Feed transit and apparent protein, phosphorus and energy digestibility of practical feed ingredients by Senegalese sole (*Solea senegalensis*)

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Abstract

A study was conducted with Senegalese sole (*Solea senegalensis*) to determine the apparent digestibility coefficients (ADCs) of protein, phosphorus and energy in practical feed ingredients. The digestible energy (DE) content was also evaluated. Test ingredients were anchovy fishmeal, solvent-extracted soybean meal, corn gluten and wheat meal. Due to their low cohesion, sole faeces were collected directly in posterior intestine by dissection. The feed transit time along the gastrointestinal tract was assessed in Senegalese sole (body weight: 140 g) reared at 19ºC by means of a radiographic technique, following a single meal of a radio contrast agent, barium sulfate. Feed digesta accumulated in the posterior intestine 10-12 hours after the single meal and its total egestion was achieved at 24 hours following the meal. Dry matter digestibility of the various test ingredients was high (ranging from 88 to 93%), with lowest values being found for soybean meal and corn-gluten. Protein digestibility was high (above 91%) for fishmeal and corn gluten, intermediate for soybean meal (87%) and moderate for wheat meal (59%). Energy digestibility varied between 88 and 93% for soybean meal, corn gluten and anchovy fishmeal and was reduced in wheat meal (73%). The DE (MJ/kg) content varied from 16.2 for anchovy fishmeal, 15.1 for corn gluten, 13.5 for solvent-extracted soybean meal and 10.1 for wheat meal. Phosphorus digestibility was highest in fishmeal (58%) and greatly reduced in vegetable ingredients (28 to 33%). In general, our data shows that flatfish species, Senegalese sole, despite its high dietary protein requirement, digests vegetable ingredients relatively well, opening the opportunity for the development of practical feeds with high levels of plant-protein sources.

Keywords: Senegalese sole; apparent digestibility; ingredients; transit time; barium meal.

1. Introduction

The Mediterranean aquaculture industry has grown steadily over the last decades, but few marine fish species, namely gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*), and turbot (*Scophthalmus maximus*), have contributed to this growth. As a strategy to diversify and ensure sustainable growth, part of the industry has devoted great efforts to find new candidate species for Mediterranean aquaculture. Given its high price and market demand, Senegalese sole (*Solea senegalensis)* has long been recognized as promising new flatfish species for Mediterranean marine fish farming. Over recent years, major advances in the weaning techniques and larvae nutrition of sole have been accomplished (Dinis et al., 1999; Morais et al. 2006; Conceição et al., 2007) and contributed decisively for today's progressive establishment of large scale commercial farming of sole in Portugal and Spain. Progress has also been achieved in establishing the nutritional requirements of Senegalese sole juveniles (Conceição et al., 2008; Rema et al., 2008; Silva et al., 2008; Borges et al., 2009). The potential of using high levels of plant protein sources in sole diets is currently under investigation (Silva et al., 2009).

Knowledge about the ontogeny of the digestive system and its detailed characterization (gastric and intestinal pH changes over developmental stage, activity of digestive enzymes, gut morphology) is available for both sole larvae and juveniles (Arrelano et al., 1999; Ribeiro et al., 1999; Martínez et al., 1999; Conceição et al., 2007; Yúfera and Darías, 2007). Nevertheless, quantitative information on the digestion of major nutrients and energy from various feed ingredients is still nonexistent for this species. Selection of potential ingredients for feed formulation for any fish species requires knowledge of the apparent digestibility coefficients (ADCs) of energy yielding nutrients. Feed formulations incorporating such data allow a fine tuning of the dietary nutrient supply on a biological and economical basis. They are also of significant interest to reduce the emission of suspended solids associated with the undigested feed fraction and reduce the environmental burden of fish farming activities.

The collection of Senegalese sole faeces has proven a challenging task. Throughout our previous works with this species (Dias et al., 2004; Rema et al., 2008), we have tried to collect the faeces by means of a faeces settling column according to the Guelph system (Cho et al., 1982). However, the extremely low cohesion of faecal material, even when tested with diets containing high levels of alginates, made such methodology unreliable to assess the apparent digestibility of nutrients. In this study we have chosen to collect Senegalese sole faeces by dissection. We needed therefore, to evaluate the time course of faeces egestion to determine the exact sampling time.

The main objective of the present study was to determine the apparent digestibility coefficients (ADCs) of protein, phosphorus and energy and the digestible energy (DE) content of some commonly used feed ingredients by Senegalese sole. Given the methodological constraints associated to the faeces collection method by dissection, the gastrointestinal evacuation time of a given meal was estimated and the exact sampling time identified.

2. Material and Methods

2.1. Ingredients and experimental diets

Practical feed ingredients tested were anchovy fishmeal (FM), solvent-extracted soybean meal (SBM), corn gluten (CG) and wheat meal (WM). Their proximate composition, gross energy content and supplier are given in Table 1.

Recommended position of Table 1

Based on known nutritional requirements of Senegalese sole, a practical basal mixture was formulated (Table 2). This basal mixture had 1% chromic oxide (Cr_2O_3) incorporated as an inert digestibility marker. A reference diet consisted of 100% of the basal mixture (REF diet). Four additional test diets were subsequently produced by mixing 70% of the basal mixture and 30% of each test ingredient. One additional diet, identical to the REF diet but containing 2.5% of barium sulfate (at the expenses of wheat) as an opaque contrast medium for x-ray examination of feed transit along the gastrointestinal tract (diet BAR) was also manufactured. Ingredients were finely ground (<600 µm), mixed in a horizontal helix ribbon mixer (model Mano, 100 L capacity, CPM, San Francisco, USA) and pelleted dry without steam using a laboratory pellet press (CPM, C-300 model, San Francisco, USA) with a 4 mm die. The diets were dried at 37ºC for 24h in an oven and stored in a refrigerator until use.

Recommended position for Table 2.

2.2. Fish and rearing conditions

Experiments were directed by trained scientists (following FELASA category C recommendations) and were conducted according to the European Economic Community animal experimentation guidelines Directive of 24 November 1986 (86/609/EEC).

Trial 1 – Assessment of feed transit time

The assessment of feed transit time in sole was performed in 36 fish (average body weight of 140 g) stocked in a rectangular PVC tank (bottom area 0.2 m^2, water column 20 cm; volume: 40 L; water-flow rate: 3.6 $L \cdot min^{-1}$), supplied with recirculated seawater (18.7 ± 1.6ºC; salinity: 36.2 ± 1‰). A natural photoperiod cycle was adopted. Fish were fed *ad libitum* for 10 days with the experimental diet containing 2.5% of barium sulfate (BAR diet) to allow an adaptation period. Following a 12 hours starvation period, fish were then fed one single meal, in excess. One hour after feeding, the rearing tank was thoroughly clean to eliminate all remaining feed. Six fish were randomly sampled, anesthetized (150 $mg \cdot L^{-1}$ of phenoxyethanol) and x-rayed (Mini X-ray HF80, MinXRay Inc., Northbrook, USA) at 2, 4, 8, 12, 18 and 24 hours after the meal. After each sampling time, x-rayed fish were placed in a separate tank. Observation of radiographs was used to qualitatively assess the progress of feed digesta along the gastrointestinal tract and establish the time following the meal required to reach the posterior intestine.

Trial 2 - Apparent digestibility measurements

The apparent digestibility coefficients (ADC) of experimental diets were determined by the indirect method, using 1% chromic oxide as a dietary inert tracer. One hundred and twenty Senegalese sole (average weight: 180±23 g) were cultured at the CCMAR Experimental Research Station (Faro, Portugal). Five homogenous groups of 24 fish were allotted to rectangular raceway tanks (bottom area 0.3 m^2, water column 15 cm; volume: 45 L; water-flow rate: 4.2 $L \cdot min^{-1}$), supplied with recirculated seawater (19.1 ± 1.2ºC; salinity: 35.1‰). During a 2-week period fish were adapted to experimental conditions and test diets, which were supplied in excess once a day (09:00 h). On the sampling day, individual fish were forced-fed the diets at 2% body weight ration. Force-feeding was performed in anesthetized fish by means of inserting a flexible silicone tube filled with the feed pellets in the fish mouth, which were then gently pushed into the mouth. Adoption of the force-feeding approach is justified by the fact that data obtained in the feed transit time trial, clearly demonstrated that voluntary feed intake was highly variable in sole, with several fish showing no feed intake. Given that faeces collection was done by the dissection technique, we needed to ensure that all fish had eaten prior to their sampling. After being forced-fed, fish were returned to the rearing tanks. Regurgitation of feed pellets was minimal, but when occurred, the feed pellets were eliminated from the rearing tanks. Force-feeding of each experimental treatment was performed in separate days. For each experimental treatment, the 24 fish were force-fed in less than 10 minutes. Twelve hours after being force-fed (time established previously, as required for digesta to reach the posterior intestine), fish were killed by lethal anesthesia (2 g·L^{-1} of phenoxyethanol) and dissected. The posterior intestine (from the ileocaecal valve to the anus) was identified and its contents carefully collected into an aluminum container. No scrapping of intestinal walls was performed to minimize the contamination of faeces with epithelial cells. Faeces from 12 individual fish per treatment were pooled, generating thus two faeces samples for each dietary treatment. Pooled feces were frozen and freeze-dried prior to analysis.

The apparent digestibility coefficients (ADCs) of nutrients and energy for the reference and test diets were calculated according to Maynard et al. (1979) as follows:

$$ADC\ (\%) = 100 \times \left(1 - \frac{dietary\ Cr_2O_3\ level}{faeces\ Cr_2O_3\ level} \times \frac{faeces\ nutrient\ or\ energy\ level}{dietary\ nutrient\ or\ energy\ level} \right)$$

ADC of dry matter was calculated according to the following formula:

$$ADC\ (\%) = 100 \times \left(1 - \frac{dietary\ Cr_2O_3\ level}{faeces\ Cr_2O_3\ level} \right)$$

The apparent digestibility coefficients of the test ingredients were estimated as proposed by Forster (1999):

$$ADC\ ingredient\ (\%) = \frac{(ADC_{TEST\ diet} \times nutrient_{TEST\ diet}) - (0.7 \times ADC_{REF\ diet} \times nutrient_{REF\ diet})}{0.3 \times nutrient_{INGREDIENT}}$$

2.3. Analytical methods

Ingredients, experimental diets and freeze-dried faeces were finely ground prior to analysis. Analytics were performed according to the following procedures: dry matter by drying at 105°C for 24 h, ash by combustion in a muffle furnace (550 °C for 6 h), crude protein (N × 6.25) in feed samples was quantified by an automatic flash combustion technique followed by a gas chromatographic separation and thermal conductivity detection (LECO FP-528, Leco, St. Joseph, USA). Faeces nitrogen content was examined in three 1 mg subsamples using an elemental analyser (Thermoquest, mod. Flash 1112), using sulphanilamide as standard. Fat was determined after petroleum ether extraction (40-60ºC) by the Soxhlet method and gross energy in an adiabatic bomb calorimeter (Werke C2000, IKA). Organic matter was calculated by the difference 100 − (moisture + ash). Chromic oxide in the diets and faeces was determined according to Bolin et al. (1952), after perchloric acid digestion. Total phosphorus was digested using Parr teflon bombs (model nº 4782, Parr) following the method described by Reis et al. (2008). The phosphorus determination was performed by atomic absorption spectrometry (SpectrAA 220 FS, Varian) using a hollow cathode lamp (model nº. 5610126000, Varian) and an absorbance of 213.6 nm.

2.4. Statistical analysis

To test differences between dietary treatments, ADC data were subjected to a one-way analysis of variance (ANOVA) and when appropriate, means were compared by the Newman-Keuls multiple range test. Prior to ANOVA, ADC values were subjected to arcsin square root transformation. Statistical significance was tested at a 0.05

probability level. All statistical tests were performed using the STATGRAPHICS Centurion XV statistical package (Statgraphics Inc., Virginia, USA).

3. Results

The proximate composition and gross energy content of the selected feed ingredients are reported in Table 1. All experimental diets had moisture contents in the range of 8 to 10% (Table 2). Protein and gross energy levels in the experimental diets varied from 42 to 59% and from 20 to 22 MJ/kg, respectively and reflected well the protein and energy contents of the test ingredients incorporated at 30% level.

Feed transit time in Senegalese sole, assessed by visual observation of the X-rayed gastrointestinal tract of fish fed a 2.5% barium sulfate diet allowed us to qualitatively establish that under our experimental rearing conditions (fish body weight: 140 g; water temperature: 19ºC) feed digesta accumulated in the posterior intestine 12 hours after fish were group fed a single meal in excess (Table 3). At 18 hours after the meal, some fish already showed total voidance of faeces, and at 24 hours all sampled fish had egested the totality of the barium sulfate meal. It is worth remembering that our objective in the present study was to determine the time period following a single meal, required to accumulate faeces in the posterior intestine of fish. Such time period (12 hours after meal) was subsequently adopted for the collection of faeces by dissection in the digestibility trial.

Recommended position for Table 3.

The apparent digestibility coefficients (ADC) of experimental diets and test ingredients are reported in Table 4. In experimental diets, ADC of dry matter averaged 90% and was little affected by dietary treatments. Digestibility of organic matter varied between 91 and 94%, with highest values ($P<0.05$) found in fish fed the reference diet and the fishmeal test diet. Digestibility of protein was high in all groups (ranging from 92 to 97%), but ADC values for the wheat meal test diet were significantly lower ($P<0.05$) than those found in all other dietary treatments. Additionally, highest protein digestibility ($P<0.05$) was found in sole fed the fishmeal test diet. Phosphorus digestibility varied between 49 and 60%, while energy digestibility was higher than 92% in all experimental diets.

Recommended position for Table 4.

Regarding the test ingredients (Table 4), ADC of dry matter was higher for anchovy fishmeal (98.6%), corn gluten (98.7%) and wheat meal (96.4%) than for solvent-extracted soybean meal (86.9%). Digestibility of organic matter was high (> 97%) for all ingredients. Protein digestibility was highest (above 91%) for fishmeal and corn gluten, intermediate for soybean meal (87%) and moderate for wheat meal (59%). Energy digestibility ranged 88 to 93% in fishmeal, corn gluten and soybean meal, and reduced to 73% in wheat meal. Phosphorus digestibility was highest in fishmeal (58%), while considerably lower values (28 to 33%) were found in vegetable ingredients. Digestible energy values found in wheat meal and soybean meal (10.1 and 13.5 MJ/kg, respectively) were lower than those observed in corn gluten and fishmeal (15.1 and 16.2 MJ/kg, respectively).

4. Discussion

Over recent years, the long announced farming potential of Senegalese sole in Mediterranean aquaculture is finally materializing with the initiation of several industrial-scale productions. To establish the production of a new fish species on a commercial basis, the nutritional adequacy of feeds during the grow-out phase is a key element. In Senegalese sole juveniles, the dietary protein requirement for optimal growth was estimated at 53%, while the requirement for maximum protein accretion (N gain) was met by a diet containing 60% crude protein level (Rema et al., 2008). Estimations of the dietary requirements of essential amino acids are also available (Silva et al., 2008; Conceição et al., 2008). Juvenile Senegalese sole tend to show a low tolerance to high dietary lipid levels (Borges et al., 2009). Knowledge about the nocturnal feeding behavior (Boluda-Navarro et al., 2009) and ability to select macronutrients (Rubio et al., 2009) through the use of self-feeding devices also exists. Senegalese sole, similarly to what is observed with other flatfish species such as turbot, plaice and Atlantic halibut, present a high crude protein requirement for optimal growth, generally above 50% (Cowey et al., 1972; Hatlen et al., 2005; Rema et al., 2008). Under such circumstances, promoting the use of sustainable feed formulations, through the replacement of fishmeal by plant-protein sources is a necessity (Silva et al., 2009). However, to achieve such goal, data regarding the digestibility of nutrients and energy of these ingredients is required. Ingredients tested in the current study (anchovy fishmeal, solvent-extracted soybean meal, corn gluten and wheat meal) are common raw materials in most fish feeds. In the overall, the composition of test ingredients was in agreement with product specifications and in accordance with previously published data (Glencross et al., 2005; Tibbetts et al., 2006).

The accuracy of apparent digestibility measurement in fish relies heavily on the capacity to collect faeces samples, which should be representative of those voided at the time of normal defecation. Various techniques have been used to collect faeces in fish, including: intestinal dissection, stripping, metabolic chambers, anal suction, continuous filtration of outlet water and faeces settling column according to the Guelph system (Smith, 1971; Austreng, 1978; Windell et al., 1978; Cho and Slinger, 1979; Cho et al., 1982; Choubert et al., 1982; Hajen et al., 1993; Carter and Hauler, 2000). All faecal collection methods have been shown to have advantages and disadvantages particularly in relation to the ease of sample collection, fish stress and welfare and the representative nature of faeces collected, namely in terms of nutrient leaching and contamination with endogenous losses. Throughout our previous works with this species (Dias et al., 2004; Rema et al., 2008), we have tried to collect the faeces by means of a faeces settling column according to the Guelph system (Cho et al., 1982). However, the extremely low cohesion of faecal material, even when test diets contained high levels of alginates (up to 8%), made such methodology unreliable to assess the apparent digestibility of nutrients. Stripping is also extremely difficult in Senegalese sole given the multiple S-shaped of its long intestine (Yúfera and Darías, 2007). Given these constraints, the methodology chosen to collect faeces representative of a complete digestion process in Senegalese sole was to sample the digesta material at the posterior end of the intestine, by dissection. However, problems noted with obtaining digesta prior to its natural voidance as faeces by stripping, anal suction or dissection, is the need to handle fish, sometimes with anesthesia, which can *per si* affect intestinal transit (Spyridakis et al., 1989). Despite these disadvantages, stripping or dissection have been recognized as reliable methods of collecting fecal materials for some species, where faeces are loosely bound (Vens-Cappell, 1985; Førde-Skjærvik et al., 2006).

The main factors known to influence feed transit time are temperature, fish size, feed intake, feed formulation and composition (Fänge and Grove, 1979; Jobling, 1987). The majority of studies on this subject, report that the use of feeds containing high levels of indigestible nutrients, most available data is related to dietary fibres, lead not only to a substantial increase in faecal egestion volume, but also to a delayed feed evacuation time (Dias et al., 1998; Storebakken et al., 1999; Adamidou et al., 2009). Similar to what has been reported in most gastric evacuation time experiments (Rouhonen et al., 1997; Sveier et al., 1999), a single meal experimental protocol was followed in the present trial. Furthermore, it is generally recognized that while assessing feed evacuation rate, it is important to measure the meal size of each individual fish (Bromley, 1994). During group feeding, it is extremely difficult to monitor the amount of feed ingested by individual fish. This situation is particularly true for Senegalese sole, which is a bottom feeder with a very passive reaction to feed distribution. However, the results obtained by group feeding can be less biased than when fish are fed individually or forced fed (Bromley, 1994). This was confirmed by the present results as the voluntary feed intake, among the 36 fish used to evaluate feed transit time, was highly variable, with 4 fish showing no feed intake.

Data generated in the present study allowed us to assess for the first time the feed transit time in Senegalese sole. This assessment by means of a radiography technique of fish fed a 2.5% barium sulfate diet allowed us to establish that in Senegalese sole (body weight: 140 g) reared at 19ºC, feed digesta accumulates in the posterior intestine 10-12 hours after a single meal and its total egestion is achieved at 24 hours following that meal. Such information is novel to Senegalese sole, but should be taken as a qualitative data, since absolute values would probably be different in a more practical situation of multiple meals and would also be highly depend on water rearing temperature. Barium sulfate is frequently used clinically as a radio contrast agent for X-ray imaging and other diagnostic procedures. It is most often used in imaging of the gastrointestinal tract during what is colloquially known as a 'Barium meal' (O'Connor and Summers, 2007). To our knowledge such technique relying on barium sulfate has never been tested in fish for studying feed transit time. Earlier studies report the successful use of barium carbonate as an inert digestibility marker in rainbow trout feeds (Richie et al., 1995). Despite any detrimental effects on growth performance or nutrient digestibility, the incorporation of indigestible silicate minerals as bulk agents, namely zeolites, kaolin and bentonite, can modify the faecal egestion profile and increase feed transit time in several fish species such as rainbow trout and European seabass (Lanari et al., 1996; Dias et al., 1998). Their properties such as ion-binding or water-holding capacity may have a strong influence on solubility, gelling and viscosity of feed during its passage through the gastrointestinal tract. Furthermore, stomach pH may also influence these properties and consequently, species differences in response to dietary inert fillers should be expected. In this context, a sound validation of the current proposal to the use of a barium sulfate meal as a methodological tool to assess feed transit time is still needed.

Data regarding the apparent digestibility of nutrients and energy in practical feed ingredients was inexistent for Senegalese sole and quite scarce for flatfish species in general. Dry matter digestibility of the various test ingredients was generally high (ranging from 87 to 99%), with lowest values being found for soybean meal. This range of dry matter digestibility is relatively higher than values reported for various feed ingredients in Atlantic cod and turbot (Burel et al., 2000; Tibbetts et al., 2006). Digestibility of dry matter is generally reduced by increasing levels of indigestible substrates, namely dietary fibres (Tibbetts et al., 2006). Given that solvent-extracted soybean meal contains high fibre content (mainly non-starch polysaccharides) and the 30% inclusion level was performed on top of a reference diet which already contained about 15% of such ingredient, it seems logical to find a reduction on its dry matter digestibility. On the other hand, dry matter ADC of wheat meal in Senegalese sole was considerably higher than values reported on available literature data for other species (Tibbets et al., 2006). It is however, important to mention that the carbohydrate fraction in wheat meal contains high levels of starch. Data from a previous study suggests that sole juveniles have a good ability to utilize both raw and gelatinized dietary starches (Dias et al., 2004). In the present work, digestibility of organic matter was high (> 97%) among the various feed ingredients. Digestibility of dry matter and organic matter are important elements in estimating suspended solids wastes originated from undigested feed. These criteria could be of particular importance for Senegalese sole, since its commercial farming occurs mainly in water recirculated systems.

In Senegalese sole, protein digestibility was high (above 91%) for fishmeal and corn gluten, intermediate for soybean meal (87%) and moderate for wheat meal (59%). Such values are within the range or in some cases slightly superior to those reported for turbot (Burel et al., 2000), gilthead seabream (Lupatsch et al., 1997), European seabass (Gomes da Silva and Oliva-Teles, 1998) and Atlantic cod (Tibbetts et al., 2006). Energy digestibility among the various feed ingredients was found to vary between 88 and 93% for soybean meal, corn gluten and anchovy fishmeal, and slightly reduced in wheat meal (73%). The digestible energy value (DE, MJ/kg) of the various feed ingredients in Senegalese sole varied from 16.2 for anchovy fishmeal, 15.1 for corn gluten, 13.5 for solvent-extracted soybean meal and 10.1 for wheat meal, a range which is in accordance with data reported for Atlantic cod (Tibbetts et al., 2006).

Phosphorus digestibility was highest in fishmeal (58%) and greatly reduced in vegetable ingredients (28 to 33%). Such reduction of phosphorus digestibility in plant ingredients is a common feature in fish, since phosphorus is mainly present in its phytate-bound form, which is poorly available to fish (Debnath et al., 2005).

In general, our data shows that flatfish species, Senegalese sole, despite its high dietary protein requirement, digests vegetable ingredients relatively well. In a recent study, Yúfera and Darías (2007) reported that acid digestion and proteolysis in the stomach seems to be residual in the Senegalese sole (gastric pH never decreased below 6.0). The digestion occurred primarily in its long intestine in a slightly alkaline environment. Such digestion characteristics are generally found in fish species which present omnivorous feeding habits. Therefore, the development of practical feeds with high inclusion levels of plant-protein sources seems promising in Senegalese sole.

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Table 1. Proximate composition of test ingredients.

Ingredients	Anchovy fishmeal (FM)[1]	Soybean meal (SBM)[2]	Corn gluten (CG)[3]	Wheat meal (WM)[4]
Dry matter, DM (%)	88.7	88.2	90.5	87.9
Crude protein (%DM)	73.8	54.1	68.0	12.4
Crude fat (%DM)	8.2	2.9	2.2	1.4
Ash (%DM)	17.7	8.7	5.9	1.5
Total phosphorus (%DM)	2.57	0.72	0.22	0.32
Organic matter (%DM)[5]	82.3	91.3	94.1	98.5
Gross energy (MJ/kg DM)	19.6	17.3	18.6	15.8

[1] LT-Anchovy fishmeal (PESQUERA EXALMAR SA., Lima, Peru).

[2] Solvent-extracted soybean meal from Brazil (Bunge Iberica SA., Barcelona, Spain).

[3] Glutalys (Roquette, Lestrem, France).

[4] Wheat meal, European origin (Cargill France SAS, St. Germain-en-Laye, France).

[5] Calculated as 100 − (moisture + ash).

Table 2. Formulation and proximate composition of experimental diets.

	Basal mix				
LT-Anchovy fishmeal[1]	44.5				
CPSP 90[2]	2.5				
Squid meal[3]	5.0				
Soybean meal[1]	15.2				
Corn gluten meal[1]	9.0				
Wheat[1]	16.0				
Fish oil	6.5				
Vitamin-Mineral premix[4]	0.3				
Chromic oxide	1.0				
	Experimental diets				
	REF	Test FM	Test SBM	Test CGM	Test WM
Basal mix, %	100	70	70	70	70
Fishmeal, %		30			
Soybean meal, %			30		
Corn gluten meal, %				30	
Wheat meal, %					30
Proximate composition					
Dry matter (DM), (%)	91.1	90.8	90.4	91.4	90.6
Crude protein (%DM)	55.1	58.9	53.6	57.8	42.0
Crude fat (%DM)	13.5	10.9	9.9	9.5	9.7
Ash (%DM)	14.3	13.8	9.9	9.1	8.3
Phosphorus (%DM)	1.77	2.20	1.65	1.40	1.58
Organic matter (%DM)[5]	85.7	86.2	90.1	90.9	91.7
Gross energy (MJ/kg DM)	21.7	21.4	20.9	20.8	19.6
Chromic oxide (%DM)	1.21	0.93	0.91	0.92	0.92

[1] Please see details in Table 1.

[2] Fish soluble protein concentrate (83.4% protein; 13.2% fat, Sopropêche, France).

[3] Special Super Prime without guts (84.2% protein; 3.4% fat, Sopropêche, France).

[4] Vitamin and mineral premix according to Dias et al. (2009). Supplied by SORGAL S.A, Ovar, Portugal.

[5] Calculated as 100 − (moisture + ash).

Table 3. Number of fish showing the location[1] of the feed digesta along the gastrointestinal tract of sole following single meal of a 2.5% barium sulfate diet.

	Time after the meal (hr)					
	2	4	8	12	18	24
	Number of fish					
No feed intake	1	1			2	
Stomach (> 75% of meal)	5	1				
Anterior intestine (> 75% of meal)		3	1			
Mid intestine (> 75% of meal)		1	3			
Posterior intestine (> 75% of meal)			2	6	1	
Voided of faeces					3	6

[1] Location of feed digesta was assessed by visual analysis of 6 x-rayed fish per sampling time.

Table 4. Apparent digestibility coefficients (ADC %) of nutrients and energy of experimental diets and feed ingredients.

ADC Diets (%)	REF	Test FM	Test SBM	Test CGM	Test WM
Dry matter	88.8 ± 1.7	89.3 ± 0.1	88.1 ± 0.6	89.3 ± 0.4	88.7 ± 0.1
Organic matter	93.6 ± 0.9b	94.4 ± 0.1b	91.8 ± 0.3a	92.8 ± 0.4ab	91.3 ± 0.1a
Protein	94.5 ± 0.4b	96.8 ± 0.8c	94.2 ± 0.3b	94.6 ± 0.5b	92.4 ± 0.0a
Phosphorus	60.4 ± 0.0c	54.0 ± 1.3b	49.2 ± 2.1a	54.8 ± 1.1b	49.4 ± 1.5a
Energy	97.0 ± 0.5c	94.0 ± 0.0b	92.4 ± 0.4a	94.5 ± 0.4b	92.6 ± 0.1a

ADC Ingredients (%)	FM	SBM	CGM	WM
Dry matter	98.6	86.9	98.7	96.4
Organic matter	99.8	97.6	99.3	99.8
Protein	94.4	86.7	90.6	58.6
Phosphorus	58.1	27.6	32.8	31.2
Energy	92.9	88.3	89.9	72.6
DE (MJ/kg)	16.2	13.5	15.1	10.1

Values are means ± standard deviation (n=2). Means in rows without a common superscript letter differ significantly (P<0.05).
Absence of superscript indicates no significant difference between treatments.