Faculdade de Ciências do Mar e do Ambiente

Mestrado em Biologia Marinha

Alimentação e Nutrição

Feeding plan for gilthead seabream (*Sparus aurata,* Linnaeus 1758)

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1. Diagnostic Features, Habitat and Biology of gilthead sea bream

The gilthead seabream *Sparus aurata* is a common teleost in the Mediterranean Sea (Guinea & Fernandez, 1997). The body of gilthead seabream is oval, rather deep and compressed; the head profile is regularly curved. This species presents a silvery grey colour and a large black blotch at the origin of the lateral line extending to the upper margin of the opercle where it is edged below by a reddish area. They present a golden frontal band between eyes edged by two dark areas (not well defined in young individuals), dark longitudinal lines often present on the sides of the body, a dark band on dorsal fin and its fork and tips of caudal fin are edged with black. In sea bream, the larvae are bilaterally symmetric at hatching and no changes to the symmetry occur with growth and the adults are pelagic (Aragão *et al.* 2004). It´s an euryhaline specie. Spawning occurs from October to December; maturity at 1-2 years (20-30 cm) for males, 2-3 years (33-40 cm) for females. Gilthead sea bream is mainly carnivorous (molluscs, particularly mussels which it can easily crush, crustaceans and fish), but accessorily they can be herbivorous. The maximum size is 70 cm but commonly is 35 cm.

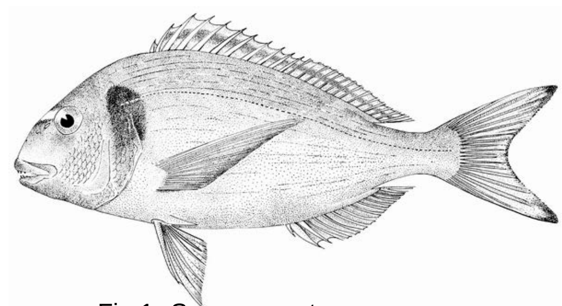

Fig.1- *Sparus aurata*

2. Significance of species for aquaculture

Gilthead seabream is a common cultured species in Southern Europe both in land-based farms and in cages at sea (Guinea & Fernandez, 1997), and together with European seabass dominates the aquaculture production in that area (Aragão *et al.* 2004). This fish reaches high prices in the market and is much appreciated locally. It can be produced with ease in the hatcheries (Sanchez-Lamadrid, 2002), being one of the most important marine fish species reared in Europe (Oliva-Teles, 2000). In fact, its aquaculture grew very fast in the

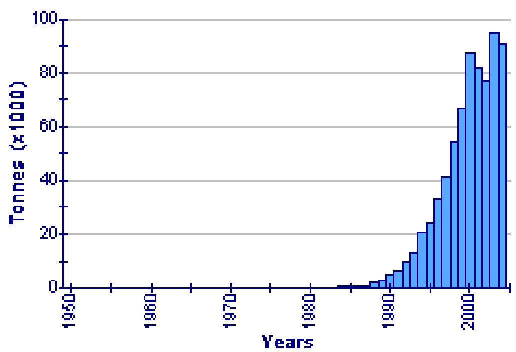

Fig. 2- Global aquaculture production for *Sparus aurata* (FAO Fishery Statistic)

Mediterranean countries, reaching 14727 metric tons in 1995 from 378 metric tons in 1984 (Sanchez-Lamadrid, 2002). In 2001, cultured seabream production was 1,053 tons, comprising 0.3% of the 342,865 tons total aquaculture catch (Sadek *et al*., 2004).

Nowadays, production of this specie is a well-controlled process but knowledge of its nutritional requirements is still incomplete compared to other fish species, particularly salmonids and carps. An adequate knowledge of the nutritional requirements is, however, of utmost importance for the formulation of high quality diets that promote optimum growth rates and feed efficiencies while minimizing feed wastes, so contributing to the sustained development of this industry (Oliva-Teles, 2000)

The extensive system is more popular, where farmers stock ponds at low densities and fish derive most of their nutrition from the natural food present in ponds. Farmers also feed seabream with wild collected fish (*Tilapia zillii*) and small size shrimp (*Palaemon* spp.) caught from northern delta lakes (Sadek 2004). In the semi-intensive system with slightly higher fish stocking density, farmers use tilapia feed, either made on the farm or bought from fish feed manufactures. At present, several governmental and private feed factories are producing pelleted fish feeds (Sadek et al. 2004).

3. Feeding habits in nature and in culture

In the wild, gilthead seabream feed on prey such as crustaceans, molluscs, polychaetes, and echinoderms using a complex heterodont system of canine-like and molariform-plated teeth, which allows the crushing of hard-textured prey (Andrew *et al.*, 2004). However, farmed gilthead seabream have been observed to chew, and occasionally eject, whole or parts of dry pellets during feeding. The ejection of intact pellets and loss of particles from crushed pellets during handling may result in an increase in feed waste and feed conversion ratios in aquaculture (Andrew *et al.*, 2003; Andrew *et al.*, 2004). In a series of food choice experiments conducted with gilthead seabream, it was shown that the incidence of chewing and ejection of food items was reduced when fish were fed soft-textured natural prey (Andrew *et al.*, 2004).

4. Nutritional requirements known

Growth and the feed required to sustain growth is of major importance in aquaculture. To improve productivity and profitability of aquaculture, we have to provide feeds that supply adequate levels of energy and protein to sustain efficient growth. Proper feed management is also crucial with regard to the aquatic environment, since feed that is not consumed or not available to the fish will be lost to the effluent surroundings and will result in nutrient enrichment of the water body (Lupatsch *et al.*, 2003). Growth optimisation is closely linked to the supply of dietary protein of appropriate quality and quantity. Growth and food conversion efficiencies can be maximised by manipulating the composition of the dietary amino acids (AA). AA imbalances in the diet cause increased AA oxidation and lead to decreased food conversion efficiencies (Conceição *et al.*, 2003).

In general gilthead sea bream nutrient base values are according to Sanchéz-Muros (2003) 45% of protein, 14% lipids and 20 % carbohydrates, with other values proposed by Oliva-Teles (2000) of 45 to 50% protein and a minimum of 9–12% lipids.

4.1. Protein

Compared to salmonids protein requirements of seabream are high, reflecting their highly carnivorous nature. Using semi-purified diets Sabaut and Luquet (1973) estimated the optimum protein requirement for maximum growth of juvenile seabream as 40%. Recently, this value was revaluated to 45–46% based on the same criteria but with practical diets. In fry protein requirement is higher, corresponding to 55% of the diet (Oliva-Teles, 2000).

The digestible protein to digestible energy ratio of the diets also affects growth rate and feed utilization efficiency. Moreover, it was also shown that seabream are able to regulate feed consumption on the basis of the protein and/or energy content of the diets, in order to fulfil nutritional needs. Available data indicates that the optimum protein to energy ratio of the diets is higher and the protein retention efficiency is lower in marine fish as compared to salmonids, suggesting that conventional energy sources have a limited protein sparing effect in marine fish. Furthermore, these differences may also have implications in the feed processing technologies and formulations involved (Oliva-Teles, 2000). The optimum protein to energy ratio of the diets for sea bass and seabream seems to be higher than for salmonids. Moreover, high dietary lipid levels do not seem to improve fish performances.

4.1.1. Amino acids

Fish do not have absolute protein requirements, but require the amino acids that compose the proteins. All fish species studied so far seem to require the same 10 essential amino acids. Data on the quantitative amino acid requirements in seabream estimated by dose-response studies is available for arginine as 5 g/16g N, for lysine as 5 g/16g N, for methionine and cysteine as 4 g/16g N and for tryptophane as 0.6 g/16g N. While specific amino acid requirements of a given fish species are not known, available data on the amino acid requirements of other species or the amino acid profile of their carcasses may be used as a guideline for practical diets formulation (Oliva-Teles, 2000).

4.1.2. Alternative protein sources

Fishmeal, due to its almost ideal nutritional composition, is the best protein source for fish diets, in spite of its high price and reduced availability in the international market making necessary to partially or completely replace dietary fishmeal by alternative protein sources. Moreover, as fishmeal quality is not standardized, fish performances are affected by fishmeal quality (Vergara *et al.* 1999, Oliva-Teles, 2000).

Different alternative protein sources for seabream have been evaluated, with the majority of these studies showing that while part of the dietary fish meal can be replaced by alternative protein sources without negatively affecting fish performances, a total replacement significantly reduces growth and feed efficiency (Oliva-Teles, 2000). This decrease in fish performance results may be attributed to an imbalance of amino acids in the alternative protein sources, the presence of anti-nutritional factors or diet unpalatability, particularly when plant feedstuffs are utilized (Oliva-Teles, 2000).

Nutrient digestibility is addictive (Oliva-Teles, 2000), thus it is important to have an adequate knowledge of the apparent digestibility coefficients (ADC) of feedstuffs for adequate diet formulation. From various studies it was concluded that the ADC of protein and lipids are generally high, while those for energy are more variable, reflecting the quality and quantity of carbohydrates in the feedstuffs. According to Spyridakis *et al*. (1988) the ADC of some amino acids is different from the ADC of crude protein of the same feedstuff. Therefore, ADC of the essential amino acids should also be known for accurate feed formulation.

4.2. Lipids

Probably all vertebrates have requirements for n-3 and n-6 fatty acids, the main differences among species being the amount of fatty acids necessary to include in the diets and the bioconversion and elongation capability of fatty acids in a given species (Sargent *et al.,* 1999). In seabream juveniles, essential fatty acid requirements were estimated to be 0.9% HUFA (highly unsaturated fatty acids) in the diets, and best growth performances were obtained with an eicosapentaenoic to docosahexaenoic (EPA/DHA) ratio of 1:2 (Ibeas *et al*., 1997). To our knowledge, essential fatty acid requirements of sea bass are not yet known.

In seabream, Marais and Kissil (1979) observed the best growth with a 9% lipid diet, the lowest value tested (9–16%). Soybean oil was used as a lipid supplement, and this may have affected the results, as fish oil is a preferred lipid source for seabream. Using fish oil as lipid source, best growth performances was attained with 15–16% lipids in the diet. A further increase of dietary lipids to 21% did not improve growth, although feed efficiency was enhanced. In seabream fed high protein diets (58%), protein-sparing effect of lipids is less evident, as no growth differences were found in fish fed diets with 9 or 15% lipids Oliva-Teles (2000). No differences in growth rate of seabream with diets including 9% lipid – 55% protein or 17% lipid –46% protein when fed to satiety. Furthermore, while under restriction feeding no differences in protein retention were observed between diets, at the highest feeding level protein retention was lowest with the high lipid diet. When high fat diets are used, special care must be taken to avoid unwanted adiposity and impaired growth performance (Oliva-Teles, 2000).

Sea bream does not seem to use high dietary lipid levels as efficiently as salmonids. Moreover, although plant oils can be used up to a certain level to replace fish oil, adequate amounts of fish oil must be incorporated in the diets to cover the EFA requirements, as fish oils are the only dietary source of n-3 HUFA (Oliva-Teles, 2000). Growth and feed efficiency are higher in gilthead sea bream fed a diet containing 1% n-3 HUFA. Further elevation of the dietary n-3 HUFA level from 1% to 1.5% resulted in worse estimations of those parameters (Ibeas *et al.*, 1996). Thus the requirement of n-3 HUFA of gilthead seabream of 1-30 g weight is at least 1% of the dry diet.

The effect of dietary lipids level on carcass quality must also be considered, as high lipid diets generally induce higher fat depots and dietary oils source also influence patterns of fatty acid in the carcass (Oliva-Teles, 2000). As fish value for human consumption is related to its high muscle HUFA levels, which can be modified by diet manipulation, special care must be taken to guarantee a high quality of the final product. Moreover, changes in carcass quality induced by the use of high-energy diets should be evaluated taking into consideration the preferences of the consumers (Oliva-Teles, 2000). It is possible that lipid source or FA composition may regulate food intake through pre- or post-absorptive mechanisms, which may cause effects on palatability, digestibility and stimulation of neuroendocrine pathways (Morais, *et al*., 2006).

4.2.1. Soybean oil

Piedecausa *et al.* 2006 results indicate that fish oil can be successfully replaced with soybean or linseed oil in sharpsnout seabream diets for a period of time of 92 days without affecting fish growth or the nutritive utilization of the diet. These results are consistent with previous studies showing the viability of partially replacing dietary fish oil with soybean oil and linseed oil in salmonids, without a negative impact on animals' growth or feed nutritive utilization. Lower growth rate values were found after six months of feeding gilthead seabream diets with an 80% soybean or linseed oil content, although no significant differences were observed with 60% replacement levels (Piedecausa *et al.* 2006).

High survival rates were found in fish that had been fed fish oil (FO) and Soybean oil (SO) experimental diets, but somewhat lower rates for linseed oil (LO). In our experience, fish fed linseed oil appear to be more sensitive to stress conditions (such as handling during sampling) than those fed fish oil and soybean oil (Piedecausa *et al.* 2006)

At the morphological level, the livers from fish on the soybean oil diet had a less consistent texture and were easier to break up than those from fish fed the other diets.

According to Caballero *et al*. (2004), linseed oil does not alter hepatic morphology because its high proportion of 18:3 (n-3) would promote hepatic lipid oxidation, yielding hepatosomatic indices similar to those of fish fed fish oil-containing diet, whereas the high percentage of 18:2 (n-6) in soybean oil would promote lipid accumulation

4.3. Carbohydrates

Fish do not use carbohydrates as efficiently as mammals and birds, as fish have a limited capability to digest and metabolize carbohydrates. Besides differences among fish species, carbohydrate digestibility is affected by the complexity of the molecule, concentration in the diets, amount ingested and technological treatments applied to the carbohydrates. Amylolitic enzyme activity in carnivorous species such as seabream is lower than in omnivorous species. The ratio of amylolitic to proteolitic enzymes is higher in seabream than in rainbow trout (*Oncorhynchus mykiss*), and this may influence carbohydrate digestion capability of these species. Differences in the metabolism of glucose after a glucose tolerance test were also found between sea bass and seabream, although the time necessary to restore glucose levels was similar in both species (Oliva-Teles, 2000). Good results have been attained with 12,5% of gelatinized starch in the feed, when compared with a superior amount of 25% crude and gelatinized starch, proving that carbohydrates while it work, aren't efficient sources of energy in sea bream.

4.4. Vitamins and Minerals

Investigation on the vitamin and mineral requirements of seabream is required. Data on vitamin requirements of marine fishes is scarce, with the majority of the work being done on yellowtail (*Seriola quinquerradiata*). In seabream knowledge was limited to pyridoxine in 2000. A requirement of vitamin C for maximum growth was also demonstrated in seabream, athough not quantified (Oliva-Teles, 2000).

Available data on the mineral requirements of marine fish is also very scarce. Of all inorganic elements, phosphorus received special attention, not only because it usually has to be supplied in the diet, as its concentration in the water is low, but also because an excess of dietary phosphorus contributes to water pollution (Lall, 1991). Therefore, it is mandatory to minimize dietary phosphorus content by formulating diets with no phosphorus surplus above requirements for maximum growth and by using feedstuffs with high phosphorus availability.

In sea bream, preliminary results showed a dietary phosphorus requirement around 0.75%, a value similar to that of other marine fish species studied so far. Apparent phosphorus digestibility of several feedstuffs was determined in sea bass (Oliva-Teles, 2000). Bioavailability of phosphorus is highly variable among feedstuffs, and is higher in animal than in plant feedstuffs. This is due to the major proportion of phosphorus in plants being stored as phytate, which is not available to animals, as they lack the enzyme phytase. However, phosphorus availability in plant feedstuffs may be improved by the inclusion of phytase to the diets (Oliva-Teles, 2000).

5. Nutritional composition of proposed feed

Several studies have reported that a standard seabream diet containing 47% crude protein and 10% lipid should be used for maximum growth (Sadek et al. 2004)

Fish meal quality may vary greatly due both to the freshness of the raw material and to the processing conditions i.e., temperature. Protein digestibility, measured in mink has been shown to be a good indicator for differences in protein digestibility due to processing conditions and shows significantly reduced values with increased processing temperatures (Vergara *et al.* 1999) commercial diets available for gilthead seabream include a the range of 17 to 28% lipid. However, most of the recent studies on the nutrition of this species report dietary lipid levels around 12% or below (Vergara *et al.* 1999).

Nutritional composition of proposed feed is represented in table 1. Proposed fed was designed with a several amount of ingredients (table 2) based on least-cost principles, in solver, Microsoft Excel.

Table 1 – Nutritional composition proposed and optimal values found in literature:

Conteudo g/100g	Dieta	Valores óptimos em publicações	
Matéria Seca	92,6		
Proteína Bruta	44,0	45 - 50	(Oliva-teles, 2000)
Gordura Bruta	15,6	9 - 12	(Oliva-teles, 2000)
Fibra Bruta	1,1		
Cinzas	10,2		
Energia Bruta	18,0	aprox 20	(Sanches *et al,* 2003)
Energia Dig.	15,2	12 - 19	
Proteina Dig	39,7	38 - 40	
Met	1,1		
Met+Cys	4,0	4,4	
Lys	3,0	1,6 - 3,25	(Gomez, 2004)
Thr	1,8	0,5 - 1,1	(Gomez, 2004)
Arg	2,7	0,8 - 1,5	(Gomez, 2004)
HUFA:		0,9	(Oliva-teles, 2000)
C18:3 ω 3	0,6	0,5	
C20:5 ω 3	1,3	1	(Oliva-teles, 2000)
C22:6 ω 3	1,5	0,5	(Oliva-teles, 2000)
Amido	8,0	8	
Fish Origin	55,0		

6. Ingredient composition of proposed feed

The ingredients chosen for the feed to be used are as in shown in table 2.

Table 2 – Ingredients of proposed feed:

Matéria-Prima
Farinha de Peixe 62
Farinha de Peixe 68
Farinha de Peixe LT
CPSP G
Bagaço Soja 48
Trigo Forrageiro
Glúten Milho 60
Premix (Vit, Min, Aditivos)
Óleo Peixe
Óleo Soja
Metionina 99%
Sêmea de Trigo

7. Formulation based on least-cost principles

The following feed composition was achieved, using the least-cost principles, thus having the nutritional requirements of gilthead sea bream met at the lowest cost possible, in order to maximize profitability.

Table 3- Ingredient composition based on least-cost principles:

Matéria-Prima	Teor %
Farinha de Peixe 62	35
Farinha de Peixe 68	8,81
Farinha de Peixe LT	2
CPSP G	3,51
Bagaço Soja 48	16,18
Trigo Forrageiro	11,16
Glúten Milho 60	5,37
Premix (Vit, Min, Aditivos)	4
Óleo Peixe	5,7
Óleo Soja	4,5
Metionina 99%	2,49
Sêmea de Trigo	1,29

The least cost obtained was of 685 € / ton of feed.

8. Feeding additives of interest

Two additives of special interest are advised: probiotics and methionine (aa). Probiotics are usually defined as live microbial feed supplements, that are administered in such a way as to enter the gastrointestinal tract and to be kept alive; this beneficially affects the host animal by improving its intestinal microbial balance and in turn its health. Most frequently, probiotics are associated with lactic acid bacteria. This is due to the fact that these bacteria often produce bacteriocins and other chemical compounds that may inhibit the growth of pathogen bacteria (Carnevali *et al.* 2004). The main strategies in the use of probiotics are to isolate intestinal bacteria with favourable properties from mature animals and include large numbers of those bacteria in the feed for immature animals of the same species. Several bacteria have been used as probiotics in the larval culture of aquatic organisms and they can be either delivered directly into the water, or via live carriers such as *Artemia* nauplii and rotifers, or else added to pelleted dry feed (Carnevali *et al.* 2004).

Supplements of methionine advance the time of feeding and lengthen ingestion phases (Sánchez-Muros, 2003).

9. Farm feeding plan for a production cycle of a farm (intensive system)

The production target of this feeding plan is 100 Ton, starting with a fry (5g), the cultured specie is *Sparus aurata*, the Growth period are 17 months with a average temp. of 19 ºC. The water is available by pumping, being saturated with oxygen. The oxygen requirements are 0.2 g Oxygen / g Feed.

The expected growth rate in every month of the production cycle was calculated, as Instantaneous growth rate (g), Relative growth rate (RGR) and Thermal unit growth coefficient (TGC). The results of instantaneous growth rate and relative growth rate were quite similar.

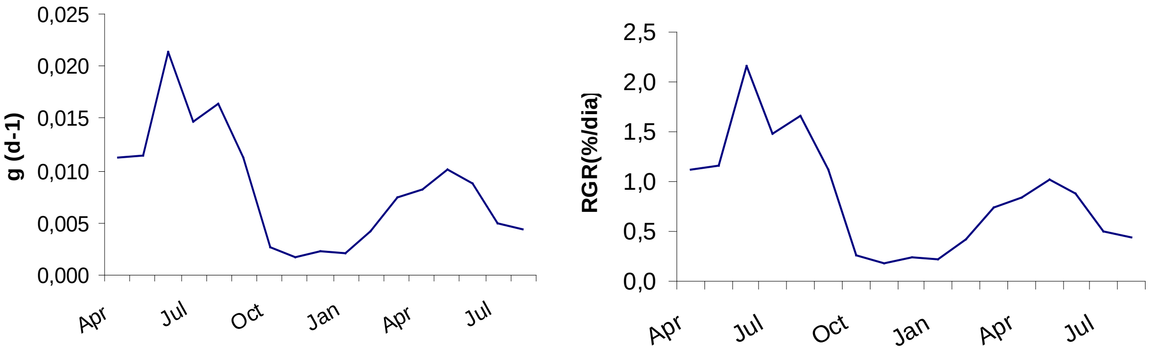

Fig 3 - Instantaneous growth rate (g) and Relative growth rate (RGR).

TGC values are not that linear, as when g and RGR go up the same happens to TGC. The lowest value of TGC is observed in the same month as the lowest values of g e RGR (November). The highest value differs from RGR and g. So, during late fall, winter spawning will occur, thus the observed decrease in growth rate, as energy is required for gonadal development. As nutrition significantly affects spawning quality, more research is needed on the nutritional requirements of broodstock (Oliva-Teles, 2000).

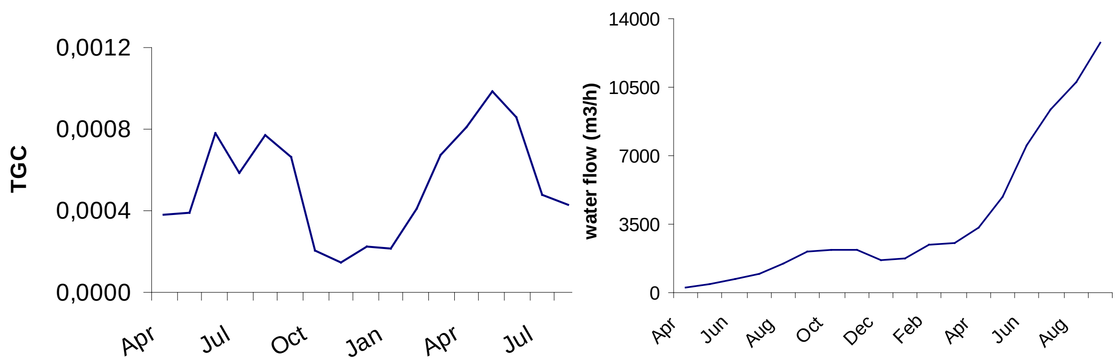

Fig.4 - Thermal unit growth coefficient (TGC) and Water flow.

With these graphics we can infer the variation of the feeding routine on a seasonal context. The feeding plan should have in consideration changes in the dissolved oxygen in water (to avoid excess oxidation).

According to Sánchez-Muros *et al.* (2003) gilthead sea bream preferentially feed during the afternoon and the evening (twice a day), and can increase food conversion and protein efficiency with demanded feeding. This study even goes on showing that the protein source has an influence in the time of feed. Optimum pellet characteristics can be obtained by extrusion, such as water stability, starch gelatinization and durability (Sadek et al. 2004).

Feed with dry pellets of known composition twice a day. The amount of food supplied should be 3% of body weight (BW) for fish of 1-10 g, 2% BW for 10-30 g and 1% BW for fish from 30 g onwards (Guinea

& Fernandez, 1997). The pellets should be fed manually ensuring they're distributed properly to all the fish in the tanks.

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