Soybean meal as a protein source in gilthead sea bream (*Sparus aurata* L.) diets: Effects on growth and nutrient utilisation.

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Abstract

The value of defatted soybean meal as a protein source for sea bream fingerlings (15.2 ± 4.4 g on average) growing to market size (300-350 g), was evaluated by feeding extruded isonitrogenous and isoenergetic diets (46% protein and 22 MJ kg^{-1}) containing 20, 30, 40 and 50% soybean meal considering two phases. On day 87, the fish weight was ranged among 66 and 81 g. Specific growth rate (SGR) of sea bream fed 50% soybean was lower (1.73 % d^{-1}) that of fish fed 20% (1.87 % d^{-1}) and 30% (1.93 % d^{-1}), but food conversion ratio (FCR) was not significantly affected, and a quadratic significant trend was observed for the feed intake (FI) in relation to dietary soybean level. At the end of second phase on day 309, fish weight was in the interval 303 and 349 g, but specific growth rate and food conversion ratio were similar for all diets, and ranged between 0.64 and 0.69 % d^{-1}, and 1.95 and 2.10 % d^{-1} respectively. Final biometric parameters were not affected by the diets, although levels of some free amino

acids in the muscle were affected. Sensory differences were detected by panellists in fish fed diet 20% as compared those fed diet 50%, which had a less marine flavour and juicy. The global growth results suggest the possibility of feeding sea bream weighing less than 80 g with 30% soybean meal, and for fish weighing more than 80 g, a 50% dietary soybean meal can be used until the fish reach commercial weight, with no negative effects on growth or feed efficiency. Nevertheless, when sensory analysis and economic aspects are considered, the maximum inclusion level of soybean was 20-22%.

Keywords: Sparids, fish nutrition, protein sources, feed formulation, free amino acids, sensory analysis, economic analysis.

Introduction

Fish meal is currently a major protein source in fish feeds, but it is a finite resource. During the past 15 years, major steps have been taken to reduce fish meal and fish oil in aqua-feeds by incorporating other animal and plant protein and lipid sources.

Alternative protein sources in feeds for gilthead sea bream (*Sparus aurata* L.), have been included, corn gluten (Robaina *et al*., 1997; Pereira & Oliva-Teles, 2003; Venou *et al*., 2003), lupin seed meal (Robaina *et al*., 1995; Pereira & Oliva-Teles, 2004), extruded peas (Pereira & Oliva-Teles, 2002), whole wheat (Gómez-Requeni *et al*., 2003), rapeseed meal (Gómez-Requeni *et al*., 2004), rapeseed protein concentrate (Kissil *et al.*, 1997, 2000), soybean meal (Lupatsch *et al*., 1997; Robaina, 1998; Isani *et al*., 2000; Ceulemans *et al*., 2003) and soybean protein concentrate (Kissil *et al*., 2000) has been the dietary replacement most widely studied. Soybean meal is a good alternative to fish meal given its high protein content, balanced amino acid profile and widespread availability, although contain some antinutritional substances (Alarcón *et al.*, 1999, 2002).

The present experiment was conducted to determine the maximum soybean meal level in extruded diets for gilthead sea bream and to describe changes in the sensorial characteristics and free amino acid levels in sea bream fillets.

Materials and Methods

Rearing system

The trial lasted 309 days (from May 2001 to March 2002) and was conducted in 8 cylindrical fiber glass tanks (1500 l) within a recirculating saltwater system (30 m^3 capacity) with a rotary mechanical filter and a 36 m^3 capacity gravity biofilter. All tanks were equipped with aeration, and the water was heated with a heat pump installed in the system. Water temperature was 22.5 ± 1.2 ºC, salinity was 33 ± 1 g l^{-1}, dissolved oxygen was 6.5 ± 0.5 mg l^{-1} and pH ranged from 7.5 to 8.5. The photoperiod was natural and all tanks had similar lighting conditions.

Fish

Sea bream fingerlings, obtained from a fish farm in Gandia (Valencia, Spain) were hold in groups of 160 in each tank. After a one month adaptation period, the experiment began with fish weighing 15.2 ± 4.4 g.

Diets and feeding

Four isonitrogenous and isoenergetic diets (46% protein and 22 MJ kg^{-1}) were formulated using commercial ingredients, such that deffated soybean was included at 200, 300 400 and 500 g Kg^{-1} (Table 1). Some essential amino acids decreased in diets according extracted soybean meal inclusion, such as lysine, which fluctuated between 5.2 and 4.49 and methionine ranged between 5.37 and 3.71 for diets 20 and 50, respectively (Table 1), and others as arginine or phenylalanine increased.

Diets were prepared as 2, 3 and 4 mm pellets by cooking-extrusion with a semi-industrial twin-screw extruder (CLEXTRAL BC-45). The processing conditions were as follows: 100 rpm screw speed, 110 ºC and 30-40 atm. Each experimental diet was assayed in two tanks, randomly assigned.

Fish were hand-fed twice a day (09.00 and 16.00) to apparent satiation and pellets were distributed slowly permitting all fish to eat. All fish were weighed individually every 30 days, using clove oil as an anaesthetic (40 ppm), to determine growth parameters. Two phases were considered, the first to day 87 and an approximate fish weight of 80 g, and a second from day 87 until the end of trial on day 309. At the end of each phase, ten fish per tank were sacrificed using clove oil to determine biometric parameters, and were stored at -30 ºC for analysing muscle and body composition and for determining sensory characteristics.

Growth, nutrient utilisation, biometric and economic indexes considered were as following:

Weight gain (%), WG = (final weight - initial weight) / initial weight

Specific growth rate (% day^{-1}), SGR = 100 x ln (final weight / initial weight) / days

Feed intake (g 100 g $fish^{-1}$ day^{-1}), FI = 100 x feed consumption (g) / average biomass (g) x days

Protein efficiency ratio, PER = weight gain (g) / protein offered (g)

Feed conversion ratio, FCR = feed offered (g) / weight gain (g)

Condition factor, CF = 100 x total weight (g) / total length ^{3} (cm)

Viscerosomatic index (%), VSI = 100 x visceral weight (g) / fish weight (g)

Hepatosomatic index (%), HSI = 100 x liver weight (g) / fish weight (g)

Mesenteric fat (%), MF = 100 x mesenteric fat weight (g) / fish weight (g)

Dressout percentage (%), DP= 100 x[total fish weight-visceral weight-head weight (g)]/fish weight (g)

Meat index (%) = 100 x meat weight (g) / fish weight (g)

Gross Energy Efficiency (%), GEE = Fish energy gain (kJ) x 100/ Energy intake (kJ)

Crude Protein Efficiency (%), CPE = Fish protein gain (g) x 100/ Protein intake (g)

Economic efficiency ratio (€ kg^{-1}), ECR = feed offered (kg) x feed cost (€ kg^{-1}) / weight gain (kg)

Economic profit index (€ $fish^{-1}$), EPI= final weight (kg $fish^{-1}$) x fish sale price (€ kg^{-1}) – ECR (€ kg $fish^{-1}$) x weight increase (kg). Sea bream sale price is calculated at 4.5 € kg^{-1}.

Chemical analysis

Chemical composition of diets, muscle and body fish were analysed following AOAC (1990) procedures: dry matter (105 ºC to constant weight), ash (550 ºC to constant weight), crude protein (N x 6.25) by the Kjeldahl method after acid digestion (Kjeltec

2300 Auto Analyser, Tecator), crude lipid by hydrolysis with 6 N HCl prior to diethylether extraction (Soxtec 1043 extraction unit, Tecator) and crude fiber by acid and basic digestion (Fibertec System M, 1020 Hot Extractor, Tecator). All analyses were performed in triplicate. The method described by Liu *et al.* (1995) was used for analysis of total amino acids in a Waters HPLC system. Approximately 60 and 50 mg of fish muscle and diets, respectively, were hydrolysed with 50 ml 6N HCl with 0.5% phenol at 115 ºC for 24 h. Aminobutiric acid was added as an internal standard before hydrolysation. The amino acids were derivatizated with AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate). Methionine and cysteine were determined separately as methionine sulfone and cysteic acid after oxidation with performic acid. The amino acids were separated by HPLC with a C-18 reverse-phase column Waters Acc. Tag (150 mm x 3.9 mm).

Free amino acids (FAA) were determined using 4 g homogenized muscle. The samples were pre-extracted with 0.01N HCl and deproteinized with acetonitrile as described Aristoy and Toldrá (1991).

Sensory analysis

The effect of diet on sensorial properties of fish fillets was studied by comparing fish fed diet 20% to fish fed diet 50%. As specified in the ISO-4120:1983 norm, the triangle test was performed in a total of seven sessions with four panellists, two men and two women, trained as ISO-8586-1:1993 norm determinates.

One fish of the group fed diet 20% and one from group 50% soybean was used in each session. Fish were thawed at 4 ºC for 24 h, and were then filleted and skinned.

The two fillets from each fish were vacuum-packed in plastic bags. Each fillet was cooked in a water-bath at 80 ºC for 10 min and was then cut into six pieces weighing 3-4 g each. The resulting 24 equally-sized pieces were coded with a three-digit number and wrapped in aluminium foil. The pieces were organised for 8 triangle tests per session (two for each panellist) and were stored at 40 ºC in thermo-regulated boxes for the duration of the sessions. Samples from the same portion were compared in each test to ensure that any registered differences could not be attributed to the fillet portion.

As a difference, one piece should be identified in each test and the judges described the characteristic of the difference (marine flavour, texture and juicy), but the comments were recorded in case of correct differentiation.

In a triangle test, the assumption of "no difference" between treatments is rejected if the number of correct responses is greater than or equal to the critical value or a standard normal value ($z_{\alpha} = t_{\alpha,\infty}$). Tabled values are provided in the ISO-4120:1983 norm.

Statistical analysis

Statistical analyses of data were carried out with SAS (Statistical Analysis System Institute, 1990). Data from the evolution of weight, feed intake (FI), protein efficiency ratio (PER) and specific growth ratio (SGR) were analysed using a mixed procedure and following a repeated measures design to take into account the variation between fish and the covariation among them. Data were not transformed. Covariance structures of mixed procedure were objectively compared using the strictest criteria (Schwarz Bayesian criterion) as suggested by Littell *et al.* (1998). Data from body composition, biometric parameters, food conversion ratio (FCR), economic conversion ratio (ECR) and economic profit index (EPI) were subjected to variance analysis following the general lineal model procedure. Each group in the calculation represented the combined group of fish per single tank (duplicate tanks per treatment). The p-value for statistical significance was 0.05. Descriptive statistics are mean ± SE unless otherwise stated.

In addition, final weight was assessed by multiple linear regression analysis using the substitution level of fish meal by soybean meal, the number of days of trial, the initial weight and feed intake ratio are considered independent variables.

The feed intake (FI) was also assessed by multiple regression analysis using weight and soybean meal level as independent variables. Likewise, the economic profit index (EPI) was assessed by polynomial regression with dietary soybean level as the independent variable.

Results

Differences in fish weight (analysed with repeated measures) were observed at the end of the two phases (Table 2). On day 87 of the experiment, the weight of sea bream fed the 50% soybean diet was lower (66 g) than that of fish fed the 20% diet (77 g) and the 30% diet (80 g), but in fish fed the 40% soybean were no different from those in other treatments. Weight differences were more pronounced at the end of the experiment, and the highest weight was observed in fish fed diet 20 and 30 (349 g and

339 g, respectively). These fish were significantly heavier than fish fed diets 40 (324 g) and 50 (303 g).

On first phase of the trial, weight gain and specific growth rate (SGR) were significant lower in fish fed diet 50 than in those of fish fed diets 20 and 30, following the same trend than weight, but on second phase these indexes did not present statistical differences, therefore the growth of sea bream up 80 g was similar for all diets.

Considering the growth recorded in all samples of the first phase, a significant multiple linear regression model was obtained (Equation 1) to describe the relationship between weight and the level of soybean meal using the variables: days of trial (t), daily feed intake (FI), and soybean meal level (SL).

$$\text{Weight} = 14.12\ (\pm 4.25) - 3.99\ (\pm 2.51) \times \text{FI} + 1.10\ (\pm 0.42) \times \text{SL} - 0.018\ (\pm 0.004) \times \text{SL}^2 + 0.66(\pm 0.06) \times t$$

$(r^2 = 97.26\%)$ (Equation1)

Feed intake (FI) and food conversion ratio (FCR) in the first phase indicated no significant statistical differences between groups, although a significant multiple trend (Equation 2) was observed for FI in regards to the level of soybean meal (SL) and fish weight (W).

$$\text{FI} = 3.40\ (\pm 0.46) - 0.031\ (\pm 0.0021) \times \text{W} + 0.096\ (\pm 0.02) \times \text{SL} - 0.0012\ (\pm 0.0003) \times \text{SL}^2$$

$(r^2 = 88\%)$ (Equation 2)

Examining the second phase of the experiment (from day 87 to day 309), no differences or trends for FI or FCR were detected.

Whole body composition and biometric parameters at the end of the trial were not significantly different (Table 3), therefore soybean inclusion did not affect these variables.

Data of muscle composition reflect no differences as summarised in Table 4. The total amino acid composition of the muscle at the end of the trial was analysed, but it was not affected by the soybean level.

In the sensorial analysis, 27 of the 56 answers correctly identified the different sample, and as a consequence, the α-risk is between 5 and 1%, strong evidence that a difference is apparent (Meilgaard *et al*., 1999) between fish fed diet 20 and fish fed diet 50. Panellists described fish fed diet 50 as having less marine flavour being less juicy and having a firmer texture than those fed diet 20.

The free amino acids in the muscle of sea bream fed diets 20 and 50 at the end of the trial are shown in Table 5 (expressed as μmole g^{-1} wet weight) and the content of alanine, lysine, and proline of fish fed diet 20 was significantly higher (3.47, 2.17 and 1.59, respectively) than that of fish fed diet 50 (2.47, 0.69 and 1.14, respectively). The content of muscle glycine in fish fed diet 50 was significantly higher (8.45) than that of fish fed diet 20 (6.13). After cooking the fillets, the content of free amino acids increased considerably but significant differences were only observed in the proline content, which was higher in fish fed diet 20 (5.6 μmole g^{-1}) than in those fed diet 50 (5.0 μmole g^{-1}).

The economic parameters were evaluated (Table 6). The cost of diets was reduced with soybean replacement, but the economic conversion ratio (ECR) of the two growth phases considered was similar among groups. A "new economic profit index" (EPI) was developed to evaluate the economic viability of diets, considering both fish sale price and cost of diets, but no significant differences were observed, although EPI showed a significant trend (Equation 3) with dietary soybean meal level (SL).

$$EPI = 1.21\ (\pm\ 0.12) + 0.007\ (\pm\ 4 \times 10^{-3}) \times SL - 0.00016\ (\pm\ 9 \times 10^{-5}) \times SL^2$$

$$(r^2 = 75.01\%) \qquad \text{(Equation 3)}$$

Discussion

The results of the present experiment show that the inclusion of extracted soybean meal in diets for gilthead sea bream can vary with fish weight. In the first phase (day 87), 30% soybean meal seems the maximum inclusion level in gilthead sea bream diets without affecting growth, upper levels (40 and 50%) resulted in significantly lower final body weight and SGR (Table 2).

The effect of dietary soybean level on fish growth reduction in the first phase is corroborated in Equation 1 of the present study, which indicates a quadratic relationship of sea bream growth and soybean meal level, therefore a light increase of soybean (from 20 to 30%) improve the growth, but a major inclusion (40 and 50 %) has a negative effect on growth. When Equation 1 is derived and equalized to zero, it is possible to obtain the dietary soybean meal level, around 30.5%, for optimum sea bream fingerling growth.

$$d\,W / d\,SL = 1.1 - 2 \times 0.018\ SL = 0 \quad \text{and} \quad SL = 1.1/0.036 = 30.5\ \%$$

These results agreed with Ceulemans *et al.* (2003), who obtaining higher growth in fish weighting 47 g fed 27% soybean than in those fed 41 and 47%, but growth was similar in fish fed diet 38% supplemented with an amino acid premix. Gómez-Requeni *et al.* (2003) did not find growth differences in 14 g sea bream fed 12% and 30% dietary soybean meal. On the contrary, other authors such as Robaina *et al.* (1995) suggested that the maximum level of soybean meal in diets for juvenile sea bream was 20%.

Likewise, Kissil *et al.* (2000) noted a decrease in sea bream growth when the fish fed diets 20, 40 and 75% soybean meal. In similar experiments, Kissil & Lupatsch (2002) reported less fish growth in fish fed 80% soy protein concentrate than in fish fed with a control diet without soy protein, but when fish meal was completely replaced by a plant material mixture, the results were excellent. The fish meal substitution by other plant proteins, such as corn gluten (Robaina *et al.*, 1995) and also by plant protein mixtures (Vega-Rubin de Celis *et al.*, 2004) provided similar results to those of the present study.

Nevertheless, considering the second phase of trial, fish growth was not affected by dietary soybean meal level, because SGR and weight gain were not different (Table 2), and differences on final weight at day 309 were due to the accumulative effect from first phase. So it would be feasible to feed 70 g sea bream with diets containing soybean dietary levels between 40-50%. The results obtained in the second phase of the experiment partially agreed with Ceulemans *et al*. (2003), who tested three diets containing soybean levels of 27%, 38% and 47% in 170 g sea bream, finding no differences in SGR of fish fed experimental diets, although the 38% soybean meal diet was supplemented with amino acids. These inclusion levels of a single protein source could be increased using a mixture of plant proteins, Francesco *et al.* (2004) and Kissil & Lupatsch (2002) found no growth differences when fish meal was substituted with 75% plant proteins.

Although the variance analysis of feed intake (FI) in the first phase of the trial did not identify significant differences between treatments, a cuadratic regression with soybean levels was observed in the first phase of the experiment, which show that juvenile sea bream increased the FI related to inclusion level of dietary soybean. This results does not agree with results obtained by Robaina *et al.* (1995), Ceulemans *et al.* (2003) and Gómez-Requeni *et al*. (2003) who all reported no differences. These results contrast with those cited by Gómez–Requeni *et al.* (2004), who reported that the incorporation of plant proteins led to a progressive reduction in the voluntary feed intake of juvenile gilthead sea bream. Kissil *et al.* (2000) reported less palatability in diets which had high soy protein concentrate, which does not agree with findings of the present experiment. Then the lower growth of fish fed diet 50 was not caused by poor feed intake, but was caused by a lower efficiency, which agree wit Kissil *et al*. (2000) and Gómez-Requeni *et al*. (2003), who proved that protein retention decreased with high substitution levels of fish meal by other plant protein sources, possibly reflecting a deficiency in essential amino acids. One possible explanation is an amino acid deficiency, causing an increase in the energy cost when increasing the losses for nitrogenous excretion (Cho, 1987), since protein synthesis is efficient if amino acids in the precursor pool are proportionally correct. In the second phase, FI was similar for all experimental diets as well as in terms of protein and energy retention.

The body composition of sea bream was not affected by the dietary soybean level as most authors have reported (Robaina *et al*., 1995), although Kissil *et al.* (2000) cited a lower lipid content in fish fed a diet higher in dietary plant protein, but this effect could be caused by reduced growth. Fillet protein and lipid content, and also amino acid composition, were not affected by the dietary soybean meal level and a similar body content was cited by Grigorakis *et al*. (2003) in 400 g commercial size sea bream.

The effect of plant ingredients on sensory characteristics of sea bream has only been evaluated by Francesco *et al.* (2004), who described the effect of a plant protein mixture on sensorial properties, but they cited no differences. Sensory differences observed in the present trial could not result from fillet composition, since all parameters were similar for fish fed diets of 20% and 50% soybean level, but the free amino acids may be responsible for the characteristic taste of seafood (Fuke, 1994). In the present trial, the soybean dietary affected the level of some free amino acids (FAAs) in sea bream muscle; alanine, lysine, and proline content of fish fed diet 20 were higher than fish fed diet 50, while the opposite occurred for glycine.

Carter *et al.* (2000) suggested that the indispensable amino acid profile of the diet is correlated with the free pool indispensable amino acids of white muscle, and a low concentration of one EAA in white muscle can indicate a limited efficiency for protein synthesis. Present results partially agree with those obtained by Gómez–Requeni *et al.* (2003, 2004), who noted that a reduction of indispensable amino acids, such as lysine, in experimental diets produced a lower level in muscle. These results are similar to those reported by Yamamoto *et al.* (2002), who replaced all fish meal in diets for rainbow trout, and observed that fish fed diets based on fish meal had a higher lysine content than fish fed soybean meal diets.

The final objective of aquaculture farms is to obtain an economic profitability, then the economic profit index (EPI) is a good parameter to evaluate the diets, because it considered the effect of technical indexes as growth and food conversion, and the economic variables, market price of fish and cost of feed. An inverse quadratic trend was detected in the economic profit index, obtaining at the end of trial, related to soybean inclusion, which is showed in Equation 3. Deriving and equalizing at zero this equation, the optimum economic soybean level is 22 %.

$$d\,EPI / d\,SL = 0.007 - 2x0.00016\,SL = 0 \quad \text{and} \quad SL = 0.007/0.00032 = 21.9\ \%$$

Conclusion

From a nutritional point of view, the dietary soybean meal level can be increased until 30% for juveniles and 50% for growing without affecting growth. Nevertheless, the use of high levels of soybean meal in diets for gilthead sea bream does not improve the economic profitability considering the actual soybean and fish meal market price. The optimum economic soybean level was 22%.

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Table 1

Ingredient content and proximate composition of experimental diets.

	Diet			
Ingredients (g kg $^{-1}$)	20	30	40	50
Fish meal, herring (5-02-000)	370	312	254	195
Gluten meal (5-28-241)	40	40	40	40
Extracted soybean (5-04-604)	200	300	400	500
Wheat meal (4-05-268)	218	175	131	88
Wheat gluten	40	40	40	40
Fish oil (7-08-048)	124	125	127	129
Vitamin-Mineral Mix[a]	8	8	8	8
Analysed composition (% dry weight)				
Dry Matter (DM)	92.0	91.9	91.6	92.9
Crude protein (CP)	45.6	46.1	46.5	46.3
Crude lipid (CL)	13.7	15.9	15.8	14.5
N-free extract (NFE)	29.8	26.9	26.7	28.5
Ash	8.5	8.3	7.8	7.1
Arginine (% CP)	7.66	7.82	7.87	8.50
Lysine (% CP)	5.20	5.07	4.79	4.49
Methionine (% CP)	5.37	5.33	4.07	3.71
Phenylalanine (% CP)	5.63	5.78	6.07	6.76
Calculated values				
Crude fiber (% dw)	2.4	2.8	3.2	3.6
GE (MJ kg^{-1}) [b]	21.6	22.1	22.1	21.9
CP/GE (g MJ^{-1}) [b]	21.3	21.1	21.2	21.4

[a] Vitamin and mineral mix (values are g kg^{-1} except those in parenthesis): Premix: 25; Choline, 10; DL-α-tocopherol, 5; ascorbic acid, 5; $(PO_4)_2Ca_3$, 5. Premix composition: retinol acetate, 1000000 IU kg^{-1}; calciferol, 500 IU k^{-1}; DL-α-tocopherol, 10; menadione sodium bisulphite, 0.8; thiamin hydrochloride, 2.3; riboflavin, 2.3; pyridoxine hydrochloride, 15; cyanocobalamin, 25; nicotinamide, 15; pantothenic acid, 6; folic acid, 0.65; biotin, 0.07; ascorbic acid, 75; inositol, 15; betaine, 100; polypeptids, 12; Zn, 5; Se, 0.02; I, 0,5; Fe, 0.2; CuO, 15; Mg, 5.75; Co, 0.02; Met, 1.2; Cys, 0.8; Lys, 1.3; Arg, 0.6; Phe, 0.4; Trcp, 0.7; excpt. 1000 g (Dibaq-Diproteg).

[b] Calculated using: 23.9 kJ g^{-1} protein, 39.8 kJ g^{-1} lipid and 17.6 kJ g^{-1} carbohydrate.

Table 2

Effect of soybean meal inclusion on growth and nutrient utilisation of gilthead sea bream in the two phases of trial

Parameter	Diet				
	20	30	40	50	SEM
First phase (0-87 days)					
Final weight	77.9^{b}	80.7^{b}	73.2^{ab}	65.9^{a}	4.38
Weight gain (%)	424^{a}	440^{a}	373^{ab}	317^{b}	21.9
SGR (% day^{-1})	1.87^{b}	1.93^{b}	1.84^{ba}	1.73^{a}	0.08
FI (g 100 g $fish^{-1}$ day^{-1})	2.51	2.73	2.89	2.90	0.13
PER	1.09	0.99	0.90	0.84	0.06
FCR	1.70	1.86	2.02	2.15	0.07
Second phase (87-309 days)					
Final weight	349.4^{c}	339.0^{c}	324.1^{b}	303.0^{a}	4.38
Weight gain (%)	351	321	343	359	18,2
SGR (% day^{-1})	0.69	0.68	0.66	0.64	0.02
FI (g 100 g $fish^{-1}$ day^{-1})	0.97	0.99	1.04	0.97	0.08
PER	1.04	1.12	1.04	1.11	0.04
FCR	2.10	1.94	2.06	1.95	0.07

Data in the same row with different superscripts differ at $P < 0.05$. Covariate initial weight: SGR.

Table 3

Effect of soybean level on biometric parameters, whole body composition and nutrient retention of gilthead sea bream at the end of the trial (day 309).

Parameter	Diet				
	20	30	40	50	SEM
CF	1.8	2.0	1.9	2.0	0.10
VSI	6.5	6.3	6.6	6.2	0.35
HSI	1.2	1.1	1.1	1.0	0.08
MF	2.5	2.6	2.6	2.1	0.25
DP	75.7	75.5	75.7	75.7	0.56
Meat index	44.5	43.7	44.5	43.5	0.85
Moisture (%)	63.4	64.7	65.0	66.36	0.95
CP (% wm)	17.8	17.1	17.4	17.7	0.25
CL (% wm)	20.6	20.4	20.0	20.0	0.53
Ash (% wm)	3.3	3.3	3.4	2.5	0.26
CE (MJ kg^{-1})	12.3	12.1	12.0	12.1	0.81
GEE (%)	25.8	23.8	22.8	23.1	1.95
CPE (%)	19.6	18.9	19.0	19.8	1.33

Data in the same row with different superscripts differ at $P < 0.05$.

Table 4

Effect of soybean level on gilthead sea bream muscle composition at the end of the trial (day 309).

Parameter	Diet				
	20	30	40	50	SEM
Moisture (%)	70.7	70.6	70.7	72.0	0.58
CP (% wm)	20.1	20.3	20.4	20.0	0.16
CL (% wm)	7.9	8.2	6.5	6.7	0.63
Ash (% wm)	1.3	1.3	1.3	1.3	0.02
CE (MJ kg^{-1})	7.9	8.0	7.4	7.4	0.24
Total amino acid composition mg g^{-1} wm					
Essential amino acids					
Arginine	15.46	14.83	16.58	18.04	1.72
Histidine	6.22	5.71	6.38	6.47	0.46
Isoleucine	8.76	8.34	8.68	9.16	0.51
Leucine	13.93	13.25	14.24	14.53	0.69
Lysine	13.85	13.96	14.56	13.49	0.68
Methionine	13.98	12.17	14.59	14.11	3.78
Phenylalanine	9.36	9.74	9.55	9.61	0.21
Threonine	8.19	7.70	8.64	8.81	0.66
Valine	9.25	9.24	9.54	9.41	0.19
Non essential amino acids					
Alanine	9.51	9.56	11.43	9.33	1.50
Aspartate	15.49	14.80	16.66	14.14	1.34
Cysteine	7.96	12.29	6.62	7.86	3.34
Glutamine	22.44	21.50	24.51	24.55	2.15
Glycine	8.84	8.83	11.64	9.10	1.68
Proline	6.94	8.71	8.66	6.29	1.26
Serine	7.20	7.66	7.94	7.32	0.57
Tyrosine	8.45	7.76	8.94	10.01	1.14
EAA/NEAA [z]	1.14	1.04	1.07	1.16	0.11

Data in the same row with different superscripts differ at $P < 0.05$.

[z] EAA / NEAA: Essential amino acids / Non essential amino acids.

Table 5

Free amino acids (μM g^{-1} of wet weight) in raw gilthead sea bream fish fillets employed in sensorial trial.

	Diet		
	20	50	SE
Aspartic acid	1.23	1.33	0.18
Threonine	1.84	1.54	0.19
Serine	0.98	0.66	0.10
Glutamic acid	0.38	0.42	0.14
Glycine	6.13^{a}	8.45^{b}	0.45
Alanine	3.47^{b}	2.47^{a}	0.26
Valine	0.54	0.40	0.06
Methionine	0.24	0.21	0.02
Isoleucine	0.29	0.21	0.03
Leucine	0.54	0.41	0.06
Tyrosine	0.47	0.34	0.09
Phenylalanine	0.44	0.38	0.06
Lysine	2.17^{b}	0.69^{a}	0.24
Histidine	7.49	5.69	0.57
Arginine	8.35	5.28	1.21
Proline	1.59^{b}	1.14^{a}	0.13

Data in the same row with different superscripts differ at $P < 0.05$.

Table 6

Results of economic parameters in both phases of the experiment.

Parameter	Diet				
	20	30	40	50	SEM
Diet cost (€ kg^{-1})	0.51	0.48	0.47	0.45	
ECR[y] (€ kg^{-1}) 1st phase	0.86	0.91	0.95	0.99	0.04
ECR[y] (€ kg fish^{-1}) 2nd phase	1.07	0.93	0.97	0.88	0.03
EPI[z] (€ fish^{-1}) 2nd phase	1.28	1.28	1.21	1.15	0.03

Data in the same row with different superscripts differ at $P < 0.05$.