), of nitrogen, lipid and energy in blackspot seabream, *Pagellus bogaraveo*, fed the experimental diets over 12 weeks

	Dietary treatments				
	D20	**D30**	**D40**	**D50**	**D60**
Crude nitrogen intake	161.7 (21.5)	150.2 (15.2)	148.9 (2.8)	152.3 (0.5)	166.4 (5.9)
Digestible nitrogen intake	116.9 (15.5)	125.0 (12.6)	129.4 (2.4)	136.6 (0.4)	152.3 (5.4)
Recovered nitrogen*	19.2 (6.4)	22.6 (1.6)	24.0 (1.5)	23.1 (0.7)	23.1 (1.1)
Non-faecal excreted nitrogen†	97.8 (21.9)	102.4 (14.2)	105.5 (3.9)	113.5 (0.3)	129.2 (6.5)
Nitrogen retention efficiency (% digestible nitrogen intake)‡	16.9 (7.7)	18.2 (3.1)	18.5 (1.5)	16.9 (0.4)	15.2 (1.3)
Crude lipid intake	531.7 (70.7)^{a}	334.4 (33.8)^{b}	255.4 (4.8)^{b,c}	217.6 (0.6)^{c}	189.0 (6.6)^{c}
Digestible lipid intake	446.1 (59.3)^{a}	312.0 (31.5)^{b}	246.0 (4.6)^{b,c}	213.4 (0.6)^{c}	187.1 (6.6)^{c}
Recovered lipid*	282.1 (12.3)^{a}	227.1 (14.7)^{b}	223.8 (13.8)^{b}	203.0 (0.1)^{b}	194.1 (14.9)^{b}
Non-faecal excreted lipid†	164.0 (71.6)^{a}	84.8 (16.8)^{a,b}	22.2 (18.4)^{b,c}	10.4 (0.6)^{b,c}	− 7.0 (8.4)^{c}
Lipid retention efficiency (% digestible lipid intake)‡	64.0 (11.3)^{a}	72.9 (2.6)^{a}	91.1 (7.3)^{b}	95.1 (0.3)^{b}	103.7 (4.3)^{b}
Gross energy intake	79.0 (10.5)^{a}	50.8 (5.1)^{b}	40.0 (0.8)^{b,c}	34.4 (0.1)^{c}	33.3 (1.2)^{c}
Digestible energy intake	38.8 (5.2)^{a}	30.6 (3.1)^{b}	28.3 (0.5)^{b}	26.2 (0.1)^{b}	28.2 (1.0)^{b}
Recovered energy*	13.3 (0.2)^{a}	11.3 (0.8)^{b}	10.7 (0.1)^{b}	10.4 (0.2)^{b}	10.1 (0.7)^{b}
Non-faecal excreted energy§	2.4 (0.6)	2.6 (0.4)	2.6 (0.1)	2.8 (0.01)	3.2 (0.2)
Metabolizable energy¶	36.4 (4.6)^{a}	28.1 (2.7)^{b}	25.6 (0.4)^{b}	23.3 (0.1)^{b}	25.0 (0.8)^{b}
Total heat loss‖	23.4 (4.8)^{a}	16.8 (1.9)^{b}	15.0 (0.5)^{b}	13.0 (0.3)^{b}	14.9 (0.2)^{b}
Energy retention efficiency (% digestible lipid intake)‡	34.6 (5.1)	36.9 (1.0)	37.8 (0.9)	39.6 (0.9)	35.7 (1.1)

The values are mean (SD) ($n = 2$).
Within a row, means without a common superscript letter differ significantly ($P < 0.05$). Absence of superscript indicates no significant difference between treatments.
*Recovered nutrient = recovered nutrient in final carcass – recovered nutrient in initial carcass.
†Non-faecal excreted nutrient = digestible nutrient – recovered nutrient.
‡Nutrient retention efficiency = Nutrient recovered/Digestible nutrient intake (%).
§Non-faecal excreted energy = non-faecal excreted nitrogen × 25kJ g^{-1}.
¶Metabolizable energy = digestible energy intake – non-faecal excreted energy.
‖Total heat losses = metabolizable energy – recovered energy.

increasing dietary protein, and decreasing levels of carbohydrate (Table 4).

Nutrient and energy balance

The partition of the nutrients as nitrogen, lipid and energy is expressed in Table 5. The digestible nitrogen intake was not affected by the dietary protein content, being the recovered nitrogen similar among treatments. Crude lipid and gross energy intake showed the significant highest values when the low protein/high carbohydrate diet was used (D20). Values for recovered lipid and energy followed the very same trend. No significant differences were observed for nitrogen and energy retention efficiency values among dietary treatments ($P > 0.05$). However, the retention efficiency of lipids increased significantly

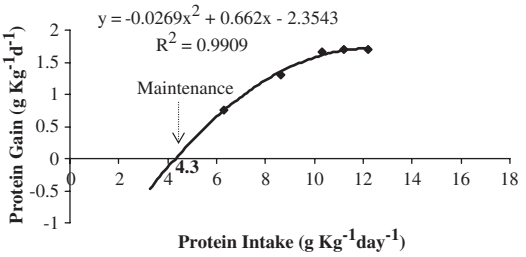

Figure 1 Relation between protein intake and protein gain is blackspot seabream *Pagellus bogaraveo* with an estimation of maintenance and growth needs by a polynomial model.

with increasing protein levels until a protein content of 40% was supplied (D40, D50 and D60). Based on daily protein gain and on protein intake data, and using a second-order polynomial regression analysis (Fig. 1), the maintenance protein intake level was estimated to be 4.3 g kg^{-1} day^{-1}, for fish weighing between 20 and 60 g.

Discussion

Few data are available on growth of blackspot seabream and these mostly refer to preliminary experiments. The DGI observed in this study (0.7–1.4) were not very good if compared with other farmed Sparidae (Santinha, Gomes & Coimbra 1996), but are higher than those previously obtained during ongrowing of juveniles (20–25 g) caught in the wild (Chereguini, Fernández-Pato & Marínez-Tapia 1990). Moreover, previous studies reported better DGIs in smaller fish (2.0 g) born in captivity (Olmedo, Linares, Ruiz, Álvarez-Blázquez, Peleteiro, Ortega & Rodriguez 1997), stressing the need of improving both reproduction and larvae culture techniques to further achieve good growth rates of juveniles.

Weight gain of young fish is usually a reliable indicator of the adequacy of the nutritional and management regimes (Cho & Kaushik 1990). In the present study, the growth performance and feed utilization displayed by blackspot seabream confirm the dependency of this fish species on a high protein diet. Both WWG and DGI of blackspot seabream improved with increasing levels of dietary protein up to 40%, but there was no further significant increase at higher dietary protein concentrations. The high voluntary feed intake of fish fed protein-poor diets (D20 and D30) showed that these fish consumed more DM in order to adjust the amount of protein ingested to their needs. Moreover, FCR has gradually improved with the increment of dietary protein up to 40%, when growth was maximal. This agrees with other studies in gilthead seabream (Santinha *et al.* 1996; Vergara, Fernández- Palacios, Robainà, Jauncey, Higuera & Izquierdo 1996) that have shown that growth and FCRs improve with high protein diets. The protein requirements for blackspot seabream growth seem to be in the same range as other marine carnivorous fish species. A number of previous studies in gilthead seabream (Santinha *et al.* 1996; Vergara, Robainà, Izquierdo & Higuera 1996), European seabass (Hidalgo & Alliot, 1988; Ballestrazzi, Lanari, D'Agaro & Mion 1994; Peres & Oliva-Teles 1999), dentex (*Dentex dentex*, Linnaeus) (Tibaldi, Beraldo, Volpelli & Pinosa 1996) and Japanese seabass (*Lateolabrax japonicus*, Cuvier) (Ai, Kangsen, Li, Zhang, Zhang, Duan, Tan, Xu, Ma, Zhang & Liufu 2004) have estimated 40–50% as the optimal dietary protein level in terms of growth performance.

The ADC for both DM and protein were slightly lower than those obtained in other fish species, like gilthead seabream (Santinha *et al.* 1996) and rainbow trout (*Oncorhynchus mykiss*, Walbaum) (Cho & Kaushik 1990), fed good-quality proteins. Nevertheless, the ADC of lipids was high for all diets and comparable with values found in other fish (Cho & Kaushik 1990; Santinha *et al.* 1996; Guillaume & Choubert 1999). In this study the ADC of DM and energy showed a concomitant increase with the reduction in dietary wheat meal, attaining maximal values with high protein diets (D50 and D60). This can be due to the relative high level of fibre in this raw material, suggesting a poor capacity of blackspot seabream to digest the carbohydrate fraction of this dietary component. The latter phenomenon might result in the deposition of a larger proportion of the recovered energy as fat. Recent studies in blackspot seabream larvae referred a high trypsine-specific activity and considerably low amylase-specific activity in this species compared with other fish (Ribeiro, Couto, Olmedo, Alvarez-Blázquez, Linares & Valente 2005), confirming the low ability of these fish to use dietary carbohydrates.

Protein utilization of diets for protein deposition decreases with increasing dietary protein levels, probably because more dietary protein is used as an energy source (Cho & Kaushik 1985). This was clearly observed in the present study, where the protein content of diet D20 was used to a minimum extent for energy purposes, as the intake of digestibility lipids was high, and was reflected in the lower non-faecal

excreted nitrogen values. Higher dietary protein levels increased the oxidation of protein to satisfy fish energy needs, resulting in a higher excretion of ammonia through the branchial system. Similar results were observed in other fish species like gilthead seabream (Santinha *et al.* 1996) and the rainbow trout (Kaushik & Luquet 1984). With diet D20 fish did not get enough protein and showed significantly lower growth compared with those fed the remaining diets. However, no significant differences were found on the nitrogen retention efficiency between diets. In growing animals, the energy retention is partitioned between protein and lipid (Cho & Kaushik 1990), but in the present study increasing dietary protein levels did not affect energy retention efficiency. Moreover, a large excess of energy intake and improper balance of protein to energy ratios with diet D20 resulted in the deposition of a larger proportion of the recovered energy as a fat. At the end of the growth trial all fish exhibited quite high fat contents ($> 18\%$), in spite of the low dietary lipid level (12%), and there was a significant and inverse effect of dietary protein level on the per cent whole body moisture and lipids. It seems that lipogenesis from carbohydrate sources might have a role in lipid deposition.

The maintenance protein requirement in blackspot seabream was estimated as 4.3 g kg^{-1} day^{-1}, being superior to those reported for rainbow trout (2.6 g kg^{-1} day^{-1}) (Kaushik & Gomes 1988), Nile tilapia (*Oreochromis niloticus*, Linnaeus) (1.9–2.2 g kg^{-1} day^{-1}) (Kaushik, Doudet, Médale, Aguirre & Blanc 1995), gilthead seabream (0.86 g $kg^{-0.7}$ day^{-1}) (Lupatsch, Kissil, Sklan & Pfeffer 1998) and European seabass (2.0–2.8 g kg^{-1} day^{-1}) (Hidalgo & Alliot 1988; Ballestrazzi *et al.* 1994). This suggests that to reduce cost, alternative sources of proteins should be investigated for these species. However, on a dietary concentration basis and considering a feeding rate of 2% day^{-1} for blackspot seabream juveniles (20–60 g), it can be estimated that the crude protein requirements for maximum weight gain would be above 40%, what is in general in accord with previous findings that suggested values of 40–60% for optimal growth in seabass and gilthead seabream (Hidalgo & Alliot 1988; Ballestrazzi *et al.* 1994; Santinha *et al.* 1996; Vergara, Robaina *et al.* 1996; Peres & Oliva-Teles 1999).

In conclusion, our data indicates that a diet with about 40% crude protein seems to be adequate for blackspot seabream simultaneously maximizing growth performance, nutrient utilization and benefit–cost ratio. However, under the assay conditions fish evidenced a lower growth rate than the ones observed in other established aquaculture species. Moreover, *Pagellus bogavaveo* showed excessive lipid deposition, regardless of the low dietary lipid level (12%). It is clear that more nutritional studies are essential to test a wider range of dietary protein/lipid combinations and different protein sources in hatchery-reared blackspot seabream, in order to improve growth, and before the species can be profitably established in intensive aquaculture.

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