Effects of dietary energy content on the voluntary feed intake and blood parameters of sea bass (Dicentrarchus labrax L.)

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ABSTRACT

Energy and protein requirements of sea bass for maximum growth and fasting energy requirements were determined by using three diets containing increasing DE levels and two fish weights.

Five hundred and sixteen sea bass were divided in two body weight (BW) classes (A: 67.7 ± 0.85g and B: 128.6 ± 0.88g, mean ± SD live weight) and randomly distributed among 24 tanks. They were fed for 12 weeks on three isoproteic diets characterized by different levels of digestible energy (DE): low energy (LE), 18.6; medium energy (ME), 19.7; and high energy (HE), 22.6 MJ kg^{-1} dry matter (DM). The entire trial lasted 113 d and was divided into two periods: a feeding trial of 83 d and a fasting trial of 30 d. Specific growth rates decreased in fish fed on the HE diet (P < 0.05), but only in fish weighing 68 g. Voluntary feed intakes and feed conversion ratios were inversely related to dietary energy contents in both weight classes. During the starvation trial, body depletion increased (P<0.05) in fish fed on high-energy diets during the feeding experiment. The gross energy requirements (per day) for maximum growth were 320 and 221 kJ kg^{-1} BW for fish weighing 68 g and 128 g, respectively. Fasting metabolisms were 60.6 and 54.1 kJ kg$^{-0.83}$ BW per day for fish weighing 68g and 128g, respectively.

It is concluded that growth performance of sea bass appear to be dependent on digestible dietary energy. Gross energy intake, net energy (production) and maintenance requirements of fish were not influenced by dietary treatments.

Key words: Sea bass, High energy diet, Energy ingestion, Growth rate, Body composition.

RIASSUNTO
EFFETTI DEL CONTENUTO ENERGETICO DELLA DIETA SUL CONSUMO VOLONTARIO E SU ALCUNI PARAMETRI EMATICI DEL BRANZINO (DICENTRARCHUS LABRAX L.)

Nella presente ricerca vennero determinati i fabbisogni energetici e proteici per il massimo accrescimento e a digiuno di spigole alimentate con tre diete caratterizzate da tre livelli energetici e utilizzando due classi di peso. Cinquecentosedici branzini, divisi in due classi di peso (A: 67,7 ± 0.85 g e B: 128,6 ± 0,88 g, peso vivo medio ± DS) e distribuiti casualmente tra 24 vasche, vennero alimentati per 12 settimane con tre diete isoproteiche caratterizzate da livelli crescenti di energia digeribile: bassa energia (LE), 18,6; media energia (ME), 19,7 e alta energia (HE), 22,6 MJ kg^{-1} SS. L'intera prova durò 113 giorni divisa in due periodi: una prova di alimentazione della durata di 83 d e un periodo di digiuno della dura-

ta di 30 d. L'accrescimento specifico risultò più basso nei pesci alimentati con la dieta HE (P<0,05) ma solo nella classe di peso A. L'ingestione volontaria di alimento e l'indice di conversione alimentare risultarono inversamente correlati con la concentrazione energetica delle diete in entrambe le classi di peso. Durante la prova a digiuno, la perdita di peso risultò maggiore nei branzini alimentati in precedenza con la dieta a maggiore concentrazione energetica (p<0,05). Il fabbisogno di energia grezza per l'accrescimento risultò pari a 320 KJ kg^{-1} peso vivo d^{-1} e 221 KJ kg^{-1} peso vivo d^{-1} per i pesci del peso rispettivamente di 68 g e 128 g. Il metabolismo a digiuno risultò pari a 60,6 KJ kg$^{0.83}$ peso vivo d^{-1} e 54,1 KJ kg$^{0.83}$ peso vivo d^{-1} per pesci del peso rispettivamente di 68 g e 128 g.

Si conclude affermando che le prestazioni produttive del branzino variano in relazione al livello di energia digeribile della dieta. L'ingestione di energia grezza, il fabbisogno di energia netta (accrescimento) e di mantenimento (digiuno) dei pesci non sono state influenzate dai trattamenti alimentari.

Parole chiave: *Branzino, Diete ad alta energia, Ingestione energetica, Accrescimento corporeo, Composizione corporea.*

Introduction

High-energy diets containing high lipid levels and optimal digestible protein (DP) contents are widely used in salmonid and marine species nutrition. They help increase growth efficiency and feed utilization, reduce the production of solid wastes and lower the environmental impact of nitrogen and phosphorus effluents leaving fish farms (Johnsen and Wandsvik, 1991). Diet composition affects the flesh quality of fish, and high-energy diets may increase body lipid content (Lanari *et al.*, 1995). Only a few studies have been carried out to determine the optimal energy and protein requirements of marine fish using high-energy diets (Curry Woods III *et al.*, 1995; Guinea and Fernandez, 1997; Lupatsch and Kissil, 1998; Lupatsch *et al.*, 2001). Recently, Lanari *et al.* (2002) reported growth performance and voluntary feed intakes (VFI) of sea bass of different weights reared under various water temperatures.

Energy and protein requirements for maximum growth are usually expressed in terms of dietary DP and digestible energy (DE) ratio (g MJ^{-1}) (Cho and Bureau, 1995), gross energy intake per kg of body weight (kJ kg^{-1} d^{-1}) and the gross energy intake per kg of weight gain (MJ kg^{-1}d^{-1}) (Kaushik and Medale, 1994). These values are obtained by *in vivo* growth and digestibility trials and by using comparative slaughter techniques (Braaten, 1979; Brown *et al.*, 1990). In 1992, Cho proposed a simple method to calculate feeding charts on the basis of the energy needs of fish, which are defined by growth rate, fish size and water temperature. According to this method, the DP/DE ratio is fixed at 22 g MJ^{-1} and energy intake changes continually to meet fish energy requirements expressed as gross body energy.

In the present experiment, energy and protein requirements of sea bass for maximum growth and fasting energy requirements were determined by using three diets containing increasing DE levels and two fish weights.

Material and methods

Growth and fasting trials were performed in the aquariums at the Villa Bruna Farm (Marano Lagunare, Italy). Five hundred and fifteen European sea bass, with initial weights of either (mean ± SD) 67.7 ± 0.85 g (A) and 128.6 ± 0.88 g (B), were randomly distributed among 24 tanks (160 l, recirculating system with a 10% water renewal per day).

Three commercial isoproteic and extruded diets differing in DE content (low, 18.6; medium, 19.7; and high 22.6 MJ kg^{-1} DM) were compared. The initial stocking densities of fish classes A and B were 9.73 kg m^{-3} (23 fish per tank) and 16.07 kg m^{-3} (20 fish per tank), respectively.

The entire trial lasted 113 days and was divided into two periods: a feeding trial of 83 d and a fasting trial of 30 d. Each tank contained a porous stone aerator and received a continuous flow of water. During the experiment, the temperature, dissolved oxygen, pH and salinity of the water were measured weekly. A photoperiod of 12 h light per day was maintained during the experiment.

Mortality was recorded daily and fish were weighed once every fortnight. Diets were distributed by means of automatic feeders set up to distribute the total amount in 10 equal meals. Feeders operated from 09.00 to 15.15 hours and distributed the concentrate for 3 min, with 38-min intervals from one meal to the next. Feed allowances were calculated on the basis of fish biomass to allow *ad libitum* feeding and approximately 20% residuals. Each tank was fitted with an apparatus for collecting feces and feed residues according to the method described by Helland *et al.* (1996) with modifications. Residuals were collected daily, separated from feces, weighed and dried. During the trial, samples of the diets were collected for proximate analysis (AOAC, 1995) and the gross energy concentration was determined using an adiabatic bomb calorimeter. At the beginning and at the end of the trial, respectively, 20 and 90 fish (five fish per tank) were sampled. Each fish was weighed (entire body, viscera and liver), minced, frozen and analyzed for chemical composition and gross energy content. The chemical composition and energy content of individual fish were determined by the same methods used for the feeds. At the end of the experiment, blood samples were collected from 120 (5 fish per tank) anaesthetized fish (50 µg g^{-1} tricaine methane sulfonate (MS222)) by caudal puncture using heparinized syringes. After centrifugation for 5 min at 3000 g, plasma was decanted and stored at -18 °C. Blood parameters (protein, urea, glucose, triglycerides, non-esterified fatty acids (NEFA), cholesterol, alanine aminotransferase (AST) and aspartate aminotransferase (ALT) were measured by an automatic analyzer (Hitachi 911, Boehringer). The experimental data recorded during the growth trial were analyzed for each weight class and subjected to one-way analysis of variance, with the comparison between means being effected with the least significant difference test (LSD) (Snedecor and Cochran, 1982).

Results and discussion

Chemical composition, gross and digestible energy contents of diets are presented in table 1. Diets were isoproteic (48% DM), whereas crude fat and gross energy, respectively, increased from 17.2% DM and 20.1 MJ kg^{-1} DM in the LE diet to 30.3% DM and 24.6 MJ kg^{-1} DM in the HE diet. DP/DE ratios ranged between 21.8 g MJ^{-1} in the LE diet and 19.05 g MJ^{-1} in the HE diet.

Growth and feeding performance are shown in table 2. During the experiment, oxygen levels were always near saturation, the mean ± SD water temperature was 24.8 ± 0.49 °C, pH was 7.5 ± 0.64 and water salinity was 20 ± 1 P.S.U.. Specific growth rates decreased in fish fed on the LE diet (P < 0.05), but only in the fish weighing 68 g. Starved sea bass weighing 68 g lost 15.5% of their body weight after four weeks in the HE treatment, compared with 11.5% in the ME and LE treatments (P < 0.05). No significant differences were observed in fish weighing 120 g. VFI and feed conversion ratios (FCR) were, in both weight classes, inversely related to dietary energy content. This trend was more apparent in smaller fish. In both weight classes, the lowest values of VFI were observed in fish fed on the HE diet. VFI ranged from 1.39% to 1.85% body weight.

Moisture, protein, lipid and energy contents of

Table 1.   Chemical composition, gross and digestible energy content of the diets.

		Diets		
		Low energy (LE)	Medium energy (ME)	High energy (HE)
Dry matter	%	89.2	88.9	90.1
Crude protein	% DM	48.3	48.1	47.8
Ether extract	"	17.2	23.5	30.3
NFE	"	24.1	18.7	13.9
Gross energy	Mj kg^{-1} DM	20.1	22.4	24.6
Digestible energy	"	18.6	19.7	22.6
DP/DE	g MJ^{-1}	21.8	20.5	19.0

the initial and final whole fish bodies are shown in table 3. In both weight classes, body lipid contents at the end of the experiment were higher than those of the initial sample. The increase in lipogenesis was influenced by the dietary energy levels. Fish fed on the HE diet showed the highest lipid and energy contents (P < 0.01) and conversely the lowest protein content.

The total protein and energy retentions are presented in table 4. There were no marked differences in protein retention between fish on different diets and between weight classes. In both weight classes, gross lipid and energy retention (%) and energy retention per kg of gain increased significantly in fish fed on the HE diet (P < 0.05).

Gross energy intake, net energy (production),

Table 2.   Growth performance of sea bass fed *ad libitum* and after starvation.

		Weight class 68g				Weight class 128g			
		Diets				Diets			
		LE	ME	HE	SE 9 DF	LE	ME	HE	SE 9 DF
Initial weight	g	67.5	66.6	68.5	3.30	128.8	129.5	128.7	2.37
Final weight	"	136.6[b]	152.1[a]	157.7[a]	3.20	252.9	241.1	241.1	14.76
SGR		0.84[b]	0.98[a]	1.0[a]	0.02	0.76	0.79	0.74	0.05
Weight after starvation	"	115.5[b]	139.5[a]	139.5[a]	29.8	212.0	225.3	222.3	20.6
SGR		-0.51[a]	-0.88[a]	-0.39[b]	0.002	-0.42[a]	-0.35[ab]	-0.25[b]	0.002
Voluntary feed intake	"	1.75	1.85	1.69	0.003	1.56[a]	1.53[ab]	1.39[b]	0.003
FCR		1.47[A]	1.40[A]	1.33[B]	0.003	1.58[a]	1.32[b]	1.33[b]	0.003
PER		1.51[B]	1.59[A]	1.69[A]	0.09	1.46[b]	1.68[a]	1.63[a]	0.010
HSI		3.1	2.8	3.4	0.41	3.1	2.6	2.9	0.11
HSI after starvation		1.6	1.7	1.8	0.06	1.3	1.4	1.5	0.02

[a,b]: P<0.05; [A,B]: P<0.01

$$SGR\ (specific\ growth\ rate) = \frac{(Ln\ final\ weight - Ln\ initial\ weight)\ x\ 100}{number\ of\ days}$$

FCR = feed ingestion / fish weight gain
PER = fish weight gain / protein intake
HSI (Hepatosomatic index) = (liver weight / empty fish weight) x 100
DF = degrees of freedom

Table 3.   Initial and final whole body composition of sea bass.

		Weight class 68 g					Weight class 128 g				
			Diets					Diets			
		Initial sample	LE	ME	HE	SE 57 DF	Initial sample	LE	ME	HE	SE 57 DF
Dry matter	%	39.5±1.59	38.2	40.7	40.6	0.0002	40.8±2.73	40.3	39.8	41.4	0.0002
Crude protein	% DM	43.8±1.85	44.8[A]	43.8[A]	41.8[B]	3.46	41.2±1.90	45.2	44.9	43.7	2.05
Crude fat	"	35.6±2.17	41.2[B]	41.2[B]	46.5[A]	6.63	37.2±1.98	40.9[B]	41.7[B]	45.9[A]	6.92
Gross energy	MJ Kg⁻¹ DM	26.0±0.86	26.66	26.66	28.2	0.58	26.88±0.90	27.5	27.2	28.4	0.93

[a,b]: P<0.05; [A,B]: P<0.01

fasting metabolism and metabolic fasting metabolism are shown in table 5. The gross energy intake was influenced by weight class only and not by the dietary treatments. (Net energy production (NEp) / Gross energy intake (GEI) and Net energy fasting (NEf) / Gross Energy Intake (GEI) values

increased in larger fish. No significant differences were observed between dietary treatments.

Blood parameters measured in sea bass at the end of the experiment are shown in table 6. Plasma concentrations of total protein, globulin and urea were not influenced by either dietary

Table 4.   Gross energy, protein and lipid retention in sea bass.

		Weight class 68g				Weight class 128g			
		Diets				Diets			
		LE	ME	HE	SE 57 DF	LE	ME	HE	SE 57 DF
Energy retention	%	24.47[b]	31.60[a]	32.8[a]	1.34	37.5[b]	36.9[b]	39.4[a]	14.8
Protein retention	"	21.81	22.42	22.0	3.53	25.9	25.1	28.8	0.39
Lipid retention	"	46.89[b]	47.67[b]	55.52[a]	6.88	70.07	60.28	61.24	14.9
Energy retention per Kg of weight gain	MJ Kg[-1]	11.60	13.08	14.15	1.71	12.92	12.72	14.46	1.69
Protein retention per Kg of weight gain	g Kg[-1]	196.7	199.7	188.7	11.7	208.9	186.4	185.6	24.8

a,b: P<0.05; A,B: P<0.01
Energy retention = (energy retained per fish X 100)/ (energy given per fish x100)
Protein retention = (protein retained per fish X 100) / (energy given per fish x 100)
Lipid retention = (lipid retained per fish X 100) / (lipid given per fish x 100)

Table 5.   Energy partitioning in sea bass diets

		Weight class 68g				Weight class 128g			
		Diets				Diets			
		LE	ME	HE	SE 9 DF	LE	ME	HE	SE 9 DF
Gross energy intake	KJ Kg[-1] BW	324	323	320	13.41	211	221	206	18.0
NEp	"	79	81	85	10.45	79	82	87	6.82
NEp / NEI	%	24.4	25.0	26.6	0.19	37.4	36.8	42.2	1.48
NEf	KJ Kg[-1] BW	82.5	81.5	84.4	19.91	79.0	69.3	72.9	7.67
NEf / NEI	%	25.5	25.2	26.0	5.44	37.4	31.4	35.4	3.11
Metabolic fasting energy	KJ/Kg BW[0.83]	58.2	58.5	60.6	13.90	61.8	54.1	59.1	5.57

a,b: P<0.05; A,B: P<0.01
NEp: net energy production; NEI: net energy intake; NEf: net energy fasting.

treatments or weight classes. Albumin concentrations decreased with high-energy diets (P < 0.05). Glucose increased (P<0.05) whereas triglyceride and cholesterol concentrations decreased in fish fed on the LE diet but only in the fish weighing 68g. AST concentration increased significantly (P < 0.05) in fish fed on the LE diet in both weight classes.

The optimal DP/DE values in sea bass diets reported in the literature are quite variable (Garzia-Alcazar *et al.*, 1994; Perez *et al.*, 1997; Lanari *et al.*, 1999). They range between 18.9 g and 28.8 g MJ⁻¹ kg DM. This large variation is due to several methodological and biological factors occurring during the experimental trials. Criteria used for evaluating optimal DP/DE ratios are the maximum weight and protein deposition and the highest protein and energy efficiencies (Kaushik and Medale, 1994). An additional parameter used for evaluation purposes is the body fat deposition at the end of the grow-out phase (Lupatsch and Kissil, 1998). In the present study, maximum growth rate was achieved at the highest dietary energy level in fish weighing 68 g, whereas, in fish weighing 130 g, the HE diet led to a decrease of specific growth rate even with an adequate level of dietary protein. Several studies (Kaushik and

Medale, 1994; Lupatsch and Kissil, 1998; Lupatsch *et al.*, 1998; Lupatsch *et al.* 2001) have shown that the optimal DP/DE ratio for rainbow trout and sea bass may be lowered from the current value of 22 to 25 g MJ⁻¹ kg DM to 17 to 20 g MJ⁻¹ kg DM. In line with these findings, the results of the present study suggest that, for sea bass farming, diets having a DP/DE ratio of 19 g MJ⁻¹ kg DM should be used for fish weighing 70 g and a DP/DE ratio of 20.5 g MJ⁻¹ kg DM for fish weighing 130 g.

Daily growth is regulated by both energy and protein intake. It is well known that most fish, among them sea bass, may regulate feed intake according to the dietary energy content (Kaushik and Medale, 1994). In the present research, sea bass were able to compensate for a high-energy diet by reducing voluntary feed intake. During the trial, the availability of specific devices to measure feed intake allowed a precise assessment of data. Lupatsch *et al.* (2001) found a tendency for a decrease in feed intake by sea bass and sea bream fed to satiation when dietary DE level increased. The same trend was also observed by Kaushik and Oliva-Teles (1986) in rainbow trout. Since the early work of Lee and Putnam (1973) in rainbow trout, it has been well recognized that the inclusion of high levels of fat in fish diets leads to ben-

Table 6. Plasmatic levels of the main blood parameters measured in sea bass.

| | | Weight class 68g | | | | Weight class 128g | | | |
| | | Diets | | | | Diets | | | |
		LE	ME	HE	SE 57 DF	LE	ME	HE	SE 57 DF
Total protein	g/l	72.0	68.0	64.4	8.94	63.6[a]	65.2[a]	58.8[b]	10.2
Globulins	"	17.6	16.0	15.7	2.30	15.4	16.4	14.6	3.09
Albumins	"	56.8[a]	52.0[b]	50.2[b]	8.50	48.0[a]	48.8[a]	43.9[b]	11.26
Urea	mmol/l	1.7	1.5	1.4	0.24	1.4	1.4	1.3	0.21
Glucose	"	4.2[a]	3.7[b]	3.5[b]	0.97	3.4	3.6	3.3	0.19
Triglycerids	"	9.1[b]	9.5[ab]	10.0[a]	1.97	9.5	9.5	9.0	1.41
NEFA	"	0.83	0.93	1.11	0.20	0.82	0.90	1.0	0.15
Cholesterol	"	9.7[b]	10.1[b]	12.6[a]	2.05	9.8	10.1	10.7	2.32
AST	U/l	47.7[a]	35.7[b]	27.2[c]	14.1	33.0[a]	20.17[b]	17.1[b]	10.5
ALT	"	3.6	3.4	2.0	1.5	3.2	3.0	2.1	1.4

[a,b]: P<0.05; [A,B]: P<0.01

eficial effects on FCR. Subsequent work has confirmed that the improvement in protein utilization is due to an increasing contribution of non-protein energy sources to overall energy expenditure (Lanari *et al.*, 1995). This, in turn, causes a reduction in nitrogen excretion (Lanari *et al.*, 1995). The average chemical composition of whole sea bass showed significant differences in fat with the HE diet. Similar results were also observed in other species (Steffens *et al.*, 1999).

The net energy requirement for fasting (NEf) was calculated by using the gross energy loss during starvation. A comparative slaughter technique was employed to measure the caloric value of the tissues utilized during feed deprivation. The average loss of sea bass was 71.24 kJ kg$^{-0.83}$ for fish weighing 68 g and 66.02 kJ kg$^{-0.83}$ for fish weighing 130 g at the temperature of 25 °C. According to Huisman (1976), the fasting metabolism reflects only 50% of the net energy requirements for maintenance and it is affected by water temperature. Beck and Gropp (1995) introduced the addition of 30% to the fasting metabolism calculation to account for the reduction of energy turnover during starvation. The digestible energy lost during starvation by sea bream was 42.5 kJ kg$^{-0.83}$ at 23 °C (Lupatsch *et al.*, 1998) and by rainbow trout was 42 kJ kg$^{-0.83}$ at 20 °C (Cho and Kaushik, 1990). In sea bream, the energy maintenance requirement was 55.8 kJ DE kg$^{-0.83}$. The energy partitioning of diets during fish metabolism showed a marked effect of size on NEp/GEI ratios. These values were higher for larger fish because of the lower gross energy intake. Watanabe *et al.* (2000) estimated in yellow tail a Nep/GEI of 34.5%, which is similar to the average value of 35% observed in sea bass weighing 130 g.

Protein requirement per kg of weight gain was not influenced by fish size. Values ranged from 188.7 g for sea bass fed on the HE diet to 208.9 *g* for fish on the LE diet weighing 130 g. Lupatsch *et al.* (1998) reported an average value of 179 g for gilthead sea bream weighing 30.1 g and 92.5 g.

Feed efficiency, protein and energy retention were inversely related to the dietary energetic levels.

Profile tests for marine fish, compared with salmonids and domestic animals, are at an early stage of development, although the extent of the literature is increasing. Blood chemistry varies more in fish than in mammals (Payne and Payne, 1987) because of the very sensitive responses by fish to the environment. In the present study, metabolite blood levels were similar to those reported for the European sea bass in the literature (Echevarrìa *et al.*, 1997; Cerdà-Reverter *et al.*, 1998; Meton *et al.*, 1999; Robaina *et al.*, 1999; Marino *et al.*, 2001). The higher carbohydrate concentration in the LE diet gave higher blood glucose and lower triglyceride and cholesterol concentrations in fish. These findings are consistent with previously reported studies (Payne and Payne, 1987; Robaina *et al.*, 1999). Albumins and AST concentrations increased with the low energy diet. ALT and AST enzymes are quantitatively the most important aminotransferases in the teleost fish liver (Cowey and Walton, 1989). The increased concentration of AST enzyme that was observed when fish were fed on the LE diet may denote a more efficient use of amino acids for growth. Similarly, Meton *et al.* (1990) found in sea bass that fish fed on diets of lower energy content had the highest enzyme activity. Shikata *et al.* (1994) reported a sharp reduction in amino acid degrading enzymes (GOT, GPT and arginase) in carp after they were fed carbohydrate-supplemented diets.

Conclusions

The present study was carried out with the aim to quantify the effects of digestible dietary energy. Maximum growth rate was achieved at the highest dietary energy level in fish weighing 68 g, whereas, in fish weighing 130 g, the high energy diet led to a decrease of specific growth rate even with an adequate level of dietary protein. Gross energy intake, net energy (production) and maintenance requirements of sea bass were not influenced by dietary treatments. Metabolite blood levels were similar to those reported for the European sea bass in the literature. The higher carbohydrate concentration in the low energy diet gave higher blood glucose and lower triglyceride and cholesterol concentrations in fish.

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