THE EFFECT OF DIETARY-PROTEIN LEVEL AND SOURCE ON GROWTH, BODY-COMPOSITION, TOTAL AMMONIA AND REACTIVE PHOSPHATE EXCRETION OF

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The effect of dietary protein level and source on growth, body composition, total ammonia and reactive phosphate excretion of growing sea bass (*Dicentrarchus labrax*)

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Abstract

Three hundred sea bass (75.8 ± 5.8 g) were randomly distributed among 12 tanks and fed for 168 days, at 1.0% live weight, with one of 6 experimental diets in a 3×2 factorial design [3 protein levels: 44, 49 and 54% crude protein (CP) on a dry matter basis; 2 protein sources: herring meal and herring meal plus corn gluten meal] with 2 replicates for each treatment. Final live weight and relative weight gain were affected by the protein level ($P < 0.05$) but not by the protein source. The moisture and crude protein of fish increased with dietary protein level while fat and energy contents decreased accordingly ($P < 0.05$). Fish fed the 44% CP herring meal diet had the highest energy, protein and lipid retention. Total ammonia excretion increased linearly and significantly with the protein level of the diet. Diets containing corn gluten gave lower reactive phosphate concentration in the effluent water than those with herring meal (55.1 vs. 113.9 mg PO_4/kg/day).

Keywords: *Dicentrarchus labrax*; Feeding and nutrition — fish, feed composition; Growth — fish; Ammonia

1. Introduction

Although fish are able to utilise dietary protein more efficiently than homeothermic animals (Lovell, 1989) they use a large percentage of the protein for energy ($\approx 40\%$ according to Walton, 1985; 30–70% according to Kaushik and Cowey, 1991), and as a consequence they excrete a considerable amount of nitrogenous waste. Since the excretion of nitrogen (N) is proportional to the quantity of ingested protein (Rychly, 1980; Kaushik

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and Cowey, 1991), the optimisation of fish production requires research into feeding techniques which promote good performance and at the same time reduce the quantity of waste products released into the water. Kim and Kaushik (1992) have shown that it is possible to obtain nutrient retention indices for protein, lipid and energy of 40%, about 100% and 40–50% respectively. However, little information is available for marine fish species on this subject and the efficiency values reported are definitely lower: retained protein ≈ 20%, retained lipid ≈ 50%, retained energy 25–30% (Spiridakis, cited by Dosdat, 1992; Lanari et al., 1993). These differences may be due to species differences as well as a general lack of knowledge about the nutrient requirements of marine fish.

In order to reduce the pollution of effluent water, attention should also be given to phosphorus (P). Due to the variability of the mineral content of water and the ability of fish to absorb minerals from their "medium" (Prosser, 1986), mineral requirements of fish have not been well defined and even the Ca/P ratio recommended in feeds can vary from 1:1 to 1:2 (Sakamoto andYone, 1973; Phillips et al., 1959). According to Lall (1991), the phosphorus requirement of fish ranges from 0.5 to 0.8% of the feed, a value lower than once adopted. These recent developments have led to a re-appraisal of the role of animal protein sources (fish meal, meat and bone meal) in fish feeds, with more attention being given to the bioavailability of the various forms of P (Ketola, 1985; Tacon, 1990). The use of vegetable protein sources has also been studied; ingredients having lower P levels than animal meals or inorganic forms of phosphorus can be used to reduce P discharge, although attention must be paid to the total available P of these diets, since P bioavailability of plant products is generally lower than in animal meals and inorganic sources (Lall, 1991).

The need to improve nutrient utilisation and simultaneously reduce the quantity of solid and soluble wastes has led to profound changes in the formulation and production techniques of fish diets in recent years (Cho, 1993). An initial approach to the themes listed above for sea bass may centre upon identifying the optimum level of crude protein to assure a high growth rate, at the same time limiting the amount of waste released into the water. This species, like other carnivorous fish, requires feeds with a high protein content. Luquet and Kaushik (1981) estimated it to be 50% for yearling sea bass (Alliot et al., 1974). Metailler et al. (1981) obtained higher growth rates and better feed conversion in 75-g sea bass fed diets containing 60% crude protein on dry weight basis.

Another approach would be to substitute part of the protein meals of animal origin, rich in P, with plant protein containing lower levels of this element. The research described here was conducted to evaluate the effect of diets formulated as described above on the performance of sea bass and to determine the amounts of N and P released into the effluent water.

2. Material and methods

Growth trial

The growth trial, which lasted 168 days, was performed at the "Villa Bruna" *valle da pesca*, located in the Marano lagoon. Six experimental diets with 3 protein levels (44, 49 and 54% crude protein, on a dry matter basis) were prepared, with the protein being supplied either by fish meal or by a mixture of fish meal and corn gluten meal. Dietary lipid level

was constant and the concentration of nitrogen-free extract decreased with increasing protein level (Table 1).

Three hundred 10-month-old sea bass (75.8 ± 5.8 g) were randomly distributed among 12 fibreglass, cylindrical conical tanks (160 litres) according to a 3 × 2 experimental design (3 protein levels, 2 protein sources). Each tank received a constant flow of water (3.0 l/min), with a temperature ranging between 17.8 and 25.6°C, dissolved oxygen between 6.3 and 12.3 mg/l and salinity between 15 and 20‰ NaCl.

Fish were fed simultaneously, 10–12 times a day, using automatic dispensers which were centrally controlled. The average level of intake was 1.04% live weight, without significant differences among treatments (Table 2). The fish were weighed every 14 days and mortality was recorded daily. Samples of each diet were collected periodically and submitted for chemical analysis according to AOAC (1990) methods. Ether extract was determined after acid hydrolysis (Sanderson, 1986) while the gross energy content was obtained by adiabatic calorimeter (IKA C400) and phosphorus with a photometric method (AOAC, 1990).

Twenty fish were sacrificed at the beginning of the experiment and 16 fish per treatment at the end. The gastrointestinal contents were removed and then each fish, refilled with its emptied gut, was minced and freeze-dried. The chemical composition and gross energy of the fish were determined as described above.

Data were submitted to a two-way analysis of variance. The comparison between means was performed with the LSD test (Snedecor and Cochran, 1982).

Excretion trial

At the end of the growth trial 6 groups of 5 sea bass with similar weight (600 ± 30 g total biomass/tank) were held for 6 weeks in 6 fibreglass tanks supplied with a constant flow (1.0 l/min) of seawater (T = 23.5–27.5°C, dissolved oxygen = 5.5–6.8 mg/l). Water salinity during this experiment increased to 27–29‰ due to a reduced input of fresh water into the lagoon following a dry period. Fish were fed the experimental diets at 1.0% live weight in a single meal, according to a 6 × 6 latin square design (6 diets, 6 periods), with a 6-day adaption phase followed by a 24-h period during which water samples were taken every 90 min. Similarly fish were maintained unfed for 6 days with a 24-h water sampling phase (with the same time intervals) afterwards. For every water sample, temperature (portable thermometer HI 8053), salinity (Atago SC28 salinometer), dissolved oxygen (YSI oxymeter model 57), pH (Orion 250A pH-meter) and content of ammonia and soluble phosphorus were measured (Strickland and Parson, 1972). The quantity of N and P excreted was calculated according to the formula used by Kaushik (1980). Data were subjected to statistical analysis according to the method suggested by Snedecor and Cochran (1982) for latin square design.

3. Results

The partial substitution of herring meal with corn gluten meal held the P level within 1% of dm (dm = dry matter basis) without variations related to the protein content. As expected, in the herring meal diets the P level was higher than 1% dm and increased with protein level (Table 1).

Table 1
Ingredient content (g/kg diet), proximate analysis and gross energy content (mean ± s.d.) of the experimental diets containing herring meal (HM) or herring meal + corn gluten meal (HM + G) and 3 different crude protein levels

Ingredient	Diets					
	HM44	HM49	HM54	HM + G44	HM + G49	HM + G54
Herring meal	390	480	560	225	280	325
Blood meal spray dehy.	70	70	70	70	70	70
Yeast brewers dehy.	70	70	70	70	70	70
Corn gluten meal (60 CP)	0	0	0	230	285	330
Fish oil	70	65	60	85	85	80
Wheat flour shorts	200	120	75	170	50	0
Precooked starch	110	105	75	60	70	35
Cellulose	50	50	50	50	50	50
Carboxymethyl cellulose	20	20	20	20	20	20
Vitamin mineral premix	20	20	20	20	20	20
Proximate analyses						
Moisture (%)	10.2 ± 1.2	9.0 ± 1.2	9.0 ± 1.2	9.5 ± 1.0	8.9 ± 0.7	9.1 ± 1.1
Crude protein (% dry wt)	43.9 ± 1.1	48.6 ± 0.8	53.6 ± 0.9	44.5 ± 1.0	49.2 ± 1.1	55.6 ± 0.6
Ether extract (% dry wt)	12.2 ± 0.6	12.3 ± 0.5	12.5 ± 0.3	12.3 ± 0.6	12.9 ± 0.2	12.4 ± 0.3
N-free extract (% dry wt)	30.2 ± 1.8	25.3 ± 1.0	20.1 ± 1.1	31.7 ± 1.7	27.2 ± 1.6	21.0 ± 0.5
P (% dry wt)	1.3 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
Gross energy (kJ/g dry wt)	21.8 ± 0.5	22.0 ± 0.4	22.1 ± 0.4	22.5 ± 0.6	22.8 ± 0.3	23.1 ± 0.4

Vitamin and mineral premix (values are g/kg premix except for those in parentheses): vit. A, (830 000 IU); vit. D_3, (250 000 IU); vit. E, 60.00; vit. K, 2.00; thiamin HCl, 4.00; riboflavin, 4.00; pyridoxine HCl, 4.00; calcium-*d*-pantothenate, 22.22; niacin, 30.30; biotin 10.00; folic acid, 0.50; vit. B_{12}, 0.02; vit. C, 60.00; choline chloride, 200.00; inositol, 20.00; $CaHPO_4$, 157.00; $KHPO_4$, 300.00; MgO, 11.54; MnO, 2.58; $FeCO_3$, 3.55; ZnO, 2.50; $CuSO_4$, 0.38; KI, 0.09; Na_2SeO_3, 0.02; ethoxyquin, 15.00; dextrin to 1000.00.

Mortality (average value 2% for the whole trial) was low and independent of the experimental treatments. The final weight and relative weight gain of fish improved as dietary protein level increased from 44 to 49% ($P < 0.05$), without any further improvement at the highest protein content. Feed conversion efficiency and protein efficiency ratio were not affected. The protein source did not modify any of the growth variables (Table 2).

The viscerosomatic (VSI) and hepatosomatic indices (HSI) decreased significantly with the increase of the protein level ($P < 0.05$, Table 3). The percentage of mesenteric fat (MF) also decreased accordingly. Diets containing corn gluten meal gave slightly higher values of VSI, HSI and MF than those observed with diets containing only herring meal (Table 3).

The body composition of fish was also affected by the experimental diets (Table 3). As dietary protein level increased, body moisture increased and fat and energy content decreased. Fish fed the corn gluten diets had lower moisture and body protein and higher fat content than those fed herring meal diets.

Fish fed the 44% CP herring meal diet had the highest energy, protein and lipid retention (Table 4). There were no difference in these parameters for fish fed the diets containing corn gluten.

Table 2
Growth and feed efficiency data of sea bass fed diets containing herring meal or herring meal + corn gluten meal and 3 different crude protein levels

	Diets					
	HM44	HM49	HM54	HM + G44	HM + G49	HM + G54
Ration[1] (% lw)	1.12	1.02	1.00	1.14	1.03	0.96
Initial average weight (g)	75.2	77.5	77.6	74.0	76.2	74.2
Final average weight (g)	199.0^{c}	224.4^{a}	230.3^{a}	203.1^{b}	220.1^{ab}	232.5^{a}
Relative weight gain[2] (%)	163.6^{c}	189.4^{abc}	194.1^{ab}	171.3^{bc}	188.7^{abc}	213.6^{a}
FCE[3]	0.52	0.57	0.58	0.51	0.56	0.60
PER[4]	1.32	1.28	1.21	1.27	1.18	1.19

Means within the same row not sharing a common superscript letter are significantly different ($P < 0.05$).
[1] Ration = (g/100 g fish weight × day) × 100.
[2] Relative weight gain = (fish weight gain, g) × 100/(initial average weight, g).
[3] Feed conversion efficiency = (fish weight gain, g)/(feed intake, g).
[4] Protein efficiency ratio = (fish weight gain, g)/(protein intake, g).

Daily ammonia excretion of fasted sea bass was 160 mg/kg, with a slight increase during the morning before decreasing linearly during the remaining hours of the day (Fig. 1). In fed fish, nitrogen excretion increased with increasing protein level (Table 4). The excretion was affected by meal time, with a rapid increase from the basal value to values 3–4 times higher 3–4.5 h after the meal, and a lengthening of the excretion period with an increase in dietary protein (Fig. 1). Total ammonia excretion was not significantly different among fish receiving the herring meal diets, whereas in fish fed the diets containing corn gluten, a clear increase with protein level could be detected. A statistical analysis performed using

Table 3
Viscerosomatic index (VSI), hepatosomatic index (HSI), mesenteric fat (MF) and proximate composition (% dry weight) of sea bass fed diets containing herring meal or herring meal + corn gluten meal and 3 different crude protein levels

	Initial	Final					
		HM44	HM49	HM54	HM + G44	HM + G49	HM + G54
VSI[1] (%)	6.2	9.1^{ab}	8.6^{b}	7.1^{c}	9.6^{a}	9.2^{ab}	8.5^{b}
HSI[2] (%)	2.4	2.5^{a}	1.9^{b}	1.6^{c}	2.3^{a}	2.3^{a}	2.0^{b}
MF[3] (%)	3.6	5.3^{ab}	5.4^{a}	4.4^{b}	6.1^{a}	5.7^{a}	5.2^{ab}
Body composition							
Moisture (%)	64.4	64.7^{b}	65.2^{ab}	66.0^{a}	63.9^{b}	64.49^{b}	64.8^{b}
Crude protein (% dry wt)	52.1	53.2^{ab}	54.0^{a}	54.3^{a}	51.1^{b}	52.3^{ab}	53.4^{a}
Ether extract (% dry wt)	33.9	30.8^{b}	30.7^{b}	28.4^{c}	33.0^{a}	31.3^{ab}	31.0^{b}
Gross enery (kJ/g dry wt)	25.3	25.2^{ab}	25.2^{ab}	24.6^{c}	25.6^{a}	25.3^{ab}	24.8^{bc}

Means within the same row not sharing a common superscript letter are significantly different ($P < 0.05$).
[1] Viscerosomatic index = (viscera weight, g) × 100/(empty fish weight, g).
[2] Hepatosomatic index = (liver weight, g) × 100/(empty fish weight, g).
[3] Mesenteric fat = (mesenteric fat weight, g) × 100/(empty fish weight, g).

Table 4
Gross energy, protein and lipid efficiency; ammonia excretion and reactive phosphate released by sea bass fed diets containing herring meal or herring meal + corn gluten meal and 3 different crude protein levels

	Diets					
	HM44	HM49	HM54	HM + G44	HM + G49	HM + G54
GEE[1] (%)	24.1[a]	26.8[d]	24.4[ab]	26.0[cd]	25.5[bc]	26.0[cd]
GPE[2] (%)	25.3[bc]	26.3[c]	23.5[a]	25.2[bc]	24.2[ab]	23.6[ab]
GLE[3] (%)	50.1[ab]	55.3[b]	46.8[a]	57.9[b]	53.3[ab]	57.5[b]
Total NH_3 excretion[4] (mg/kg BW day)	255.6[ab]	254.0[ab]	266.6[ab]	228.3[a]	289.3[bc]	332.7[c]
Metabolic N losses[5] (%NI)	34.1	32.0	30.9	34.4	33.4	32.2
Reactive phosphate[6] (mg/kg BW day)	106.7[b]	110.8[b]	124.3[b]	58.2[a]	55.0[a]	52.1[a]
Leached phosphorous[7] (%PI)	24.3[ab]	28.2[b]	29.3[b]	18.7[a]	18.6[a]	16.7[a]

Means within the same row not sharing a common superscript letter are significantly different ($P < 0.05$).
[1] Gross energy efficiency = (fish energy gain, kJ) / (energy intake, kJ) × 100.
[2] Gross protein efficiency = (fish protein gain, g) / (protein intake, g) × 100.
[3] Gross lipid efficiency = (fish lipid gain, g) / (lipid intake, g) × 100.
[4] Branchial and urinary NH_3 losses.
[5] %NI = total N-NH_3 excreted (mg/kg BW/day) × 100/N intake (mg/kg BW/day).
[6] Reactive phosphate = faecal phosphorous leached in the water.
[7] %PI = reactive P-phosphate in the water (mg/kg BW/day) × 100/P intake (mg/kg BW/day).

all the excretion data showed a significant relationship between the protein level of the diet and ammonia excretion. No difference was observed between metabolic losses, expressed as percent of N intake (Table 4).

The amount of reactive phosphate leached in the water was not affected by protein level but by protein source, since corn gluten diets significantly reduced P leached in comparison

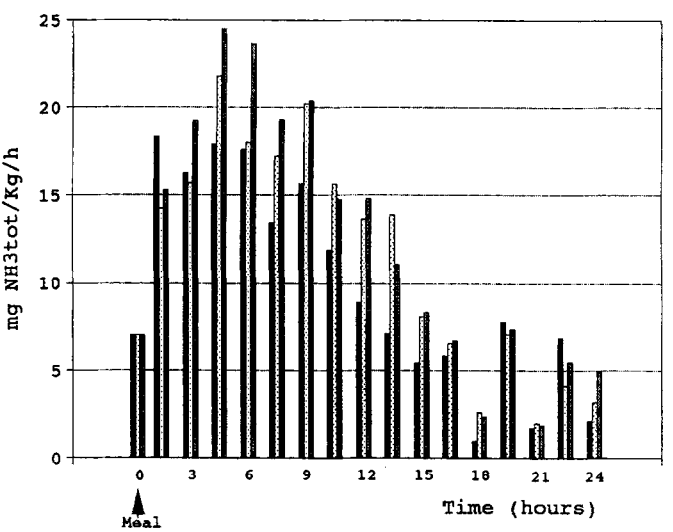

Fig. 1. Postprandial patterns of NH_3 excretion in sea bass fed diets with growing crude protein content. Left column = 44% CP dm; middle column = 49% CP dm; right column = 54% CP dm.

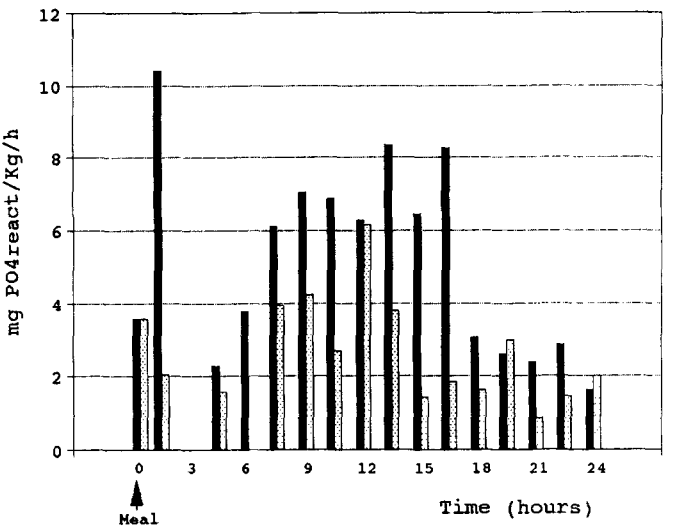

Fig. 2. Postprandial patterns of reactive phosphate production by sea bass fed diets with different protein source. Left column = herring meal; right column = herring meal + corn gluten meal.

to the herring meal diets (Table 4). The same trend was noted, as expected, for the leached/ingested phosphorous ratio. The phosphorus discharged into the water during the day was affected by the meal time, with a trend for the P levels to increase in the morning and late afternoon (Fig. 2).

4. Discussion

According to Hidalgo and Alliot (1988), the sea bass protein requirement varies with the parameter used to estimate it: 50% dm if based on growth rate and 40% dm if based on the utilisation of nutrients and energy. The results of the present experiment suggest that a level of 49–50% crude protein (dm) is sufficient for good growth rates and feed conversion ratios.

PER values were similar to those obtained by Hidalgo and Alliot (1988) with diets based on fish meal fed to sea bass reared at 20°C.

Our results confirm the possibility of using high percentages (20%) of corn gluten meal in diets for sea bass without negative effects on growth rate, as previously reported for 5.5-g fingerlings by Alliot et al. (1979).

Considering the effect of dietary protein level on body composition, the increases in moisture and protein were similar to those observed by Metailler et al. (1981) in sea bass fed diets with higher protein content (53.5–65% dm). However, Metailler et al. (1981) did not observe the effect of dietary protein on fat and energy content noted in the present work probably because these authors analysed the fish carcass and not the whole body. Finally, it is worth mentioning that the substitution of part of the fish meal with corn gluten significantly increased the fat level and decreased the protein content of the whole fish, as already reported by Alexis et al. (1985) for rainbow trout.

The daily excretion of ammonia by sea bass unfed for 6 days, (160 mg/kg/day) was intermediate between the values reported by Guerin-Ancey (1976) (69–75 mg NH_3/kg/day) and those of Spiridakis (cited by Dosdat, 1992) (275 mg N-NH_3/kg/day). This latter value is higher than that observed in the present work and it can be attributed to the smaller size of fish used by this author (30–40 vs. 130 g). The difference between our data and those of Guerin-Ancey (1976) is probably due to the facilities used: this author confined single fish in small tanks (1–10 litres), thus controlling voluntary activity, while in our experiment the fish were not subjected to any limitation of space.

As previously observed in salmonids and cyprinids (Brett and Zala, 1975; Kaushik, 1980; Heinsbroek et al., 1993), the excretion of NH_3 in farmed sea-bass undergoes considerable variations during the day as a function of the meal time, reaching the maximum excretion 4.5 h after a meal. The total amount excreted increased with dietary protein level, from 242 to 272 and 300 mg/kg/day, with a significant linear relationship ($r = 0.4$, $n = 36$) as already observed in the same species by Vitale-Lelong (1989) and on trout by Beamish and Thomas (1984). The linear relationship between ingested N and excreted NH_3 was clearly demonstrated by Rychly (1980), at least for fish receiving up to 160 mg N/100 g/day, while, according to Kaushik (1980), the relationship may be linear or logarithmic in both trout and carp. In the present trial, the N-NH_3 excretion, expressed as a percentage of the ingested N, was higher than that reported by Porter et al. (1987) for sea-bream ($\approx 30\%$ N ingested), but these authors noted a high proportion of N excreted as dissolved organic N (DON = 30%). Fish fed diets containing vegetable protein had a higher N-NH_3 excretion than those receiving fish protein only. The probable reason for this is related to the amino acid profile of corn gluten, characterised by a high leucine level. The content of this amino acid, estimated using tables of food composition (National Research Council, 1981), was 4.7, 5.5 and 6,1% for the 3 diets containing corn gluten meal, in comparison to an average value of 3.6% for herring meal diets. These values are from 2–4 times higher than the estimated requirement for leucine in a carnivorous species such as chinook salmon (Wilson, 1989).

The reactive phosphate leached into the effluent water clearly showed a reduction in P discharge by using corn gluten in sea bass diets. The use of plant protein as a means to reduce P content in effluent water, already suggested by Lall (1991) and Cho et al. (1991, 1993), could be further improved by using microbial phytase to increase the availability of phytic P of plants, as shown by Rodehutscord and Pfeffer (1994) in trout. To pursue this, more research on seabass P requirements and the effect of plant protein on ammonia excretion are neeeded.

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