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Regulation of feed intake, growth, nutrient and energy utilisation in European sea bass (Dicentrarchus labrax) fed high fat diets

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Abstract:

Three practical isoproteic (54% protein) diets were formulated to contain graded levels of crude fat (diet L: 10%, diet M: 20% and diet H: 30%). Each diet was assigned unrestrictedly to three and restrictedly to two replicate groups of fish (IBW 243 g). In unrestricted groups, increasing the dietary lipid level led to a significant decrease in voluntary feed intake without affecting growth rate. In the feed-restricted groups, daily growth rates increased with increasing dietary fat levels. There was a significant and inverse effect of the dietary fat content on whole body moisture and fat levels, with highest lipid (ca. 20%) and lowest moisture (ca. 58%) contents in sea bass fed diet containing the highest lipid level; muscle lipid concentration was however not affected. Nitrogen retention was significantly increased by an increase in lipid concentration in the diets, with better efficiencies observed in unrestricted (ca. 31%) than in restricted groups (ca. 27%). Nitrogen loss was significantly affected by both the feeding level and the diet composition, with lowest values (ca. 60 g kg^{-1}) in groups fed diet H unrestrictedly and highest values (ca. 90 g kg^{-1}) in groups fed diet L at a restricted level. Soluble phosphorus excretion in H groups was less than half that in L groups, regardless of the feeding level.

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Keywords: Dicentrarchus labrax; European sea bass; Digestible Energy; Lipid; Feed intake; Phosphorus excretion

1. Introduction

High energy diets are widely used in salmon and trout farming, given the significant benefits of high levels of non-protein energy (fats or digestible carbohydrate) on improved protein retention and reduced nitrogen excretion (Lee and Putnam, 1973; Kaushik and Oliva-Teles, 1985; Cho and Kaushik, 1990; Cho et al., 1994). Quite surprisingly, such a trend in diet formulation for salmonid species has had little impact on diet formulation for European sea bass, this despite the demonstrated benefits of decreasing the DP/DE ratios (Dias et al., 1998a; Kaushik, 1998). Close control of feed intake and reduction of nutrient losses at the source is crucial for an environmentally sustainable development (Cho and Bureau, 1998; Kaushik, 1998) of fish farming. This is especially important in open sea cage farming, as is the case for the majority of sea bass farms where any treatment of wastewater is impossible.

A number of fish species have been reported to regulate their feed intake in relation to the energy content of the diet (see de la Higuera, 2001). There is also abundant literature on the effect of feed intake on growth of sea bass (Carrillo et al., 1986; Tibaldi et al., 1991; Santulli et al., 1993; Ballestrazzi et al., 1994; Garcia - Alcázar et al., 1994; Pérez et al., 1997; Dias et al., 1998a; Peres and Oliva-Teles, 1999, 2001, 2002; Lupatsch et al., 2001, 2003). Except in a few cases (Kaushik et al., 2003; Paspatis et al., 2003), in most of the studies on the effect of feed intake on growth of sea bass, fish were fed a fixed amount of food, or, in some cases, hand fed to satiation, so that the capacity of European sea bass to regulate their feed intake in relation to the digestible energy (DE) content of the diet remains little explored.

This study was conducted to evaluate the effects of increasing dietary DE levels through incorporation of graded levels of fat on voluntary feed intake and the correlated growth response in fish fed unrestrictedly in comparison to fish fed restrictedly. In addition,

the consequences of increased dietary energy level on tissue chemical composition, nutrient utilisation and hepatic lipogenic capacities, nitrogen and phosphorus excretion were investigated.

2. Material and methods

Three practical diets designated as L (low), M (medium) and H (high), were formulated to be isoproteic and to contain a graded level of lipid (Table 1). They were extruded (twin-screw, post-coating) into 3.5 mm pellets by a commercial feed company (FK, Norway). All diets also contained yttrium oxide as an inert tracer for the determination of apparent digestibility coefficients (ADC). Each diet was randomly assigned to five groups of fish. Of these five groups, three were equipped with a feed dispenser filled in excess every day so that feed was always available on-demand. The others were equipped with a feed dispenser filled at the same time of day but with a feed ration equivalent to the average feed intake of the triplicate groups of fish fed without restriction with diet H during the previous 24 h.

The animals used were 20 month-old farmed European sea bass *Dicentrarchus labrax* originating from the same parental stock (Mediterranean area). At Day –30 (D_{-30}), fish were acclimated to the experimental environment and fed on-demand a commercial diet (15% lipid, 46% protein, 20.4 $kJ.g^{-1}$ DM gross energy). At D_0, fish were individually weighed and randomly divided among 15 groups with 50 individuals in each (individual initial fish weight comprised between 220 and 260 g, mean 243 g). They were reared in 1 m^3 indoor tanks supplied with 1 m^3 h^{-1} of fresh filtered seawater in a flow-through system. Water salinity was 38.8 ‰, and oxygen concentration was always above 6.2 mg l^{-1}. Temperature was maintained at 22 ± 0.7°C with artificial lighting of 450 lux at the water surface (16h/8h L/D cycle with 30

mn artificial twilight, light on at 6:00 h).

Feed was available on-demand by means of electronic self-feeders. This feeding system previously described (Boujard et al., 1992) was designed in such a way that each time a fish activates a rod, an electric pulse is generated and, through a relay, triggers an electric feeder that delivers a predetermined amount of feed (between 1.7 and 1.9 g, i.e. 1 pellet per fish). The rods were positioned below the water surface and were protected with a plastic ring in order to prevent any unintentional triggering of the feeders (Covès et al., 1998). Time and date of each impulse from the demand detectors (the rods) were read online by a microcomputer (Husky Hunter, Husky computers Ltd) and stored on disk. Each day, the feeding system was turned off between 09:00 h and 10:00 h in order to replenish each feed dispenser and to check for the presence of uneaten pellets in the sediment traps located at the outlet of each tank.

At D_{21}, D_{41}, D_{61} and D_{91} (last day of the trial), feed was withheld for 24 h, then fish were individually weighed. A representative sample of whole fish and tissues (dorsal muscle, liver and digestive tract, this last being sampled with the perivisceral fat) were withdrawn at D_0 and from each treatment group at D_{91} and kept frozen (-20°C) until analyses of body composition and tissue lipid content. Additional samples of liver were taken, frozen in liquid nitrogen and stored at –80°C for lipogenic enzyme assays.

Whole fish bodies were pooled (10 fish at the start of the experiment, then 5 fish per replicate at the end), ground and freeze-dried before chemical analyses. Ingredients, diets, feces and whole body samples were analysed following standard procedures (AOAC, 1995): dry matter after drying at 105°C for 24 h; starch by the glucose-amylase-glucose-oxidase method (Thivend et al., 1972), protein (N × 6.25) by the Kjeldahl method after acid

hydrolysis; gross energy in an adiabatic bomb calorimeter (Parr); fat after extraction with petroleum ether by the Soxhlet method. From fish fed to satiation, the muscle, liver and digestive tract samples were analysed for total lipid content according to Folch et al. (1957).

Soluble protein content of liver homogenates was determined by the method of Bradford (1976). Activity of glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), malic enzyme (ME, EC 1.1.1.40) and fatty acid synthetase (FAS, EC 2.3.1.38) were measured on frozen hepatic tissue, according to extraction and assay procedures as described by Dias et al. (1998a). Enzyme activity units (IU) defined as μmoles of substrate converted to product per min at assay temperature, are expressed per mg of hepatic soluble protein (specific activity).

For digestibility measurements (Apparent Digestibility Coefficient, ADC), fecal matter was collected in a modified version of the decantation chamber described by Cho and Slinger (1979) and directly fitted to the circular rearing tanks. Feces were collected over five consecutive days (D_{81}-D_{85}) and frozen. Fecal samples were freeze-dried before analyses of dry matter, nitrogen, energy, lipid, phosphorus and yttrium oxide. Yttrium concentrations were determined in diet and fecal samples by atomic absorption spectrophotometry using a nitrous oxide-acetylene flame, after acid digestion (2% nitric acid and 2 g l^{-1} KCl).

In order to quantify soluble nitrogen (N) and phosphorus (P) excretions, outlet water was automatically sampled in each tank during 5 consecutive days (D_{81}-D_{85}) using a peristaltic pump and collected into bottles (2 l per day) containing a small amount of chloroform following procedures used by Kaushik (1980) and Dosdat et al. (1996). Concentrations of ammonia-N and urea-N were analysed by the indophenol blue (Tréguer and Le Corre, 1975) and diacetylmonoxime methods (Aminot and Kérouel, 1982), respectively, using an autoanalyser. Soluble P (PO_4) in the water was determined by the ammonium molybdate method (Tréguer and Le Corre, 1975).

The growth performance, feed intake and feed utilisation, nitrogen and phosphorus excretions were described using the following parameters:

- Daily growth index : DGI : ($FBW^{1/3} - IBW^{1/3}$) / number of days) × 100; where FBW = final body weight and IBW = initial body weight;
- Total feed intake (TFI, g $fish^{-1}$ on a as fed basis) = (cumulative feed distributed – feed refusals) / number of fish;
- Feed intake (% of initial weight) = 100 × TFI / IBW;
- Feed efficiency (FE) = Wet weight gain / feed intake;
- Protein efficiency ratio = Wet weight gain / protein intake
- $$\text{ADC } (\%) = 100 \times \left[1 - \frac{\text{dietary } Y_2O \text{ level}}{\text{fecal } Y_2O \text{ level}} \times \frac{\text{fecal nutrient or energy level}}{\text{dietary nutrient or energy level}} \right];$$
- Nitrogen (or energy) retention (% TFI) = 100 × nitrogen (or energy) gain / total nitrogen (or energy) intake;
- Nitrogen loss (g kg^{-1} fish produced) = 1000 × (total nitrogen intake – nitrogen gain) / wet weight gain;

With total nitrogen (or energy) intake = TFI × nitrogen (or energy) content of the diet, and nitrogen (or energy) gain = (FBW × % nitrogen (or energy) of final whole body) – (IBW × % nitrogen (or energy) of initial whole body).

- Ammonia-N (or Urea-N, or soluble P-PO_4) excretion (%) = 100 × g ammonia-N (or Urea-N, or P-PO_4) released / g digestible nitrogen or available phosphorus intake.

All statistical analyses were performed using the Prism® 3.0 package (GraphPad, USA). Arcsine√ transformations of percentage data were performed to achieve homogeneity of variance. The effects of the feeding level (restricted, unrestricted), the dietary compositions (L, H, M diets) and their interactions were tested using a two-way analysis of variance

(ANOVA). When F values showed significance, individual means were compared using Tukey's multiple range test to detect intergroup significant differences. Differences between treatment groups were considered significant at $P < 0.05$. Data are presented as mean ± SD; no common letters indicate significant differences.

3. Results

ADC values (Table 1) did not differ significantly between diets. The digestible protein (DP) content was 52% in all diets while the DE content increased from 20 to 25 kJ g^{-1}; consequently the DP/ DE ratio decreased from 26 in diet L to 21 mg DP / kJ DE in diet H. Availability of phosphorus did not vary significantly (ADC phosphorus : 55 to 59%).

During the whole period of study, there were no uneaten pellets in the sediment traps located at the outlet of each tank, so that all the feed distributed can be considered eaten by the fish. Both total feed intake (g, Table 2) and feed intake (% of initial weight, Fig. 1) were significantly affected by diet composition in groups fed unrestrictedly.

Mortality was negligible during the experiment with only 2 dead fish among a total of 750 (<0.3 %). Final average body weight (FBW) was significantly affected both by the feeding level and diet composition (Table 2). However this should be taken with caution because in the unrestricted groups, initial average body weight was slightly (though not significantly) different between treatments, with highest values in groups that also showed the highest final average body weight. Of better meaning is the daily growth index, only significantly affected by diet composition in groups fed restrictedly (Table 2). Regardless of the feeding level, feed as well as protein efficiencies (Table 2) were significantly affected by diet composition, with better efficiencies in fish fed the diet containing the highest lipid (DE) level.

Data on whole body composition, retention and loss are presented in Table 3. There was a significant and inverse effect of the diet composition on the whole body moisture and lipid contents, with highest lipid and lowest moisture content in fish fed the diet containing 30% crude fat (H). A small but significant effect of the feeding level was also observed, with slightly more protein and less moisture content in groups fed unrestrictedly compared to those fed restrictedly. Concerning tissue lipid content (Table 4), an increase in liver lipid (30%) and digestive tract lipid content (13%) was observed, whereas there were no significant variations in muscle lipid, with increasing dietary lipid level.

Nitrogen and energy retention (Table 3) was significantly increased by an increase in lipid concentration in the diets, regardless of the feeding level. The feeding level also affected nitrogen retention, with better retention efficiencies in unrestricted than in restricted groups. As a result, nitrogen loss was significantly affected by both feeding level and diet composition, with lowest values (c. 60 g kg^{-1}) in groups fed unrestrictedly diet H, and highest values (c. 90 g kg^{-1}) in groups fed restrictedly diet L.

Data on nitrogen and phosphorus excretion rates (expressed per unit digestible N or P intake) are presented in Table 5. Ammonia-N and Urea-N excretion rates were both significantly affected by ration size and dietary lipid level. The higher the dietary lipid level, the lower the nitrogen excretion. Groups fed diet H restrictedly displayed 33 % less nitrogen excretion than those fed diet L. Soluble phosphorus excretion was affected in the same way as nitrogen excretion, the groups fed diet H excreting less than half the amount of phosphorus than groups fed diet L, regardless of the feeding level.

Specific activity of liver G6PD and ME decreased when dietary lipid level increased (Fig. 2). The same pattern was also observed for FAS, but the activity was significantly lower

in fish fed the diet containing 30% lipid compared to the others groups. A close negative correlation between the activity of these enzymes and the quantity of lipid intake was observed (Fig. 3). This correlation was more significant for G6PD and ME (R= – 0.84 and – 0.82, for G6PD and ME, respectively) than for FAS.

Post-mortem changes in muscle pH measured in unrestricted groups after slaughter at the end of the trial did not vary between groups (c. 7.3, 6.8 and 6.1 at 0, 2 and 18 h after slaughter, respectively). The non-destructive measure of fat content using Torry fat meter (calibrated for salmonids) did not give any reliable data that could be correlated to analytical data either of whole body or that of muscle (data not shown).

4. Discussion

Rearing density varied between 12 and 25 kg m^{-3}, below the upper limit of 35 kg m^{-3} up to which no effect on feeding behaviour and growth could be detected (Dalla Via et al., 1998; Paspatis et al., 2003).

It is remarkable that no uneaten feed was found during the entire study. In some earlier studies with sea bass fed using self-feeders, a feed reward higher than 0.6 g per trigger actuation resulted in feed loss (Paspatis et al., 2000). This was because when the number of pellets distributed exceeded the quantity that can be immediately consumed by the fish, sea bass chose to trigger the feeder rather than to follow the sinking pellets. However these observations were made with smaller fish (3 to 20 g) than in the present study (220 to 500 g), and the rod was protected with a ring that prevented unintentional feed distribution (Covès et al., 1998). In the present study the size of the pellets was larger (3.5 mm vs 0.4 to 2 mm), so that the number of pellets per amount of feed distributed was lower, and the chance of accidental trigger actuation lower, than in the study of Paspatis et al. (2000). These

differences may explain why a feed reward of c. 1.8 g per trigger actuation (about 1 pellet per fish) did not create any problem with feed wastage. Under similar rearing conditions, Kaushik et al. (2003) also did not find any wastage of feed.

Growth rates as found here are in the high range of values, comparable to those reported by Kaushik et al. (2003) in similar conditions. Interestingly, a DP/DE ratio of 20.8 for sea bass of 300 g had been already recommended in the study of Lupatsch et al. (2001). This could have been achieved with a diet of 19 kJ DE and 395 DP. In the present sudy a DP/DE ratio of 20.9 is achieved with a higher concentration of both energy (25 kJ DE) and protein (522 g DP).

When fed at restricted levels, growth is driven mainly by dietary DE content. Conversely, those fed the different diets but with free access to feed regulated their intake in relation to dietary DE level and showed similar growth performance. To the best of our knowledge, this is the first time that such a close control of feed intake has been shown in European sea bass. Physiological mechanisms related to maintaining overall energy status and control of body weight are often invoked in fish (Cho and Kaushik, 1990) as in mammals (Forbes, 1988). Kaushik and co-workers found that in rainbow trout, voluntary feed intake was controlled by the availability of energy (Kaushik et al., 1981; Kaushik and Luquet, 1984). The capacity of rainbow trout to adjust their feed intake in relation to the energy content of the diet was confirmed later in a series of works (Bromley and Adkins, 1984; Kaushik and Oliva-Teles, 1985; Beamish and Medland, 1986; Boujard and Médale, 1994; Gélineau et al., 2001). However, in hand-fed rainbow trout, the compensation of low dietary lipid levels (17.1 kJ g^{-1}) by an increase in feed intake was not sufficient to reach the same amount of energy intake as with a high dietary lipid content (21.4 kJ g^{-1}), and this resulted in a difference in growth performance (Gélineau et al., 2001). A lack of significant relation between dietary energy content and demand-feeding activity was found by Alanärä (1994) in rainbow trout

and Alanärä and Kiessling (1996) in Arctic charr. These authors suggested that the difference in energy content between the diets they used (1.2 kJ g^{-1}) was too small to induce any regulation of feeding activity. In agreement with this, Gélineau et al. (2001) observed that significant changes in feeding activity were only detected when the difference between dietary digestible energy contents was at least 2.2 kJ g^{-1}, and in the present study there was a difference of c. 5 kJ g^{-1} between the diets L and H. However, in wild young-of-the-year Atlantic salmon, the difference required in dietary energy content to obtain significant variations in voluntary feed intake was only 1.1 kJ g^{-1} DM (Paspatis and Boujard, 1996). But in the study of Paspatis and Boujard (1996), the population used was the first generation obtained with wild reproducers, and one might argue that the degree of domestication of the strain used may affect the capacity of the fish to regulate its feed intake, as it is often the case with farmed animals (Rauw et al., 1998).

A positive effect of the increase in dietary DE levels on feed efficiency was observed for the sea bass fed restrictedly (27.8 %) and unrestrictedly (34.3 %), when comparing diet H with diet L ($FE_{dietH}/FE_{dietL} - 1$). This was accompanied by a significant increase in nitrogen (17.6 and 16.5 %) and energy (27.7 and 12.2 %) retention for restrictedly and unrestrictedly fed fish, respectively. After the initial works of Lee and Putnam (1973), several authors have shown that increasing the dietary non protein energy levels leads to a better utilisation of ingested protein, because of an increased contribution of the non protein energy sources to energy expenditure (Cho and Kaushik, 1985, 1990). In juvenile sea bass, a few studies have confirmed the existence of a protein sparing effect of lipid and digestible carbohydrate (Alliot et al., 1979; Morales and Oliva-Teles, 1995; Dias et al., 1998b; Peres and Oliva-Teles, 1999, 2001, 2002). However, only in the present study has the beneficial effect of a dietary incorporation of lipid up to 30 % on the feed efficiency and protein retention been

demonstrated with sea bass of commercial size (up to 480 g). In the study by Ballestrazzi and Lanari (1996), the sea bass used were smaller than in the present study (100-200g), and because of the concomitant increase of the lipid and protein concentrations in the diets used by these authors, it is not easy to conclude that the observed increase in protein retention efficiency was due to the increase in dietary lipid concentration. Peres and Oliva-Teles (1999) found that there was a beneficial effect of increasing the dietary fat level up to 24%, beyond which protein utilisation decreased. However, in accordance with earlier data in rainbow trout with a wide range of sizes (see Jobling, 2001), this increase in protein retention efficiency was accompanied by an increase of the whole body fat content (22 % in restricted groups and 14 % in unrestricted groups), when comparing the final body composition of the sea bass fed diet H with those fed diet L. This increase in body lipid was mainly due to an increase in lipid content of liver and digestive tract, with no variation in muscle lipid content, in accordance with previous observations in juvenile sea bass (Dias et al., 1998a; Peres and Oliva-Teles, 1999) and confirm that in European sea bass, the primary sites of lipid storage are liver and peri-visceral adipose tissue. One might argue that an increase in perivisceral fat have a negative impact on the commercial value of the end product; however in the present study only a 13 % increase of the perivisceral fat content was observed between sea bass fed unrestrictedly diets L and H (Table 4). In addition, final whole body lipid content was more affected by the dietary lipid increase in restricted than in unrestricted groups (Table 2).

Whole body protein content varied little and was in the range of 17.1 ± 0.9 % as found by Lupatsch et al. (2001) for the same species. Our values of N retention (23-31 %) are well within the range of available data on sea bass (25-28 %, Dias et al., 1998a; Paspatis et al., 2003). N retention is found, in general, slightly higher in salmonids (30-35 %, Kim et al., 1998), or in turbot and in cod with values in the range of 34-39 % (Houlihan et al., 1989;

Mallekh et al., 1999; Burel et al., 2000), and slightly lower in sea bream (20-25 %, Lupatsch and Kissil, 1998). Lupatsch et al. (2003) however found subsequently that when values on lipid and protein retention were both expressed as their energy equivalents, similar values were found for European seabass and gilthead sea bream. They also clearly demonstrated that when considered above maintenance requirements, efficiency of utilization of DP was above 50% in this species. In this context, it is also worth mentioning that inter-species differences exist in N requirements for maintenance (Fournier et al., 2002).

The values of N loss estimated in the present trial varied between 61 and 91 g kg^{-1} fish gain, again well within the range of published data on sea bass (Dosdat et al., 1996; Lemarié et al., 1998; Paspatis et al., 2000, 2003). These data clearly demonstrate the potential beneficial effects of decreasing the DP/DE ratio on decreasing nitrogenous wastes and increasing N gain, as was theoretically proposed by Kaushik (1998). There are apparent discrepensies in N release when calculated from dissolved N and from the budget. However one should keep in mind that excretion was measured during 5 consecutive days, while retention and loss values are deducted from data that cover the entire experiment. It remains that our excretion data confirms experimentally, like did recently Peres and Oliva-Teles (2001), that both daily ammonia excretion and oxygen consumption were inversely correlated to dietary lipid levels and a decrease of dietary DP/DE ratio spared protein utilization for metabolism, essentially due to a decrease of non-fecal nitrogen excretion and of the heat increment of feeding.

Although whole body phosphorus content was not determined here, from literature data, it is clear that phosphorus level in the whole body of European sea bass is relatively stable at 0.6 ± 0.1% of fresh weight (Lemarié et al., 1998; Oliva-Teles et al., 1998; Kaushik et al., 2003). Data on soluble phosphorus excretion in relation to P intake are scarce in European

seabass. Ballestrazzi et al. (1994) observed that between 17 and 30% P was excreted on a daily basis depending on the dietary protein level and source. The experimental conditions differed from ours in terms of fish body weight (76 g) and temperature (23.5-27.5 °C). In land-based farming conditions (119 tons, four simultaneous batches ranging from 4.2 g and 365 g BW and held at 16.5 to 18.5 °C, Lemarié et al. (1998) estimated that soluble P excretion was c. 59% of P intake. More recently, Kaushik et al. (2003) compared the effect of different level of fish meal replacement by plant protein on P excretion under similar experimental conditions (fish BW range and temperature). P excretion significantly decreased with dietary P content while food intake and daily growth intakes (DGI) did not differ among the treatments. They found that in European seabass fed a fish meal based diet (45% crude protein, 21.6% crude fat and 1.16% phosphorus) P excretion amounted to about 42%. The variations in P excretion observed in the present study are probably due to an interaction between P intake and growth rate rather than affected directly by the lipid level. Among unrestricted groups, P excretion was positively correlated to total feed and consequently P intake for the same DGI, suggesting that P needs are correctly covered by dietary P content even for the lowest feed intake. On the other hand, among restricted groups, P excretion was inversely correlated to growth rate for a fixed P intake. In that case the higher the growth performance the greater the P retention and hence the reduced P excretion. However, several sampling periods directed to P excretion evaluation and P retention measurements would have been profitable to confirm this.

Lipogenic enzyme activities in sea bass showed that G6PD, ME and FAS were depressed when increasing dietary lipid level. This inhibitory effect of high dietary lipid level on lipogenic enzymes activities has been reported in several fish species (Lin et al., 1977; Likimani et al., 1982; Arnesen et al., 1993; Shimeno et al., 1995; Dias et al., 1998a; Gelineau

et al., 2001). However our results on FAS confirm that the regulation of lipogenesis is less controlled in fish than in mammals, as previously shown in juvenile sea bass (Dias et al., 1998a) and in rainbow trout (Corraze et al.,1999; Gelineau et al., 2001).

5. Conclusion

The outcome of this study is that sea bass can be fed diets containing up to 30 % lipids. Such high energy diet had a positive effect on nitrogen balance by increasing retention and reducing losses. Concomitantly, the use of high dietary diets decreases phosphorus excretion, simply because less fish meal per unit of fish growth is needed, thanks to the protein sparing effect of the increase of non protein energy intake. It was also demonstrated that European sea bass are able to adjust their feed intake in relation to the dietary digestible energy content, at least when they are fed using self-feeders. High dietary fat does lead to increased fat deposition in the visceral and hepatic tissues, but has no adverse effects in terms of muscle fat deposition.

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Figure captions

Fig. 1. Evolution of feed intake (% of initial weight) over time for groups of sea bass (mean values) held in groups of 50 and fed on demand with diets formulated to contain a graded level of lipid and designated as L (low), M (medium) and H (high), either unrestrictedly (full line) or restrictedly (broken line). Different letters indicate statistically different final weight gain (P<0.05, one-way ANOVA and Tukey's multiple range test). Vertical bars indicate 1 SD.

Fig. 2. Hepatic lipogenic enzyme activities in European sea bass fed different dietary fat levels. G6PD = glucose 6 phosphate dehydrogenase, ME = malic enzyme and FAS = fatty acid synthetase. Data are specific activities, expressed as UI or μUI/ mg protein. Data are presented as mean ± standard deviation, means with no common letters are significantly different ($P < 0.05$).

Fig. 3. Effects of an increase in daily fat intake on the activity of lipogenic enzymes in sea bass fed different dietary fat levels. Data are presented as mean ± standard deviation, R = Pearson's correlation coefficient.

Table 1

Ingredients, chemical composition and apparent digestibility coefficients (ADC) of the experimental diets L (low energy), M (medium energy), and H (high energy).

	L	M	H
Ingredients (g/100g dry matter)			
Fish meal, LT 94	57.5	57.5	57.5
Whole wheat	27.4	16.5	11.0
Wheat gluten	7.7	9.3	5.6
Fish oil	3.8	13.1	22.3
Potato starch	1	1	1
Mineral premix*	0.3	0.3	0.3
Vitamin premix*	0.15	0.15	0.15
Rovimix, Stay C 25 %	0.06	0.06	0.06
Betaine 97 %	0.1	0.1	0.1
Binder (suprex corn)	2	2	2
Yttrium oxide	0.008	0.008	0.008
Analytical composition			
Dry matter (%)	90.4	90.5	95.2
Protein (N×6.25) (% DM)	53.8	53.7	53.8
Fat (% DM)	11.3	21.3	30.0
Gross energy (kJ/g DM)	21.6	23.9	25.8
Starch (% DM)	17.1	11.7	5.4
Phosphorus (% DM)	1.5	1.5	1.4
ADC values (%)			
Dry matter	84.7 ±1.41	89.1 ±0.26	90.9 ± 0.08
Energy	92.0 ± 0.68	95.2 ± 0.22	96.8 ± 0.21
Protein	96.2 ± 0.24	96.7 ± 0.13	97.1 ± 0.02
Fat	95.1 ± 0.21	97.4 ± 0.38	97.6 ± 0.39
Phosphorus	54.6 ± 4.00	55.5 ± 2.70	59.2 ± 2.12
Starch	95.8 ± 0.23	99.2 ± 0.10	99.9 ± 0.03
Digestible protein (DP, % DM)	51.8	51.9	52.2
Digestible energy (DE, kJ/g DM)	19.9	22.7	25.0
DP / DE ratio (mg/kJ)	26.0	22.8	20.9
Available P, (% DM)	0.82	0.83	0.83

*Proprietary formulae, meeting recommendations of NRC (1993)

Table 2.

Initial (IBW) and final average body weight (FBW), Daily growth index (DGI), total feed intake (TFI) and Feed efficiency (FE) of groups of European sea bass fed on demand either unrestricted or restricted daily amounts of feed. Fish were fed using diets (L, M and H) with different (low, medium and high) lipid levels. Data are presented as mean ± standard deviation. Within each column, means with no common letters are significantly different ($P < 0.05$).

	IBW (g)	FBW (g)	DGI (% day^{-1})	TFI (g fish^{-1})	FE	PER
Unrestricted group						
L	233 ± 7.6	441 ± 7.5^{ab}	1.65 ± 0.12^{a}	291 ± 24^{a}	0.72 ± 0.02^{b}	1.48 ± 0.04^{b}
M	240 ± 7.2	453 ± 30^{ab}	1.66 ± 0.11^{a}	267 ± 35^{ab}	0.80 ± 0.02^{ab}	1.64 ± 0.04^{ab}
H	250 ± 5.2	482 ± 15^{a}	1.73 ± 0.08^{a}	253 ± 14^{b}	0.92 ± 0.03^{a}	1.79 ± 0.06^{a}
Restricted group						
L	244 ± 7.0	403 ± 0.7^{c}	1.30 ± 0.11^{c}	238 ± 2.7^{c}	0.67 ± 0.03^{b}	1.38 ± 0.06^{b}
M	249 ± 1.4	435 ± 4.9^{bc}	1.45 ± 0.01^{b}	236 ± 0.1^{c}	0.79 ± 0.02^{ab}	1.62 ± 0.03^{ab}
H	245 ± 7.1	448 ± 15^{ab}	1.57 ± 0.03^{a}	227 ± 12^{c}	0.90 ± 0.01^{a}	1.76 ± 0.02^{a}

Two way – ANOVA (1)	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Interaction	2.39	0.146	0.426	0.665	2.15	0.172	4.53	*	0.906	0.438	0.965	0.417
Feeding level	2.06	0.184	11.0	**	20.9	***	36.9	***	4.39	0.065	4.50	0.063
Diet composition	2.55	0.132	7.29	**	3.56	0.072	8.59	**	98.5	***	64.2	***

(1) *, ** and *** indicate when *P* levels are < 0.05, < 0.01 and < 0.001, respectively. Degrees of freedom for the F tests are 2, 1, 2 and 9 for the interactions, the feeding level, the diet composition and the residuals, respectively.

Table 3.

Final body composition, nitrogen and energy retention (% of intake), and nitrogen loss of groups of European sea bass fed on demand diets with different lipid levels either unrestricted or restricted daily amounts of feed. Data are presented as mean ± standard deviation. Within each column, means with no common letters are significantly different ($P < 0.05$).

	Final comp (% fresh weight)			Retention (% TFI)		Nitrogen loss (g kg^{-1} fish produced)
	Moisture	Protein	Lipids	Nitrogen	Energy	
Unrestricted						
L	59.8 ± 0.2^{ab}	17.1 ± 0.2^{a}	17.2 ± 0.1^{b}	26.7 ± 0.4^{ab}	47.7 ± 2.5^{bc}	79.3 ± 2.4^{b}
M	59.4 ± 0.9^{ab}	16.4 ± 0.8^{ab}	18.8 ± 1.4^{ab}	27.6 ± 3.7^{ab}	51.1 ± 3.1^{ab}	71.0 ± 4.0^{c}
H	58.4 ± 0.5^{b}	16.8 ± 0.4^{ab}	19.6 ± 1.2^{ab}	31.1 ± 2.3^{a}	53.5 ± 2.6^{ab}	61.7 ± 1.5^{d}
Restricted						
L	61.5 ± 1.2^{a}	16.4 ± 0.3^{ab}	16.8 ± 1.5^{b}	23.2 ± 0.1^{b}	43.3 ± 2.6^{c}	91.3 ± 1.6^{a}
M	60.0 ± 0.5^{ab}	16.1 ± 0.2^{ab}	18.2 ± 0.4^{ab}	25.9 ± 1.2^{ab}	51.7 ± 0.4^{b}	73.1 ± 2.6^{c}
H	58.4 ± 0.5^{b}	15.9 ± 0.1^{b}	20.5 ± 1.2^{a}	27.3 ± 0.1^{ab}	55.3 ± 0.3^{a}	66.4 ± 0.9^{cd}

Two way – ANOVA (1)	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Interaction	1.75	0.226	0.483	0.632	0,671	0,535	0.357	0.708	2.24	0.161	4.97	*
Feeding level	4.63	*	5.91	*	0,004	0,950	7.41	*	0.263	0.620	22.0	***
Diet composition	13.4	**	1.58	0.257	9,28	**	4.89	*	16.6	***	86.5	***

(1) *, ** and *** indicate when *P* levels are < 0.05, < 0.01 and < 0.001 , respectively. Degrees of freedom for the F tests are 2, 1, 2 and 9 for the interactions, the feeding level, the diet composition and the residuals, respectively.

Table 4.

Tissue lipid content in European sea bass fed unrestrictedly diets (L, M and H) with different (low, medium and high) lipid levels. Data are presented as mean ± standard deviation. Within each column, means with no common letters are significantly different ($P < 0.05$).

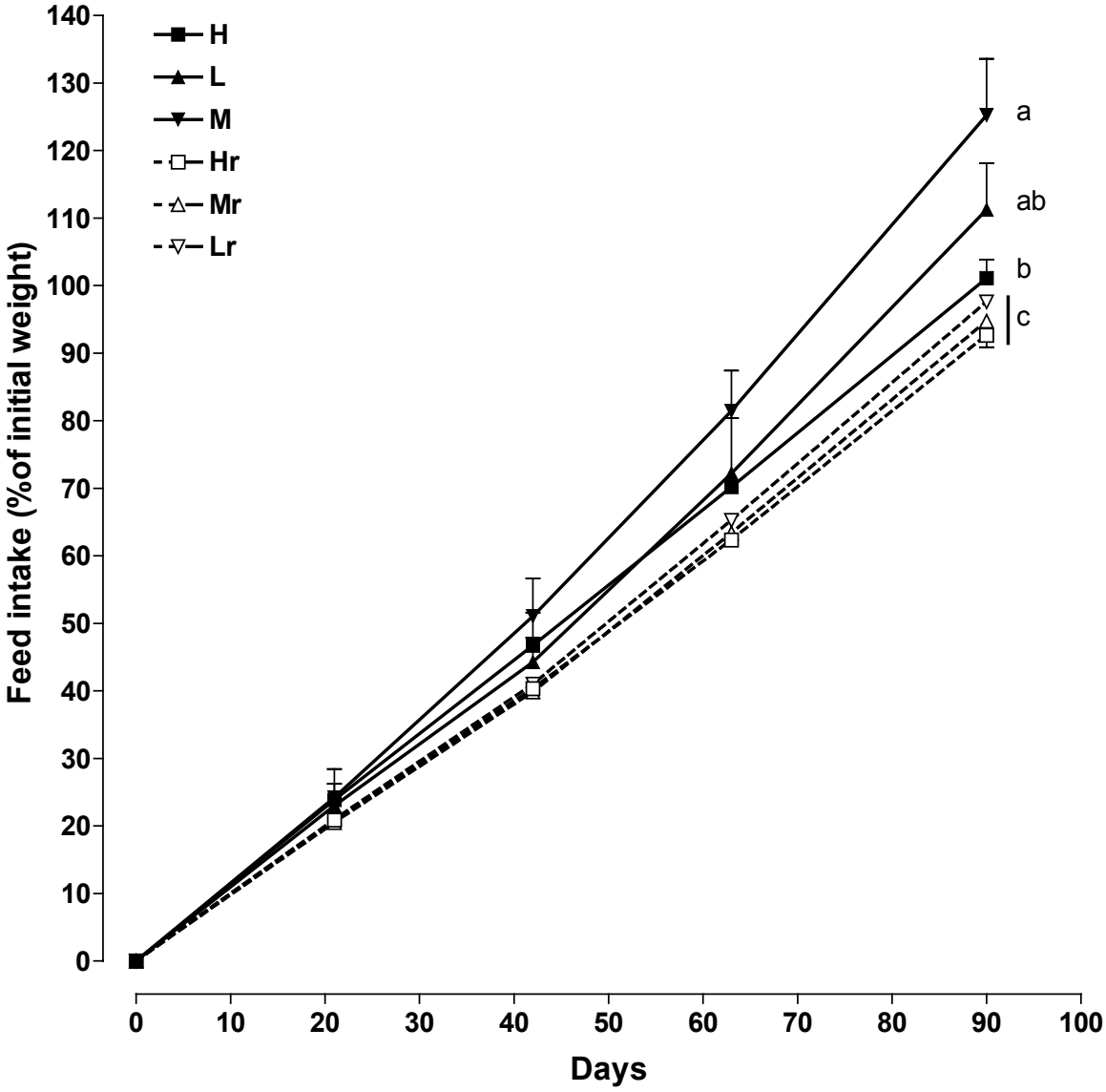
	Tissue size (% whole body weight)			Tissue lipid content (% fresh weight)		
	Muscle	Liver	Digestive tract	Muscle	Liver	Digestive tract
Initial	40.6 ± 0.8	2.6 ± 0.4	7.6 ±0.5	8.4 ± 0.3	41.4 ± 0.9	61.3 ± 2.9
L	45.4 ± 0.3	2.7 ± 0.3	6.8 ± 0.6^{b}	10.4 ± 0.3	36.9 ± 5.0^{b}	63.4 ± 1.8^{b}
M	44.2 ± 0.5	2.4 ± 0.1	7.8 ± 0.5^{ab}	10.6 ± 1.1	47.9 ± 2.5^{a}	70.1 ± 1.3^{a}
H	46.0 ± 2.7	2.7 ± 0.3	8.6 ± 0.5^{a}	11.9 ± 0.2	51.8 ± 3.4^{a}	71.7 ± 0.7^{a}

Table 5.

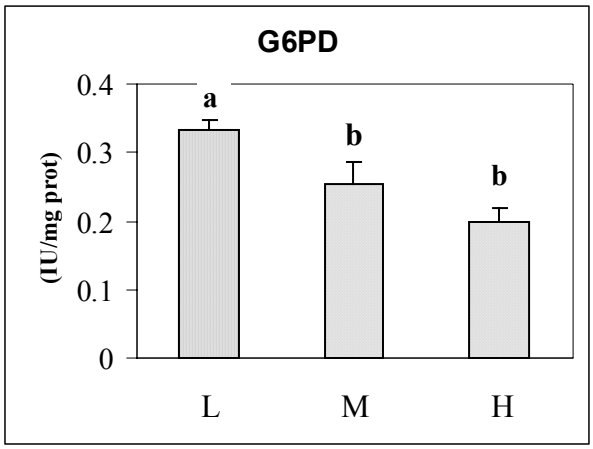
Nitrogen (Ammonia-N and Urea-N) and phosphorus (P-PO4) excretions of groups of European sea bass fed on demand either unrestricted or restricted daily amounts of feed. Fish were fed using diets (L, M and H) with different (low, medium and high) lipid levels. Data are presented as mean ± standard deviation. Within each column, means with no common letters are significantly different ($P < 0.05$).

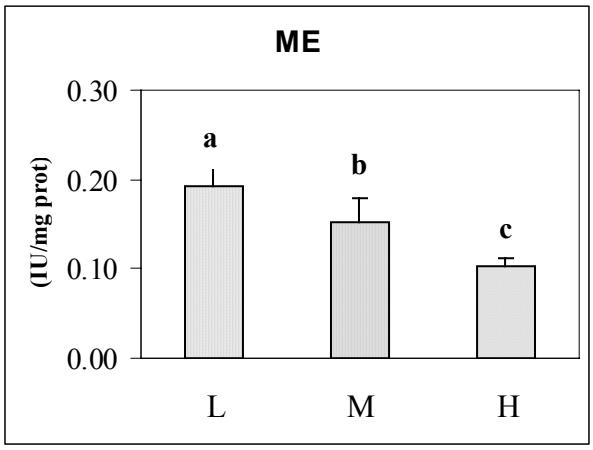
	Ammonia-N (% digestible N intake)		Urea-N (% digestible N intake)		P-PO4 (% available P intake)	
Unrestricted group						
L	44.6 ± 0.8^{c}		6.3 ± 0.3^{b}		81.5 ± 9.5^{c}	
M	40.4 ± 2.5^{ab}		5.7 ± 0.6^{ab}		58.9 ± 13.2^{b}	
H	34.4 ± 6.0^{a}		4.6 ± 1.2^{a}		32.8 ± 7.9^{a}	
Restricted group						
L	56.1 ± 0.03^{d}		7.7 ± 0.8^{c}		81.1 ± 11.7^{c}	
M	45.1 ± 0.2^{c}		6.4 ± 0.3^{b}		65.3 ± 16.9^{b}	
H	37.8 ± 2.3^{a}		5.1 ± 0.5^{ab}		38.8 ± 6.1^{a}	
Two way – ANOVA (1)	F	*P*	F	*P*	F	*P*
Interaction	2.87	0.109	0.44	0.655	0.11	0.893
Feeding level	16.85	**	5.59	*	0.32	0.582
Diet composition	26.51	***	11.06	**	16.85	***

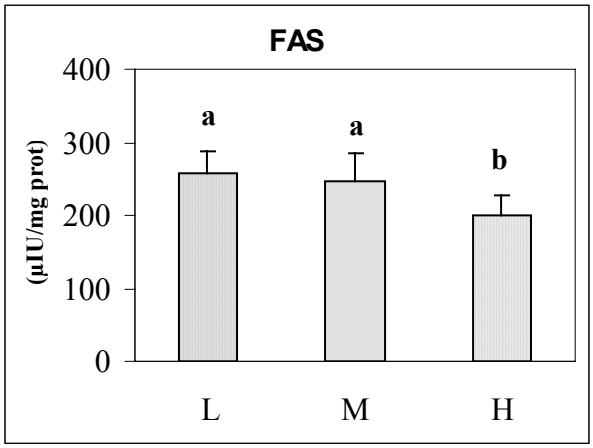
(1) *, ** and *** indicate when *P* levels are < 0.05, < 0.01 and < 0.001, respectively. Degrees of freedom for the F tests are 2, 1, 2 and 9 for the interactions, the feeding level, the diet composition and the residuals, respectively.



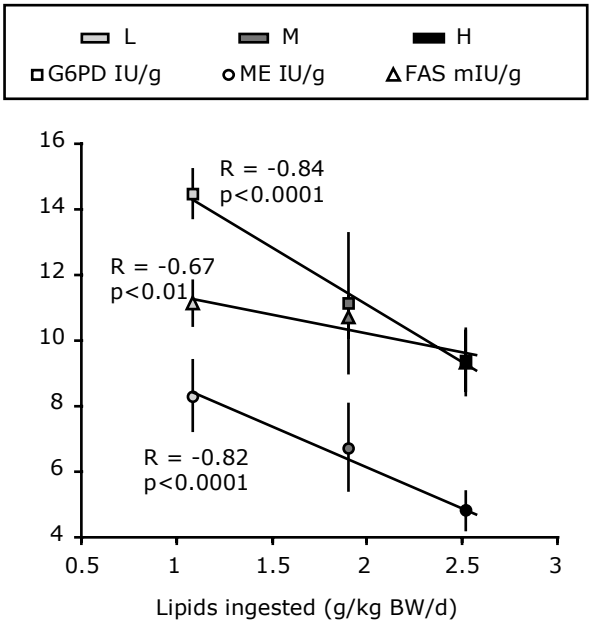
Boujard et al., fig. 1







Boujard et al., Fig 2



Boujard et al., Fig 3.