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in

Montero D. (ed.), Basurco B. (ed.), Nengas I. (ed.), Alexis M. (ed.), Izquierdo M. (ed.). Mediterranean fish nutrition

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 63

2005
pages 9-18

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SUMMARY – A novel approach to determine the protein and energy requirements in fish is described: this involves quantifying the requirements for energy and protein as the sum of the daily requirements for maintenance and growth. The requirement for maintenance is mainly a function of the size of the fish as well as the water temperature, and is proportional to the metabolic body weight. The requirement for growth depends on the weight gain and its energy and protein content. Total daily intake can therefore be calculated by incorporating the respective utilization efficiencies of energy and protein deposits. The consequence of this approach is that protein and energy needs are expressed in terms of absolute daily intake per unit of anticipated weight gain and not only as an inclusion level in the diet. Protein and energy requirements can thus be calculated for seabream and seabass at various stages of their growth.

Keywords: Energy, protein, DP/DE ratio, protein efficiency, factorial approach.

RESUME – *"Besoins en protéine et énergie chez les espèces méditerranéennes". Nous décrivons ici une nouvelle approche pour déterminer les besoins en protéine et en énergie des poissons : il s'agit de quantifier ces besoins à partir des besoins journaliers pour l'entretien et la croissance. Les besoins pour l'entretien sont principalement fonction de la taille des poissons et de la température de l'eau ; ils sont proportionnels au poids métabolique corporel. Les besoins pour la croissance dépendent du gain de poids et de sa composition en protéine et énergie. La quantité journalière d'aliment à distribuer peut donc être calculée en tenant compte de l'efficacité de dépôt de l'énergie et des protéines. Cette approche factorielle permet d'exprimer les besoins énergétiques et protéiques non seulement en termes de niveau d'incorporation dans le régime mais aussi en termes de quantité à distribuer par jour, par unité de poids et en fonction du gain de poids attendu. Cette approche permet de fournir des recommandations sur les besoins en protéines et en énergie et les rapports DP/DE optimaux de la daurade et du bar à différentes étapes de leur croissance.*

Mots-clés : *Energie, protéine, rapport DP/DE, efficacité de la protéine, approche factorielle.*

Introduction

Fish culture in the Mediterranean is presently based on two major species, gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*), and production of both species is expanding rapidly (Basurco and Abellán, 1999). At the same time interest is increasing in the culture of novel species to exploit new markets for the Mediterranean aquaculture. Given the economic importance of feed and feeding in aquaculture, the need to develop nutritionally balanced diets, in particular concerning protein and energy content is clear.

Energy and protein requirements are very complex as they are closely linked. Without protein there is no growth, but neither is there without energy. Since protein can function as an energy source in addition to its essential role for growth, the optimal balance between the supply of dietary non-protein energy and protein should be determined.

With regard to species such as gilthead seabream and European seabass, studies on the protein requirement have stated that minimum protein requirements were determined as 45% for seabream (Sabaut and Luquet, 1973) and as 50% for seabass (Alliot *et al*., 1974; Hidalgo and Alliot, 1988). More recent literature data of protein requirements and digestible protein/digestible energy (DP/DE) ratios are presented in Table 1 and for comparison some have been recalculated on a digestible protein and energy basis.

The optimal DP/DE values appear variable for both species, but this also depends upon the criterion used for evaluating optimization: using maximum weight and protein deposition or highest protein efficiency. It is difficult to say whether this variation is simply due to methodological differences or the result of real species-specific differences. However, a common feature of most of the results is that the daily growth in fish is enhanced by higher protein intake, whereas at the same time the protein efficiency decreases.

Table 1. Recent literature data on optimal DP/DE ratios in seabream and seabass

Species	Size range (g)	DP/DE ratio†	References
Seabream	0.8 - 3.3	30.2 - 27.6	Vergara *et al*. (1996a)
	5.6 - 29.8	25.9 - 22.7	Vergara *et al*. (1996b)
	42.5 - 146.3	22.4 - 20.8	Santinha *et al*. (1999)
	17.5 - 49.9	28.1 - 21.5	Company *et al*. (1999)
	209.0 - 333.0	23.8 - 23.8	García-Alcázar *et al*. (1994)
Seabass	3.1 - 18.4	23.7 - 23.7	Pérez *et al*. (1997)
	6.8 - 25.9	20.8 - 21.8	Peres and Oliva-Teles (1999)
	18.4 - 56.0	28.8 - 23.8	García-Alcázar *et al*.(1994)
	91.8 - 341.8	22.3 - 22.3	Lanari *et al*. (1999)
	130.0 - 226.0	22.9 - 18.9	Dias *et al*. (1999)

†DP/DE ratios in g/MJ for maximal growth or highest protein efficiency.

Compared to salmonids, seabream and seabass seem to require a higher protein level in the diet as reviewed by Oliva-Teles (2000). Some authors also fail to find protein sparing effect of lipids in those two species (Company *et al*., 1999), contrary to salmonids where high energy diets improve protein retention as well as the feed conversion efficiency. One of the factors affecting the dietary protein to energy ratios might be the use of fish of different weights, as protein requirements decrease with increasing fish size. A further difference between studies is the choice of feeding rate which could vary from a given proportion of biomass to *ad libitum* feeding. Since the growth rate is the criterion for which the protein and energy requirements are being established, it is reasonable that high growth rates should be achieved with high feed intake.

Factorial approach

In view of those difficulties a different approach to determine the protein and energy requirements in fish is proposed here. This involves quantifying the amounts for energy and protein in growing fish according to the daily requirements for maintenance and for growth. This method has been described in detail for seabream and seabass in recent studies (Lupatsch *et al*., 1998; 2001a,b). The significance of the factorial approach is that protein and energy needs are not expressed as percentage of the diet, but in terms of the absolute daily feed intake per unit of weight and anticipated weight gain.

Quantification of energy and protein requirement in the growing fish is the sum of the needs for maintenance and growth. The energy requirement for maintenance is mainly dependent on body size and temperature and therefore is proportional to the metabolic body weight. The requirement for growth is dependent on the amount and composition of the added gain. Thus dietary intake can be calculated by including the utilization efficiencies for deposition of new growth:

Requirement = $M \times BW\ (kg)^b + G \times growth$.

$BW\ (kg)^b$ = Metabolic body weight for energy or protein.

M and G are coefficients describing the efficiency of utilization of dietary energy and protein for maintenance or growth.

The aim of this paper is to apply the factorial model to quantify the daily energy and protein needs in growing *Sparus aurata* and *Dicentrarchus labrax*. It is based on trials described in the above mentioned publications and includes some recent data. This approach can be considered as a model or working tool for other fish species considered as promising mariculture candidates.

Feed intake and growth

A prerequisite for estimating feed requirements is accurate prediction of the growth potential of a fish species. This modelling requires growth or weight gain data from fish from past records or trials. Since "optimal growth" is generally used as the criterion for estimating the dietary energy and protein requirements, it is essential that food supply in those trials is not limiting to guarantee sufficient energy and protein intake.

In recent studies with seabream (Lupatsch and Kissil, 1998) the best curve fit to describe the weight gain (y) dependent on fish weight and temperature has been obtained using an exponential regression (equation 1). The growth data used were based on trials, where fish were weighed about every 14 days and absolute weight gain per day calculated for the period between two successive sample weighings. The corresponding body weight was the average weight of the fish during this period and average water temperatures ranged between 19-27°C. By rearranging equation (1) the weight final W_t can be predicted starting from W_0 after t days (equation 2). The same type of equation is used to describe the feed intake dependent on fish weight and temperature as well (3). Likewise, this growth curve in its general form but with species specific constants has been successfully used to describe the growth of *Dicentrarchus labrax* (equations 4 to 6). It is necessary to define these parameters for different stocks, strains or husbandry conditions, and in this study we have determined these coefficients for seabream and seabass for conditions prevailing in the Red Sea.

Sparus aurata:

$$\text{Weight gain (g/fish/day)} = 0.024 \times \text{BW (g)}^{0.514} \times e^{0.060 \times \text{Temp}} \quad (1)$$

or

$$W_t = [W_0^{0.486} + 0.01166 \times e^{0.060 \times \text{Temp}} \bullet \text{days}]^{2.058} \quad (2)$$

$$\text{Feed intake (g/fish/day)} = 0.029 \times \text{BW (g)}^{0.598} \times e^{0.057 \times \text{Temp}} \quad (3)$$

Dicentrarchus labrax:

$$\text{Weight gain (g)} = 0.0196 \times \text{BW (g)}^{0.517} \times e^{0.065 \times \text{Temp}} \quad (4)$$

or

$$W_t = [W_0^{0.483} + 0.009418 \times e^{0.065 \times \text{Temp}} \times \text{days}]^{2.070} \quad (5)$$

$$\text{Feed intake (g/fish/day)} = 0.0216 \times \text{BW (g)}^{0.588} \times e^{0.063 \times \text{Temp}} \quad (6)$$

According to the growth predictions (equations 2 and 5), seabream has a higher growth potential than seabass and can reach 380 g in 12 months from a stocking size of 1g. Seabass on average will reach just 325 g in the course of one year. The feed intake reflects the same pattern, at the same body weight (BW) feed intake is lower for seabass than for seabream. In this case the feed itself was the same for both species, averaging 450 g protein, 190 g lipid and 21.2 gross energy (GE) per kg feed.

Figure 1 below shows daily weight gain per day per fish related to the weight of the fish, based on which the growth equations are modelled for seabream and seabass.

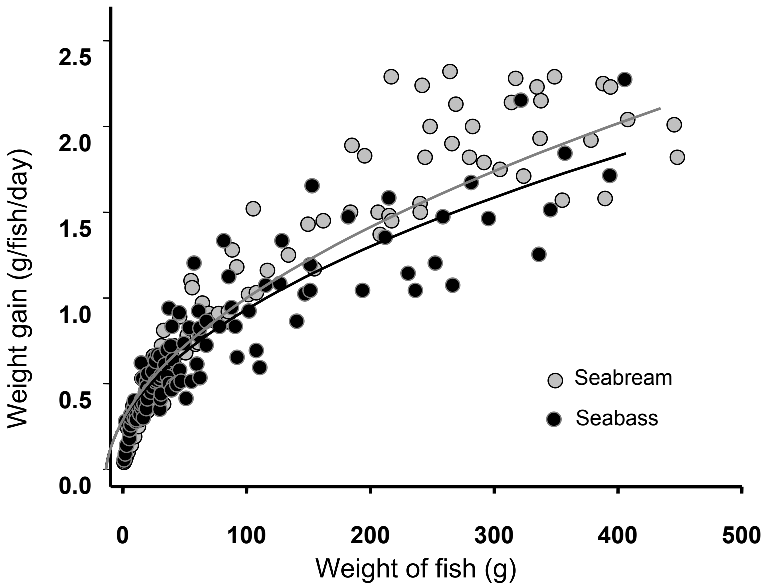

Fig. 1. Daily weight gain (g/fish) at different body weight (g) and temperatures (°C) in *Sparus aurata* and *Dicentrarchus labrax*. The curves are described by equations (1) and (4) and are calculated for a constant temperature of 23°C.

DE and DP requirements

Composition of gain

Because a large proportion of the energy and protein consumed by the fish is retained as growth, the composition of the gain is a major factor determining the subsequent energy and protein requirement of fish. When measuring whole body composition of fish at increasing sizes, each unit weight gain is assumed to equal the body composition at that specific size. In seabream and seabass the protein content was on average 176 and 171 mg/g respectively and this value did not change with fish size (Fig. 2). Dry matter and energy content are generally the most variable factors in fish and can increase dramatically specially for the growth period of smaller fish (>100g) which is depicted in Fig. 2. There is an increase in dry matter, lipid and consequently energy content in growing fish (equations 7 and 8).

Sparus aurata:

$$\text{Energy content (kJ/g)} = 4.66 \times W(g)^{0.139} \tag{7}$$

Dicentrarchus labrax:

$$\text{Energy content (kJ/g)} = 5.17 \times W(g)^{0.107} \tag{8}$$

Comparing the body composition of the fish, seabass has slightly lower energy levels than seabream. According to equation (8), energy content for a 400 g seabass is 9.82 kJ/g as compared to 10.72 kJ/g for seabream of the same size. While the energy content (in conjunction with fat deposition) may also be dependent upon the nutritional history, in these studies both fish species were fed the same diet.

Therefore, in estimating requirements for tissue deposition and growth, wide variations between species, especially in terms of energy are expected based on the differing tissue composition. Relatively energy dense seabream and seabass require more dietary energy per unit of weight gain than leaner fish.

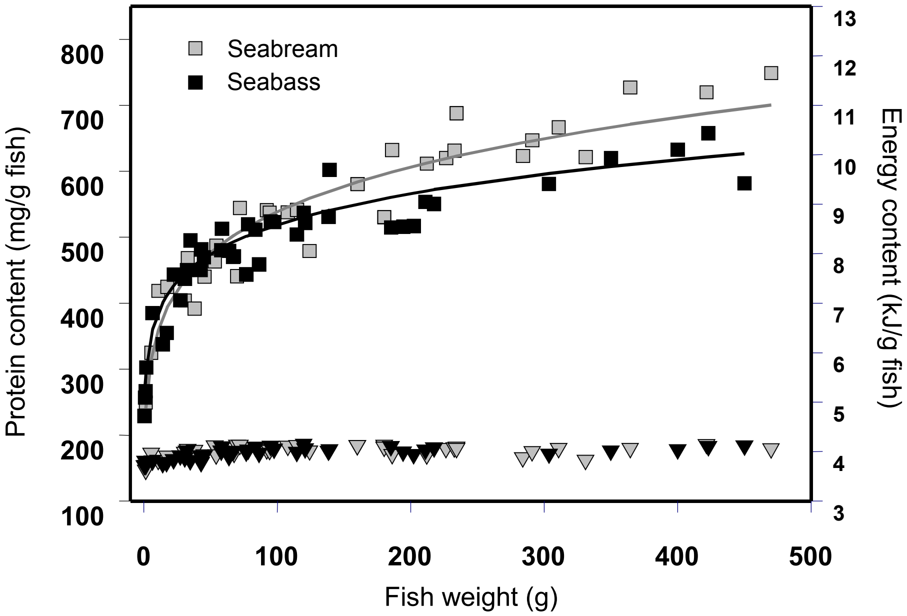

Fig. 2. Protein (mg) ▽ and energy content (kJ) ĺ per g live weight of seabream and seabass of increasing size.

Energy and protein loss at starvation

Energy loss in gilthead seabream and European seabass as depicted in Fig. 3 can be expressed depending on fish weight with the common equation of a $\times$ BW(kg)^{b} where fish weight is given in kg, and b is the exponent of the metabolic body weight. Protein loss during starvation is presented in the same manner:

Sparus aurata:

Energy loss (kJ/fish/day) = 41.5 $\times$ BW(kg)$^{0.82}$ (9)
Protein loss (g/fish/day = 0.40 $\times$ BW(kg)$^{0.70}$ (10)

Dicentrarchus labrax:

Energy loss (kJ/fish/day) = 35.3 $\times$ BW(kg)$^{0.80}$ (11)
Protein loss (g/fish/day) = 0.39 $\times$ BW(kg)$^{0.70}$ (12)

Loss at starvation, which represents basal metabolism, is increasing in absolute terms (kJ/fish/day) with fish weight, but younger fish spend more energy per unit size than bigger fish. The exponent for the energy loss-body weight relationship is b = 0.82 and 0.80 for seabream and seabass respectively. On the other hand, the best fit between protein loss and body weight in seabream and seabass was reached using a metabolic body weight with an exponent of 0.70 (equation 10 and 12). This also reflects the difference in the relationships between energy and protein and increasing body weight (Fig. 2), where protein content is generally conserved in regard to fish size. It is thus clear that protein and energy loss cannot be described by the same metabolic body weight. Moreover, the specific exponents for energy as well as for protein for the two fish species are so close in value to suggest they might be common for a number of fish species.

For poikilothermic fish, in addition to weight, temperature has a significant effect on their metabolism, although its importance may be species-specific. In this studies with gilthead seabream and European seabass energy and protein loss at starvation was measured at an average temperature of 23.5 ± 2.3°C (19 to 27°C) and at this range the temperature effect was found to be very small compared to the size effect. Therefore the metabolic rate can be described only for this range.

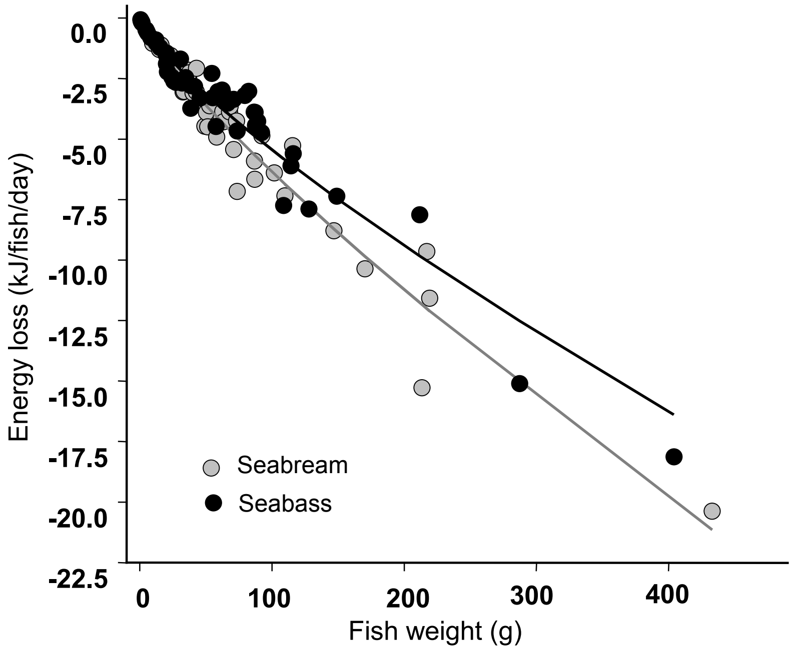

Fig. 3. Daily energy loss (kJ/fish) in seabream and seabass after starvation (corresponding fish weight is geometric mean between the initial and final weights).

Efficiency of energy and protein utilization

It should be noted that energy and protein loss at starvation are only approximations of the requirements for maintenance, allowance must be made for the efficiency of utilization of the dietary energy and protein.

Feeding *Sparus aurata* and *Dicentrarchus labrax* of different sizes on graded levels of DE resulted in both fish showing linear responses to retention (Fig. 4). The relationship between daily DE intake (x) and energy gain (y) for seabream and seabass each referring to their respective metabolic weights of $kg^{0.82}$ and $kg^{0.80}$ can be described by the following linear equations:

Sparus aurata:

$$y = -34.0 + 0.67x \qquad (13)$$

Dicentrarchus labrax:

$$y = -31.3 + 0.69x \qquad (14)$$

Maintenance requirement (DE_{maint}) is found where energy gain equals zero (y = 0) and the efficiency of DE for growth is defined by the slope of the line (Fig. 4). For seabream the required energy intake is calculated from equation (13) as DE_{maint} = 50.8 kJ/BW(kg)$^{0.82}$/day. Accordingly, DE_{maint} for seabass is calculated from equation (14) as 45.4 kJ/BW(kg)$^{0.80}$/day. The efficiencies of utilization of DE for growth were determined as k_{DEg} = 0.67 and 0.69 for seabream and seabass respectively. The reciprocal values 1/0.67 = 1.50 and 1/0.69 = 1.44 are a measure for the requirement (as kJ DE) to deposit one unit of energy (kJ).

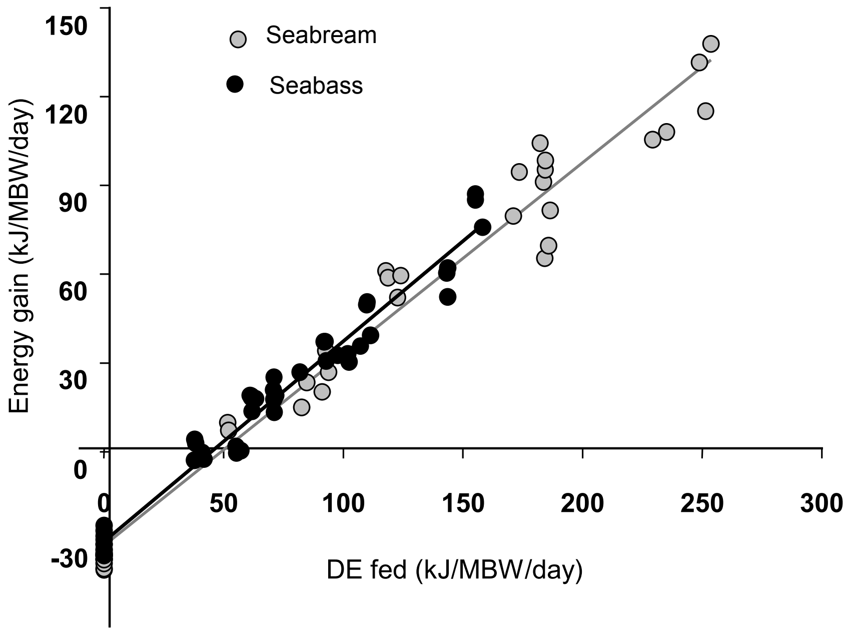

Fig. 4. Daily energy retention per unit metabolic weight in seabream and seabass fed increasing levels of DE.

The relationship between dietary DP intake and protein retained showed different responses in seabream and seabass. For seabass the relationship between DP intake and protein gain was linear (Fig. 5) and can be described by the following equation:

Dicentrarchus labrax:

$$y = -0.32 + 0.52\,x \quad (15)$$

In this case the efficiency of utilization of protein can be defined again by the slope of the line and amounts to $k_{DPg} = 0.52$ and maintenance requirement for protein can be calculated as $DP_{maint} = 0.61 g/BW(kg)^{0.70}/day$. In seabream however the relationship of DP intake over protein gain was not linear and was best described by an exponential curve (Fig. 5).

Sparus aurata:

$$y = 2.19\,[\,1 - e^{(-0.26\,(x - 0.54))}] \quad (16)$$

The difference between these responses lies in the higher feed intake of seabream as seen in Fig. 5. With the higher feed intake more protein was ingested, however, the efficiency of protein deposition was diminished. Apparently, the dietary energy to protein balance was not optimal for seabream at the higher intake levels. As depicted in Fig. 2, the energy content of seabream is higher than for seabass, increasing the dietary energy demand compared to protein. The protein consumed at the high feeding levels was used for additional energy purposes other than protein synthesis. At maintenance level however the DP demand is similar to seabass and amounts to $DP_{maint} = 0.62 g/BW(kg)^{0.70}/day$ (Fig. 5).

As the relationship of DP intake and protein gain was not linear in seabream, a constant value for protein efficiency for growth could not be determined. Therefore, the efficiency of utilization of protein k_{DPg} for growth above maintenance for each of the levels of DP intake was calculated as follows:

$$k_{DPg} = \text{Protein gain}/(\text{DP fed} - DP_{maint}) \quad (17)$$

DP_{maint} = requirement of dietary protein for maintenance = 0.62 g $DP/kg^{0.70}/day$

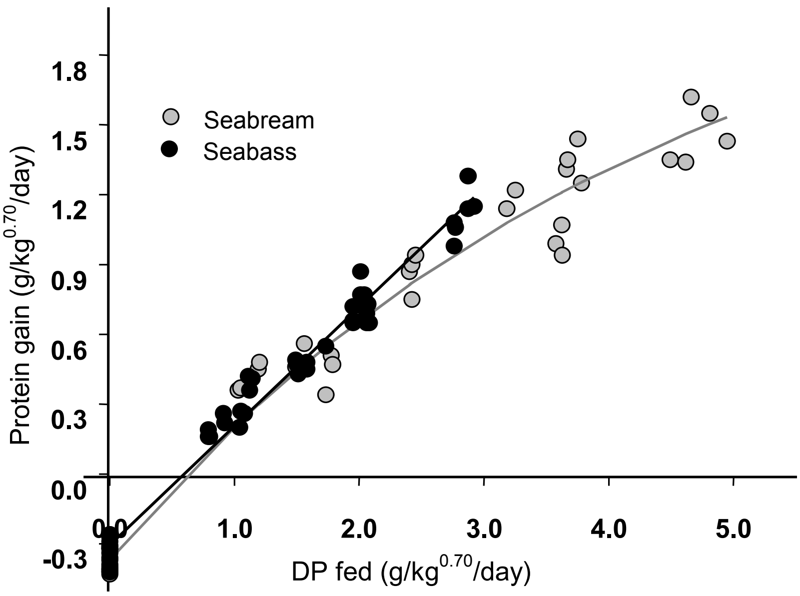

Fig. 5. Daily protein retention per unit metabolic body weight of $kg^{0.70}$ in seabream and seabass fed increasing levels of DP.

The efficiency of protein utilization calculated for the increasing levels of DP intake covered a wide range between k_{DPg} = 0.33 up to 0.80 as Fig. 6 illustrates. At low DP intake, close to maintenance requirements, the protein efficiency is the highest at k_{DPg} = 0.80, because it is limiting. With increasing DP intake the efficiency reaches a level as low as k_{DPg} = 0.33. At the inflection point of the response curve protein gain versus DP intake an optimal protein efficiency value of k_{DPg} = 0.47 was estimated as demonstrated in Fig. 6. The value of k_{DPg} = 0.47 therefore characterizes the protein efficiency for the optimal protein gain, although higher protein efficiencies can be reached at reduced overall gain. The cost in units of DP to deposit one unit of protein gain would therefore be the reciprocal value of 2.13.This value however is only appropriate for a dietary protein with a balanced amino acid profile, like fish meal. In practical diets, that might be limiting in one or two amino acids the protein efficiency will be reduced.

DP/DE ratios in feed

Daily DE and DP requirements for gilthead seabream and European seabass can therefore be quantified according to the common equation:

Requirement = M $\times$ BW $(kg)^b$ + G $\times$ growth

Sparus aurata:

Energy (kJ/fish/day) = 50.8 $\times$ $(kg)^{0.82}$ + 1.50 $\times$ energy gain
Protein (g/fish/day) = 0.62 $\times$ $(kg)^{0.70}$ + 2.13 $\times$ protein gain

Dicentrarchus labrax:

Energy (kJ/fish/day) = 45.4 $\times$ $(kg)^{0.80}$ + 1.44 $\times$ energy gain
Protein (g/fish/day) = 0.61 $\times$ $(kg)^{0.70}$ + 1.92 $\times$ protein gain

With the adequate growth predictions (equations 1 and 4) daily protein and energy requirements as well as DP/DE ratios for different fish sizes can be recommended for seabream and seabass at varying DE contents of the diets (Table 2).

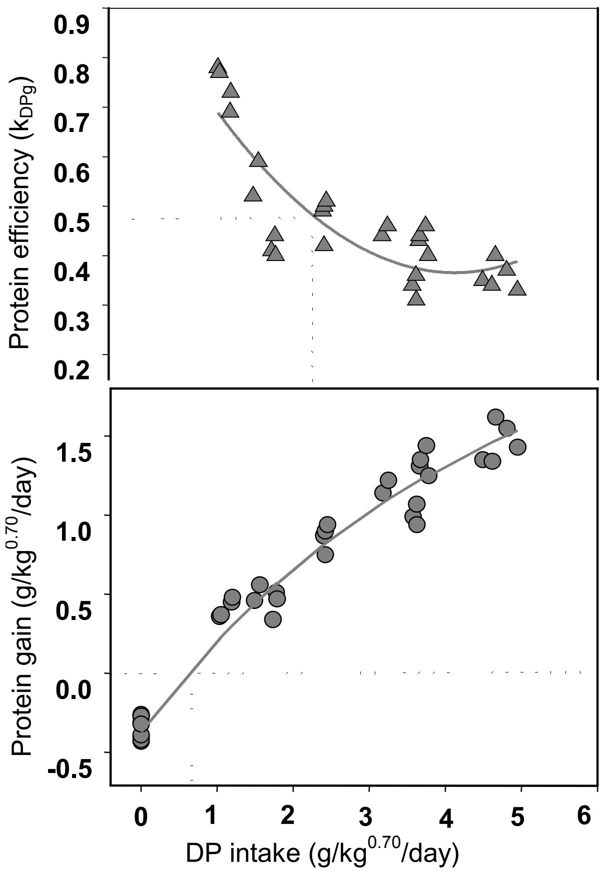

Fig. 6. Relationship between protein efficiency values of growth (k_{DPg}) and protein gain in *Sparus aurata* fed increasing levels of DP.

Table 2. Recommendations of dietary energy and protein supply for seabream and seabass

	Seabream				Seabass			
Body weight (g/fish)	50		300		50		300	
Growth (g/fish/day)	0.71		1.79		0.66		1.56	
Energy								
DE_{maint} (kJ/fish/day)	4.36		18.93		4.13		17.33	
DE_{growth} (kJ/fish/day)	8.58		27.64		7.47		21.38	
DE_{tot} (kJ/fish/day)	12.93		46.57		11.61		38.71	
Protein								
DP_{maint} (g/fish/day)	0.076		0.267		0.075		0.263	
DP_{growth} (g/fish/day)	0.267		0.671		0.217		0.512	
DP_{tot} (g/fish/day)	0.340		0.940		0.292		0.775	
Feed formulation								
DE content of feed (MJ/kg)	15	18	15	18	15	18	15	18
Feed intake (g/fish/day)	0.86	0.72	3.10	2.59	0.77	0.64	2.58	2.15
DP content of feed (g/kg)	395	472	303	363	379	456	300	360
FCR (feed/gain)	1.21	1.01	1.73	1.45	1.17	0.98	1.65	1.38
DP/DE ratio (g/MJ)	26.3	26.3	20.1	20.1	25.3	25.3	20.0	20.0

Using the factorial approach described here, daily intake for energy and protein in growing seabream and seabass can be calculated for different body weights (Table 2). The total feed requirement and therefore the resulting FCR and dietary protein inclusion level for same size fish will change according to the selected DE content of the diet (DE content of 15 or 18 MJ/kg, Table 2). According to the ability to ingest high amounts of feed, lower energy diets could be fed, which then contain lower dietary protein levels, since, based on the calculation from Table 2, the same amount of protein per day per fish would be consumed. Thus comparing fish of different sizes, the FCR increases with fish size as the proportion of the maintenance requirement changes. In contrast optimal dietary DP/DE ratios decrease due to the increasing energy/protein ratio in the carcass.

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