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Dietary protein requirement of sharpsnout sea bream (*Diplodus puntazzo*, Cetti 1777) juveniles

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ABSTRACT

A trial was undertaken to estimate the protein requirement of sharpsnout sea bream (*Diplodus puntazzo*) and the protein sparing of dietary lipids. Ten diets were formulated to contain 5 protein levels (ranging from 15 to 55%) and two lipid levels (12 and 18%). Each diet was assigned to duplicate groups of 15 fish with a mean individual body weight of 49.3 g. A quadratic model was used to adjust weight gain and N retention (g kg ABW^{-1} day^{-1}) to dietary protein levels. Based on that model, optimum dietary protein requirement was estimated to be 42.9% for maximum weight gain and 43.8% for maximum N retention, corresponding to a protein intake of 7.68 g kg ABW^{-1} day^{-1}. Protein requirement for maintenance was estimated to be 0.71 g kg ABW^{-1} day^{-1}. Dietary lipid level improved protein utilization efficiency but did not affect protein requirement. Whole-body protein content increased with dietary lipids and protein content, but no other relevant differences in body composition were noticed. Hepatosomatic index increased with dietary starch and lipid levels and was directly correlated to liver glycogen content. Diet composition affected plasma glucose clearance and cholesterolaemia but not plasma protein and triglyceride levels.

1. Introduction

The increase of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) production in the last decade led to a price drop that reduced economic return of aquaculture in the Mediterranean region. To overcome market saturation problems and maintain industry profitability there is a need to diversify production by exploring new species. Among candidates, breams of the *Diplodus* genus are considered as having great potential, given their good acceptability by consumers, high market price, easy adaptation to captivity, acceptable growth and farming characteristics similar to that of gilthead sea bream (Hernandez et al., 2001a).

Sharpsnout sea bream (*Diplodus puntazzo*) appears as one of the most promising bream species, as it has growth rates similar to sea bass (Divanach et al., 1993) and a consumer acceptance that rival that of gilthead sea bream (Piedecausa et al., 2007; Rondan et al., 2004). Main positive attributes of sharpsnout sea bream are its appearance, flavor, texture, juiciness and flesh fat level (Hernandez et al., 2001b). Unlike sea bass and sea bream, which are carnivorous, sharpsnout sea bream is omnivorous and its natural diet consists mainly of algae and sponges (Sala and Ballesteros, 1997). Given its efficient use of lipids and carbohydrates as energy sources (Hernandez et al., 2001a), its tolerance to high levels of dietary vegetal protein sources, such as soybean meal (Hernandez et al., 2007) and its high potential for digesting polysaccharides (Tramati et al., 2005), it is expected that it will use commercial diets more cost-effectively than carnivorous species.

Despite sharpsnout sea bream omnivorous habits, it was reported in several macronutrient selection studies its preference for protein-rich diets (63 to 67% protein) (Almaida-Pagan et al., 2006, 2008; Vivas et al., 2006) though in another study a target protein intake of 47% was determined (Atienza et al., 2004).

Therefore, the present study aimed to estimate the dietary protein requirement of sharpsnout sea bream juveniles and the potential protein-sparing effect of dietary lipids.

2. Material and methods

2.1. Diets

Ten diets were formulated to contain 5 protein levels (ranging from 15 to 55%) and 2 lipid levels (12 and 18%). Diets were formulated using fishmeal and fish oil as protein and lipid sources. All dietary ingredients were finely ground, well mixed and dry-pelleted in a laboratory pellet mill (CPM, California Pellet Mill) through a 2-mm die. Diets were dried at 50 °C for 24 h and stored in plastic bags until used. Ingredients and proximate composition of the experimental diets are presented in Table 1.

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2.2. Growth trial

The trial was carried out at the experimental facilities of the Marine Zoology Station, Faculty of Sciences, Porto University, in a thermo-regulated water semi-recirculation system equipped with 20 cylindrical fiberglass tanks of 300 l water capacity, supplied with a continuous flow of filtered seawater.

Sharpsnout sea bream (*D. puntazzo*) juveniles were provided by IPIMAR/CIMSul, at Algarve. After transportation to the experimental facilities the animals were submitted to a quarantine period of two weeks and then acclimated for a month to the rearing systems. During this period the fish were fed a commercial diet. Then, 20 groups of 15 fish with a mean body weight of 49.3 g were established and diets were randomly assigned to duplicate groups of these fish. During the trial, fish were fed to apparent visual satiety twice a day, 6 days a week. Utmost care was taken to assure that all feed supplied was consumed. The trial lasted 11 weeks and during this period, water temperature was maintained at 22 ± 0.5 °C, salinity averaged 33 ± 2‰ and nitrogenous compounds were maintained at levels within limits recommended for marine species. A natural photoperiod was adopted.

2.3. Sampling

During the trial, fish of each tank were bulk-weighted every three weeks under slight anesthesia (ethylene glycol monophenyl ether, 0.3 ml l^{-1}), after one day of feed deprivation. A random sample of 8 fish from the initial batch and of 3 fish per tank at the end of the trial were taken, killed by lethal anesthesia (ethylene glycol monophenyl ether) and pooled for whole-body composition analysis. Based on whole-body composition analysis, N retention (g N kg ABW^{-1} day^{-1} or % N intake) and E retention (kJ kg ABW^{-1} day^{-1} or % E intake) were estimated as follows:

$$N\ retention\left(g\ N\ kg\ ABW^{-1}\ day^{-1}\right) = (FBW \times FBN) - (IBW \times IBN)/(((IBW + FBW)/2) \times nb\ days)$$

$$N\ retention(\%NI) = ((FBW \times FBN - IBW \times IBN)/NI) \times 100$$

$$E\ retention\left(kJ\ E\ kg\ ABW^{-1}\ day^{-1}\right) = (FBW \times FBE - IBW \times IBE)/(((IBW + FBW)/2) \times nb\ days)$$

$$E\ retention(\%EI) = ((FBW \times FBE - IBW \times IBE)/EI) \times 100$$

where ABW was average body weight; IBW and FBW were initial and final body weights; IBN and FBN or IBE and FBE were initial and final body N or E content, respectively; NI or EI were nitrogen or energy intake.

Another 3 fish per tank were randomly sampled, immediately bled and liver and viscera removed. Individual weight, liver and viscera weights of these animals were recorded for determination of hepatosomatic and visceral indexes and liver samples were then stored in a freezer at −20 °C until analysis. Blood samples were collected from the caudal vein using heparinised syringes; blood was immediately centrifuged at 3000 rpm for 10 min and the plasma stored at −20 °C until analysis.

2.4. Chemical analysis

Chemical analysis of the experimental diets and whole fish were conducted as follows: water content, by drying samples in an oven at 105 °C until constant weight; ash, by incineration in a muffle furnace at 450 °C for 16 h; protein (N × 6.25), according to the Kjeldahl method after acid digestion using a Kjeltec system; lipid, by petroleum ether extraction in a SoxTec System HT apparatus; energy, by direct combustion of samples in an adiabatic bomb calorimeter (PARR Instruments, model 1261). Whole-fish was dried and homogenized before analysis.

Commercial kits from Spinreact, S.A. (Gerona, Spain) were used for plasma glucose (Kit, cod. 1001190), triglycerides (Kit, cod. 41031) and cholesterol (kit, cod. 1001090) determination. Plasma protein was determined according to Bradford (1976), using a commercial Kit (Sigma protein Kit, cod. B6916).

Table 1
Ingredient composition and proximate analysis of the experimental diets.

Diets	L12					L18				
	P15	P25	P35	P45	P55	P15	P25	P35	P45	P55
Ingredients (% dry weight)										
Fish meal[a]	14.6	28	41.3	54.6	68	14.6	28	41.3	54.6	68
CPSP[b]	5	5	5	5	5	5	5	5	5	5
Cod liver oil	9.9	8.8	7.7	6.7	5.6	15.9	14.8	13.7	12.7	11.6
Gelatinized starch[c]	67.5	55.2	43	30.7	18.4	61.5	49.2	37	24.7	12.4
Choline chloride (50%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix[d]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral premix[e]	1	1	1	1	1	1	1	1	1	1
Binder[f]	1	1	1	1	1	1	1	1	1	1
Proximate analysis (% dry weight)										
Dry matter (%)	90.1	95.0	92.0	97.5	97.7	91.9	91.4	96.1	98.1	92.6
Crude protein	16.4	26.4	36.0	45.8	55.4	15.5	26.2	35.4	45.8	55.9
Gross lipid	11.4	11.5	11.9	11.3	11.3	16.9	17.4	17.1	17.1	18.0
Ash	4.5	6.5	8.5	10.7	13.2	4.4	6.3	8.6	10.8	14.1
NFE[g]	67.7	55.7	43.7	32.3	20.1	63.2	50.1	39.0	26.3	12.0
Gross energy (kJ g^{-1})	18.0	19.4	19.5	20.1	20.5	19.2	20.4	21.2	21.2	22.6
P/E (g MJ^{-1})	9.1	13.6	18.5	22.8	27.0	8.1	12.8	16.7	21.6	24.7

[a] Pesquera Diamante, Steam Dried LT. Austral Group, S.A. Peru (crude protein: 73.6% DM; gross lipids: 11.5% DM).
[b] Soluble fish protein concentrate. Sopropèche, France (crude protein: 79.4% DM; gross lipids 19.7% DM).
[c] Pregelatinized maize starch. C-Gel Instant, 12016, Cerestar, Mechelen, Belgium.
[d] Vitamins (mg kg^{-1} diet): retinol, 18.000 (IU kg^{-1} diet); cholecalciferol, 2000 (IU kg^{-1} diet); α-tocopherol, 35; menadion, 10; thiamine, 15; riboflavin, 25; Ca pantothenate, 50; nicotinic acid, 200; pyridoxine, 5; folic acid, 10; cyanocobalamin, 0.02; biotin, 1.5; ascorbyl monophosphate, 50; inositol, 400.
[e] Minerals (mg kg^{-1} diet): cobalt sulfate, 1.91; copper sulfate, 19.6; iron sulfate, 200; sodium fluoride, 2.21; potassium iodide, 078; magnesium oxide, 830; manganese oxide, 26; sodium selenite, 0.66; zinc oxide, 37.5; dibasic calcium phosphate, 8.02 (g kg^{-1} diet); potassium chloride, 1.15 (g kg^{-1} diet); sodium chloride, 0.44 (g kg^{-1} diet).
[f] Aquacube. Agil, UK.
[g] Nitrogen free extract = 100 − (crude protein + gross lipids + ash).