



# The optimum inclusion level of defatted soybean meal (SB) in experimental diets for meagre (*Argyrosomus regius*) for growth maximization and amino acid retention

Idah P. A, Yuan Yongming and Nwankwo Ifannyi

Research Group in Aquatic Resources, Institute of Animal Science and Technology,  
Polytechnic University of Valencia, Valencia, Spain.

## Abstract

The meagre (*Argyrosomus regius*) is a carnivorous fish which requires diets with higher protein content, causing an increment in diets cost. A way to diminish this cost is to use vegetable meals like soybean meal (SB). So the aim of this trial was to determine the optimum inclusion level of defatted soybean meal in experimental diets for this species. 800 fishes (165 g) were distributed in 8 tanks, two replicates per treatment. Four isoproteic (50% CP) and isolipidic (17% CI) diets were formulated with four levels of soybean meal inclusion, 0, 15, 30 and 45%. The trial lasted 107 days. Meagre fed diets 15 and 30% obtained the highest final weight. There were no significant differences among treatments in the feed conversion rate (FCR) and the protein efficiency (PER). According to the quadratic regression, the optimum SB inclusion to maximize thermal growth coefficient (TGC) was 26.4% and for FCR was 27.6%. No significant differences were observed in energy, protein and amino acid retention among diets. The inclusion of SB in meagre diets can generate a decrease in the use of fish meal and in turn reduce the cost of producing meagre Mediterranean aquaculture industry.

**Keywords:** *Argyrosomus regius*, fish meal replacement, defatted soybean meal, economic analysis.

## INTRODUCTION

The species belonging to the Scianidae family and selected for this present experiment is *Argyrosomus regius* known as Meagre, is a good candidate for the diversification on commercial aquaculture in Mediterranean and Eastern Atlantic for its good flesh and growth rate (El-Shebly et al., 2007; Roo et al., 2010).

The meagre produced in floating cages has shown good management (Jiménez et al., 2005) and high growth rate, reaching 1 kg in 10 to 13 months (Calderón et al., 1997; Roo et al., 2010). Limited information about the optimal feeding nutritional requirements of meagre is available, it only exists a recent study of dietary lipid requirements (Chatzifotis et al., 2010). Likewise the effects of different levels of plant proteins on the on growing of meagre have been studied very recently (Estévez et al., 2010).

Increase of aquaculture production around the world depends upon the development of sustainable protein sources to replace fish meal in aquafeeds. Fish meal is generally incorporated at levels between 30 and 60% in feeds for carnivorous marine fish (Wang et al., 2006a). Aquaculture production demands more and more alternative proteins to substitute fish meal. These meals should not have good amino acids profiles, but also lower prices than fish meal to reduce the production cost. The alternative meals should be highly digestible protein sources of plant and/or animal origin that support similar fish performance and concurrently have no adverse effects upon the environment (Murray et al., 2010). Defatted soybean meal (standard toasted and solvent-extracted, SB) is the most used vegetable meal in aquafeeds, because is a widely available, economical protein source with relatively high digestible protein and

energy contents and good amino acid profile (Wang et al., 2006b). The use of defatted soybean protein as a dietary protein has been examined for many commercial important marine fish species such as cobia (*Rachycentron canadum*) (Zhou et al., 2005), Mediterranean yellowtail (*Seriola dumerili*) (Tomás et al., 2005), European sea bass (*Dicentrarchus labrax*) (Tibaldi et al., 2006), Sharpshout seabream (*Diplodus puntazzo*) (Hernández et al., 2007) and gilthead sea bream (*Sparus aurata*) (Martínez-Llorens et al., 2009).

Soybean meal has a different acceptance in other carnivorous sciaenid species, both qualitatively and quantitatively, but there is no information available on meagre. In the Sciaenidae family, SB meal has been tested in different species; *Nibea miichthioides* has a limited ability to utilize SB as a protein source in practical feeds (Wang et al., 2006b) and *Sciaenops ocellatus* gained much weight with diets containing 50% of protein from soybean meal (McGoogan and Gatlin III, 1997; Reigh and Ellis, 1992). These results indicate a considerable variation in the ability of different species of the same family to utilize SB protein as an alternative to fish protein in the diet.

The aim of this trial was to determinate the optimum inclusion level of defatted soybean meal (SB) in experimental diets for meagre (*A. regius*), to maximize growth, feed efficiency parameters and amino acid retention and relate it with economic analysis.

## MATERIALS AND METHODS

### Experimental setup

The trial was conducted in 8 octagonal concrete tanks (4000 L) inside a recirculated seawater system at the aquaculture laboratory of Animal Science Department at the Polytechnic University of Valencia, (Valencia, Spain). The tanks were set up in a marine water recirculation system (65 m<sup>3</sup> of capacity) with a rotary mechanic filter and a gravity biofilter of around 6 m<sup>3</sup> capacity. All tanks were equipped with aeration and water was heated by a heat pump installed in the system. The equipments used to control water parameters were an oxy-meter (OxyGuard, Handy Polaris V 1.26), a refractometer with 0 to 100 g L<sup>-1</sup> range (Zuzi, A67410) and a kit using the colorimetric method to determinate nitrate, ammonia and nitrite concentrations. The kits were obtained from AquaMerck (Merck KGaA, Darmstadt, Germany). During the trial, the water temperature (23 ± 1° C) and dissolved oxygen (7 ± 0.5 mg L<sup>-1</sup>) were measured daily. Salinity (33 ± 1 g L<sup>-1</sup>), pH (7.3 ± 0.5), NH<sub>4</sub><sup>+</sup> (0.0 mg L<sup>-1</sup>), NO<sub>2</sub><sup>-</sup> (0.34 ± 0.2 mg L<sup>-1</sup>) and NO<sub>3</sub><sup>-</sup> (46.1 ± 3.7 mg L<sup>-1</sup>) were measured three times a week. Photoperiod was natural throughout the experimental period, and all tanks had similar lighting conditions.

### Fish and experimental design

The fish were transported to the experimental facilities of Polytechnic University of Valencia from a commercial hatchery localized in France. The fish were acclimated to the experimental conditions and fed a commercial diet (47% of crude protein (CP), 20% of crude lipid (CL), 5.8% Ash and 1.5% crude fibre (CF),

Skretting, Spain).

A group of 800 fishes, 165 g in mean weight, were distributed in 8 tanks; two replicates per treatment were randomly selected. The experiment finished when fish doubled the initial weight. All fishes were weighed every 5 to 6 weeks, approximately. Previously, fish were anaesthetised with 30 mg L<sup>-1</sup> of clove oil (Guinama<sup>®</sup>, Valencia, Spain) containing 87% of eugenol. The fishes were not fed for 24 h before weighing.

The trial lasted 107 days (from December 2009 to March 2010). At the beginning, 16 fishes per tank and the end 10 fishes per tank, were slaughtered by a thermoshock in a melting ice bath, to determine body composition and biometric parameters and were stored at -30°C to determine proximate and amino acid body composition.

### Diets and feeding

Four isoproteic (50% CP) and isolipidic diets (17% CL) were formulated using commercial ingredients (Table 1), in which defatted SB was included at 0, 15, 30 and 45% (Table 2). Diets were prepared by cooking-extrusion processing with a semi-industrial twin-screw extruder (CLEXTRAL BC-45, St. Etienne, France). Processing conditions were as follows: 100 rpm speed screw, 110°C temperature, 30 to 40 atm pressure and 3 and 6 mm diameter pellets, according to fish size. Each experimental diet was tested in duplicate tanks. Fishes were fed by hand twice a day to apparent satiation from Monday to Saturday. Pellets were distributed slowly, allowing all fishes to eat.

### Proximate composition and amino acid analysis

Chemical analyses of the dietary ingredients were determined prior to diet formulation. Diets and their ingredients as well as the whole fishes were analysed according to AOAC (1990) procedures: Dry matter (105°C to constant weight), ash (incinerated at 550°C to constant weight), crude protein (N × 6.25) by the Kjeldahl method after an acid digestion (Kjeltec 2300 Auto Analyser, Tecator Höganäs, Sweden), crude lipid extracted with methyl-ether (Soxtec 1043 extraction unit, Tecator) and crude fibre by acid and basic digestion (Fibertec System M., 1020 Hot Extractor, Tecator). All analyses were performed in triplicate.

The amino acid content in diets and whole body were determined after acid hydrolysis with HCL 6N at 110°C for 23 h. as previously described Bosch et al. (2006), through a Waters (Milford, MA, USA) HPLC system consisting of two pumps (Mod. 515, Waters), an autosampler (Mod. 717, Waters), a fluorescence detector (Mod. 474, Waters) and a temperature control module. Aminobutyric acid was added as internal standard after hydrolysis. The amino acids were derivatised with AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) and separated with a C-18 reverse-phase column Waters Acc. Tag (150 mm × 3.9 mm). Methionine and Cystine were determined separately as methionine sulphone and cysteic acid respectively after performic acid oxidation followed by acid hydrolysis.

### Economic analysis

The price of each diet was determined by multiplying the respective contributions of each feed ingredient by their respective costs per kg and summing the values obtained for all the ingredients in each of the formulated diets. The used raw material prices were the average prices in FAO GLOBEFISH (January, 2010), Instituto Técnico y de Gestión Ganadero, S.A and "Mercados Agroalimentarios" (Official FOB prices). The price of each ingredient (January, 2011) was: Fish meal=1.38 € kg<sup>-1</sup>; Defatted soybean meal=

**Table 1.** Proximate composition of ingredients used in experimental diets.

Ingredient	Fish meal, herring	Wheat	Soybean meal
International Feed N <sup>o</sup>	(5-02-000)	(4-05-268)	(5-04-604)
Dry matter (%)	91.9	87.7	89.35
Crude protein (% DM)	72.4	10.6	45.28
Crude lipid (% DM)	9.6	1.5	1.61
Crude fibre (% DM)	0.3	4.2	5.6
Ash (% DM)	15.8	1.6	7.7
NFE (% DM)	1.9	82.1	39.81

\*NFE was calculated as = 100 - %CP - %CL - %Ash - %CF.

0.321 € kg<sup>-1</sup>; Wheat meal=0.154 € kg<sup>-1</sup>; Fish oil=0.780 € kg<sup>-1</sup>; Vit-Min Mix=7.50 € kg<sup>-1</sup>.

The Economic Conversion Ratio (ECR) was used to evaluate the diets from an economic point of view and it was calculated following the expression:

ECR (€ kg<sup>-1</sup> fish) = feed conversion ratio (kg diet kg<sup>-1</sup> fish) × price of diet (€ kg<sup>-1</sup> diet)

### Statistical analysis

Growth data and nutritive parameters were treated using multifactor analysis of variance (ANOVA), introducing the initial live weight as covariate (Snedecor and Cochran, 1971). Newman-Keuls test was used to assess specific differences among diets at a significance levels of  $P < 0.05$  significance levels (Statgraphics, Statistical Graphics System, Version Plus 5.1, Herndon, Virginia, USA).

Quadratic regression analyses were applied, where specific growth rate (SGR) and feed conversion ratio (FCR) were a function of soybean meal (SB) dietary level using the expression:

$$Y = a + b(SB) + c(SB)^2$$

Optimum soybean meal dietary level was obtained by deriving this equation and equalising to zero. All experiments were carried out according to the rules or protocols of the Animal Welfare Commission at the Polytechnic University of Valencia.

## RESULTS

The composition of test diets including dry matter, CP, CL, ash and gross energy (GE) was similar (Table 2). The essential amino acid (EAA) profiles of the diets were variable (Table 2), Arg, His, Met, Phe and Thr decreased according to SB increased in the diets and the opposite trend was observed in the Iso and Leu content. The Lys content was similar in all diet. In relation to non essential amino acids (NEAA), the dietary content of Asp and Glu were increased according to dietary level of SB. The relation EAA/NEAA also diminished with dietary soybean meal level until 0.86.

No significant difference was observed in final survival (that it was around 84% ± 10.61). The meagre did not present adaptation problems nor exhibit stress behaviour.

At the end of the trial, a significant effect of soybean

meal inclusion was observed on fish growth (Table 3). Meagre fed diets 15 and 30 obtained the highest final weight (380 and 385 g, respectively). The final weight of fish fed diet 45 was also higher (360 g), than fish fed diet 0 (333 g). Likewise, fish fed diet 15 and 30 obtained significantly higher Thermal Growth Coefficient (TGC) ( $3.10 \times 10^{-3}$  and  $3.15 \times 10^{-3}$ , respectively) than fish fed 45 diet ( $2.87 \times 10^{-3}$ ) and fish fed the 0 diet that obtained the lowest TGC ( $2.56 \times 10^{-3}$ ).

Regarding nutritional parameters, the daily feed intake, the feed conversion rate (FCR) and the protein efficiency (PER) ratio were not different for all the diets (Table 3).

With the aim to determining the SB dietary levels that maximize the fish growth, a second-order polynomial regression analysis was assessed and the equation that describes the relationship between TGC and the dietary SB level is expressed in Figure 1. Based on the above polynomial equation, the point maximum of this quadratic curve is the dietary SB level to maximize the TGC (26.4% SB). Likewise, Figure 1 shows the second-polynomial regression between FCR and dietary level SB and the SB level that obtain the minimum FCR resulted 27.6% SB.

Biometric parameters and body composition were not affected by experimental diets (Table 4). Significant differences were not observed in whole body composition. The energy retention (GEE) results are similar in all tested diets with values between 24 and 25.7%. Neither, significant differences were observed among diets in the efficiency of protein (CPE), between 27.6 and 30.6%.

The ingestion of essential amino acids (expressed as g AA × 100g<sup>-1</sup> of fish and day) did not showed significant differences with the diets (Figure 2). Overall, the Met intake was the lowest (from 0.8 to 1.24 g AA × 100g<sup>-1</sup> of fish and day) followed by the His intake (from 1.9 to 2.1g AA × 100g<sup>-1</sup> of fish and day) and the Lys and Leu intake were higher than the others amino acids intake (upper to 3 g AA × 100g<sup>-1</sup> of fish and day in the four experimental diets). Figure 3 shows the retention efficiency (%) of essential amino acids of fishes fed with the experimental diets at the end of the experiment. The His was the amino acids with the lowest retention efficiency (25% of average) and the Thr presented the highest retention

**Table 2.** Formula and proximate composition of the experimental diets.

Ingredients (g kg <sup>-1</sup> )	Diet			
	0	15	30	45
Fish meal, herring (5-02-000)	660	576	493	407
Soybean meal (5-04-604)	0	150	300	450
Wheat (4-05-268)	216	144	70	0
Fish oil (7-08-048)	104	110	117	123
Vitamin–mineral Mix	20	20	20	20
Analysed composition (% dry weight)				
Dry matter	91.9	91.31	90.09	90.41
Crude protein (%CP)	50.43	50.13	50	50.98
Crude lipid (%CL)	17.78	17.94	18.09	17.42
Ash (%)	11.17	11.22	10.95	10.55
Crude fibre (%CF)	1.11	1.62	2.12	2.64
Calculated values				
NFE	19.51	19.09	18.84	18.41
GE (MJ kg <sup>-1</sup> ) <sup>†</sup>	22.57	22.48	22.34	22.36
CP/GE (g MJ <sup>-1</sup> )	22.35	22.3	22.38	22.8
Essential amino acid content calculated (g 100 <sup>-1</sup> g)				
Arginine	4.19	4.13	4.07	4.00
Histidine	1.82	1.75	1.68	1.61
Isoleucine	2.43	2.44	2.45	2.46
Leucine	3.90	3.92	3.94	3.95
Lysine	3.00	3.00	3.01	3.00
Methionine	1.10	1.04	0.99	0.92
Phenylalanine	3.38	3.29	3.21	3.11
Threonine	2.45	2.39	2.33	2.27
Valine	2.66	2.65	2.64	2.62
Non essential amino acid content calculated (g 100 <sup>-1</sup> g)				
Alanine	3.03	2.94	2.84	2.74
Aspartate	4.31	4.59	4.87	5.14
Cystine	0.73	0.72	0.71	0.69
Glutamine	6.66	7.09	7.51	7.93
Glycine	3.65	3.48	3.31	3.13
Proline	4.93	4.60	4.27	3.92
Serine	2.09	2.14	2.20	2.25
Tyrosine	2.41	2.28	2.16	2.03
EAA/NEAA	0.90	0.88	0.87	0.86

\*NFE calculated: 100-%CP-%CL-%Ash-%CF. <sup>†</sup>GE: Gross energy: Calculated using: 23.9 kJ g<sup>-1</sup> proteins, 39.8 kJ g<sup>-1</sup> lipids and 17.6 kJ g<sup>-1</sup> carbohydrates.

(around of 35%) following by Arg (34%) and Lys and Met (32%). There were no significant differences of amino acids efficiency retention among diets. Ratio between dietary EAA level of experimental diets and EAA in the carcass was calculated (expressed as %EAA<sub>diet</sub>/<sub>%EAA<sub>fish</sub></sub>) and the results are shown in Figure 5. The His presented the upper value of this ratio (upper to 130%), and Arg, Lys, Met and Thr presented the ratio below to 100%. No differences were observed in ratio %EAA<sub>diet</sub>/<sub>%EAA<sub>fish</sub></sub> in relation to diets, with a exception of Met that presented

the lowest ratio in diet 30 and 45 and the highest in the 0 diet.

Regarding to economic analyses of the diets, the cost of diets was reduced with the increase of soybean meal in diets (Table 5). Significant differences were showed in the economic conversion ratio (ECR) that was higher in control diet (diet 0) than in the others diets.

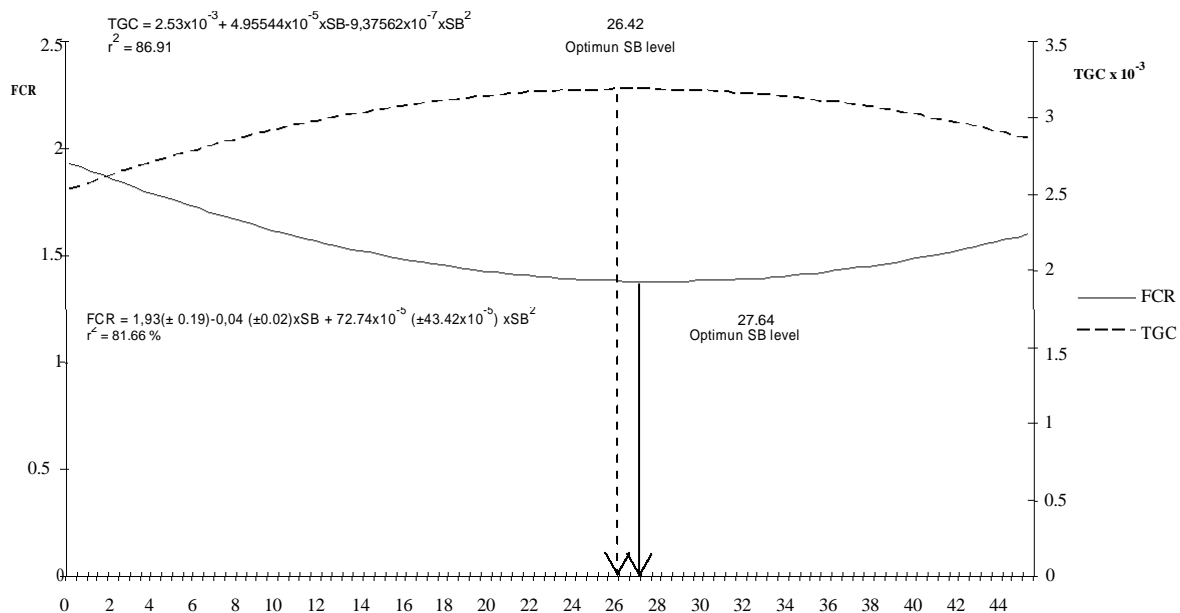
## DISCUSSION

The results of this present trial show that the meagre exhibits a high growth, with TGC around  $3.00 \times 10^{-3}$ , greater than other marine species such as gilthead sea bream with TGC average of  $1.72 \times 10^{-3}$  (Mayer et al., 2008) and others scianids as *Argyrosomus japonicus* (Pirozzi et al., 2009) feed with commercial diets ( $1.46 \times 10^{-3}$ ). Likewise the TGC in present trial were also higher than TGC recalculated by the growth results obtained by Calderón et al. (1997) with *A. regius* feeding with pelletized diets ( $2.02 \times 10^{-3}$ ), Estevez et al. (2010) feeding *A. regius* with experimental diet with extruded commercial diet ( $1.73 \times 10^{-3}$ ) and by El-Shebly et al. (2007) ( $1.78 \times 10^{-3}$ ) with feed based in tilapia and shrimp. The diets with 15 or 30% of SB obtained the best final weight and TGC (385 g and  $3.15 \times 10^{-3}$ , respectively) but the optimum soybean inclusion in diets for *A. regius* was 27% for both, growth and feed conversion. Figure 4 shows the TGC obtained in this experiment compared with different marine species that were feeding with different levels of plant proteins in diets. Despite the fact that knowledge of meagre nutrition is limited there are a few feeding studies in fishes of the scianids family. The studies made by Wang et al. (2006b) recommended soybean meal dietary level below 10% (40% of fish meal substitution) in diets for cuneate drum, since exceeding this level produced a detriment in the fish growth. Higher levels of dietary soybean meal were recommended for other scianids as *Sciaenops ocellatus*, for example McGoogan and Gatlin III (1997) obtained a good growth results when soybean meal was included at 66% in diets and in the same species, Reigh and Ellis (1992) also observed that the 70% of soybean meal dietary inclusion did not affected negatively to fish growth. Regarding to replaces fish meal with a vegetable protein mixtures, Estevez et al. (2010) fed *A. regius* with four experimental diets with two inclusion levels (42 and 52%, those represented the 315 and 38% of total protein of diets) of mixture plant protein (soy cake, corn gluten, soy protein concentrate and sunflower cake) with or without fish protein hydrolysates and observed that the fish growth was significantly reduced by the inclusion of plant protein, although the growth of fish fed diets with 42% of plant protein dietary inclusion obtained similar growth to fish fed control diet. The quadratic regression analysis was recommended by Shearer (2000) because it was used to obtain optimum levels of ingredients or nutrients

**Table 3.** Main performances of meagre fed increasing levels of dietary soybean meal.

Parameter	Diet				SEM
	0	15	30	45	
Initial weight (g)	164	166	166	165	3.84
Final weight (g)	333 <sup>c</sup>	380 <sup>a</sup>	385 <sup>a</sup>	360 <sup>b</sup>	1.2
TGC × 10 <sup>-3</sup> *	2.56 <sup>c</sup>	3.10 <sup>a</sup>	3.15 <sup>a</sup>	2.87 <sup>b</sup>	0.144
FI (% day <sup>-1</sup> ) †	0.79	0.90	0.95	0.89	0.03
FCR ‡	1.30	1.34	1.43	1.45	0.05
PER §	1.53	1.49	1.40	1.36	0.05

Means of duplicate groups. Data on the same row not sharing a common superscript letter are significantly different (P < 0.05). SEM: Pooled standard error of the mean. Initial weight was considered as covariable for final weight and TGC. \*TGC = 1000 × [Final weight (g)<sup>1/3</sup> - Initial weight (g)<sup>1/3</sup>] / (T° - minimum T° to feed). †Feed intake (g/100 g fish<sup>-1</sup> day<sup>-1</sup>) FI = 100 × feed consumption (g) / average biomass (g) × days. ‡Feed conversion ratio, FCR = feed offered (g) / weight gain (g). §Protein efficiency ratio, PER = Weight gain (g) / protein offered (g).



**Figure 1.** Optimum dietary soybean meal level for TGC and FCR depending on dietary soybean meal obtaining by quadratic regression.

(Martínez-Llorens et al., 2009; Sánchez et al., 2007). According to the polynomial regression showed that the dietary soybean meal obtained for a maximum growth was 26.4%. Similar results were reported in other marine species, such as gilthead sea bream that recommended between 20.5% (Martínez-Llorens et al., 2009) and 30.5% (Martínez-Llorens et al., 2007) of dietary soybean meal level for maximum growth, and likewise Tomás et al. (2005) recommended from 20 to 30% of defatted soybean meal dietary inclusion for maximum growth of *Seriola dumerili*. Chou et al. (2004) for juvenile cohiba (*Rachycentron canadum*) estimated by quadratic regression a growth optimum at 16.9% replacement of fish meal protein by soybean meal protein.

Although daily feed intake, FCR, and PER do not

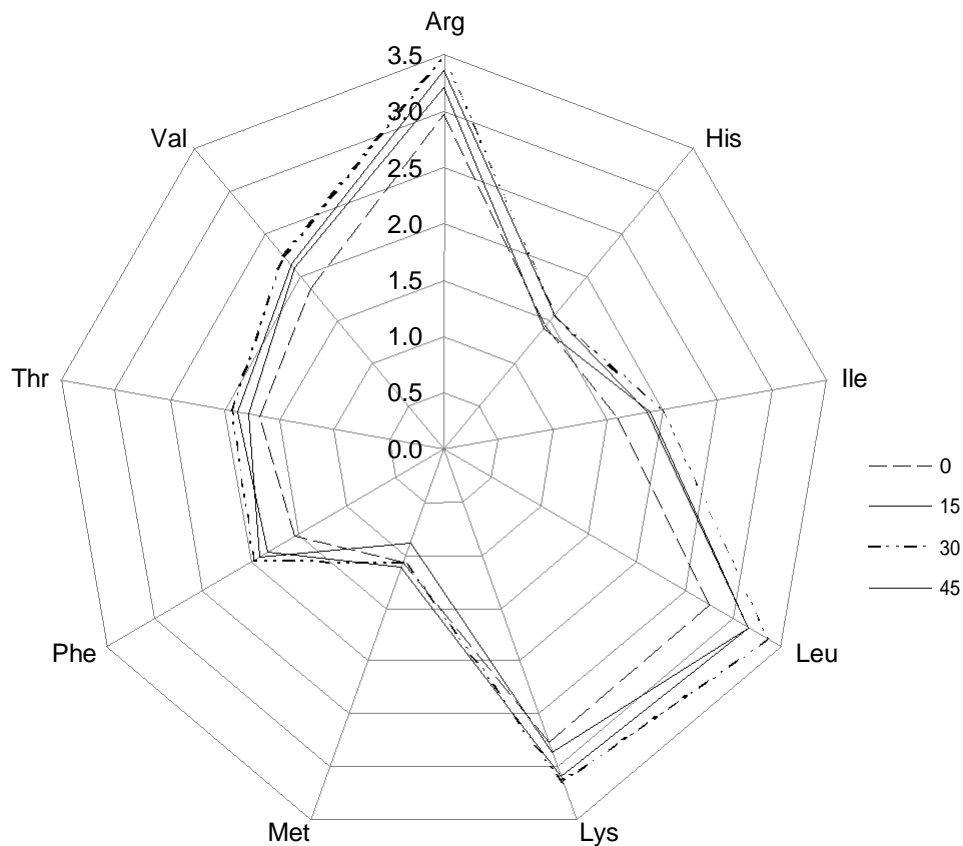
statistically differ between diets, they do not appear so close. Daily feed intake of diet 0, in particular, is 13% lower than the value recorded for diets 15 and 45, and 17% lower than diet 30. To provide evidence that the may be caused by the nutrient imbalances besides to palatability properties of plant proteins.

In relation to biometric parameters, no effect of diet was observed and similar indexes were obtained by Poli et al. (2003); 1.04 condition factor, 44% fillets, 6% VSI, but the mesenteric fat obtained in present trial (2.4%) were higher than the obtained by Poli et al. (2003), and the cause of this differences probably could be to that meagres in this present experiment not formed gonad. There was not significant effect on the whole body composition of meagre by the experimental diets and

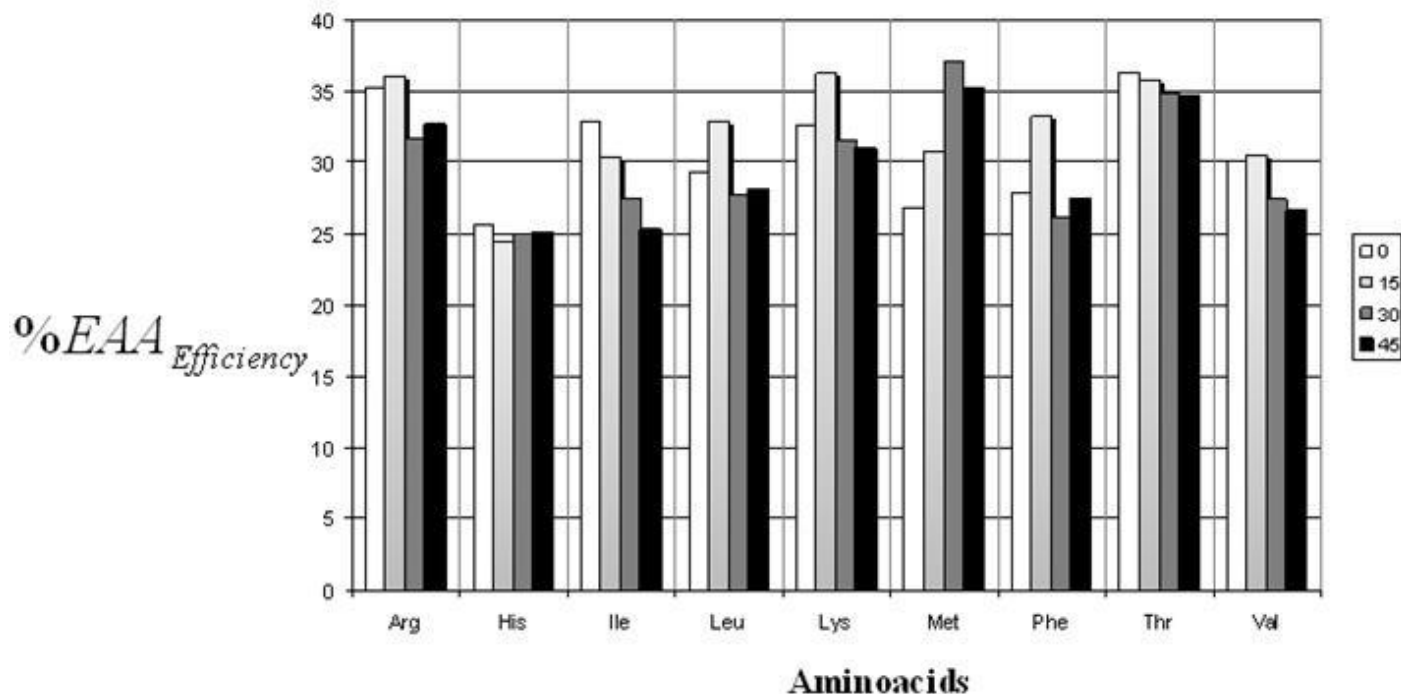
**Table 4.** Biometric indices and proximate composition (expressed as percentage of wet weight) of *A. regijsted* increasing levels of soybean meal.

Parameter	Diet				SEM
	0	15	30	45	
CF	1.11	1.18	1.17	1.16	0.02
VSI (%) <sup>†</sup>	6.12	5.77	5.49	6.02	0.24
HSI (%) <sup>‡</sup>	1.96	1.6	1.58	1.61	0.11
MF (%) <sup>§</sup>	2.43	2.53	2.33	2.48	0.16
DP (%) <sup>¶</sup>	70.33	70.9	70.56	69.17	0.84
MI (%) <sup>  </sup>	58.76	60.75	58.39	59.39	1.44
Moisture (%)	74.05	72.62	71.8	71.9	0.55
Crude Protein (% ww)	17.37	17.95	18.6	17.37	0.37
Crude Lipid (% ww)	6.5	6.63	6.81	7.64	0.25
Ash (% ww)	2.45	2.65	3.12	2.57	0.19
CPE (%) <sup>**</sup>	29.7	30.4	30.6	27.6	1.83
GEE (%) <sup>††</sup>	24.0	24.8	25.4	25.7	1.33

The data are the mean (n=10) ± SEM. Data in the same row with different superscripts differ at P < 0.05. \*Condition factor CF = 100 × total weight (g)/total length<sup>3</sup> (cm). <sup>†</sup>Viscerosomatic index (%) VSI = 100 × visceral weight (g)/ Fish weight (g). <sup>‡</sup>Hepatosomatic index (%) HIS = 100 × liver weight (g) / Fish weight (g). <sup>§</sup>Mesenteric fat (%) MF = 100 × mesenteric fat weight (g)/fishweight (g). <sup>¶</sup>Dressout percentage (%) DP = 100 × [total fish weight (g)-visceral weight (g)-head weight (g)]/ fish weight (g). <sup>||</sup>Meat index (%) MI = 100 × meat weight (g)/fish weight (g). <sup>\*\*</sup>Crude protein efficiency (%) CPE = Fish protein gain (g) × 100/ protein intake (g). <sup>††</sup>Gross energy efficiency (%) GEE = Fish energy gain (kJ) × 100/energy intake (kJ).



**Figure 2.** Ingestion of essential amino acids (EAA) in each experimental diet expressed as g per 100 g<sup>-1</sup> of fish and day. Each value is the mean of duplicate groups. Different superscripts indicated differ at P < 0.05.



**Figure 3.** Retention efficiency (%) of essential amino acids in *A. regius* fed with the experimental diets at the end of the experiment. Each value is the mean of duplicate groups. Different superscripts indicated differ at  $P < 0.05$ . Retention of ingested protein (%) = Fish amino acid gain (g)/ ingested amino acids (g)  $\times 100$ .

**Table 5.** Global results of economic parameters at the end of the experiment.

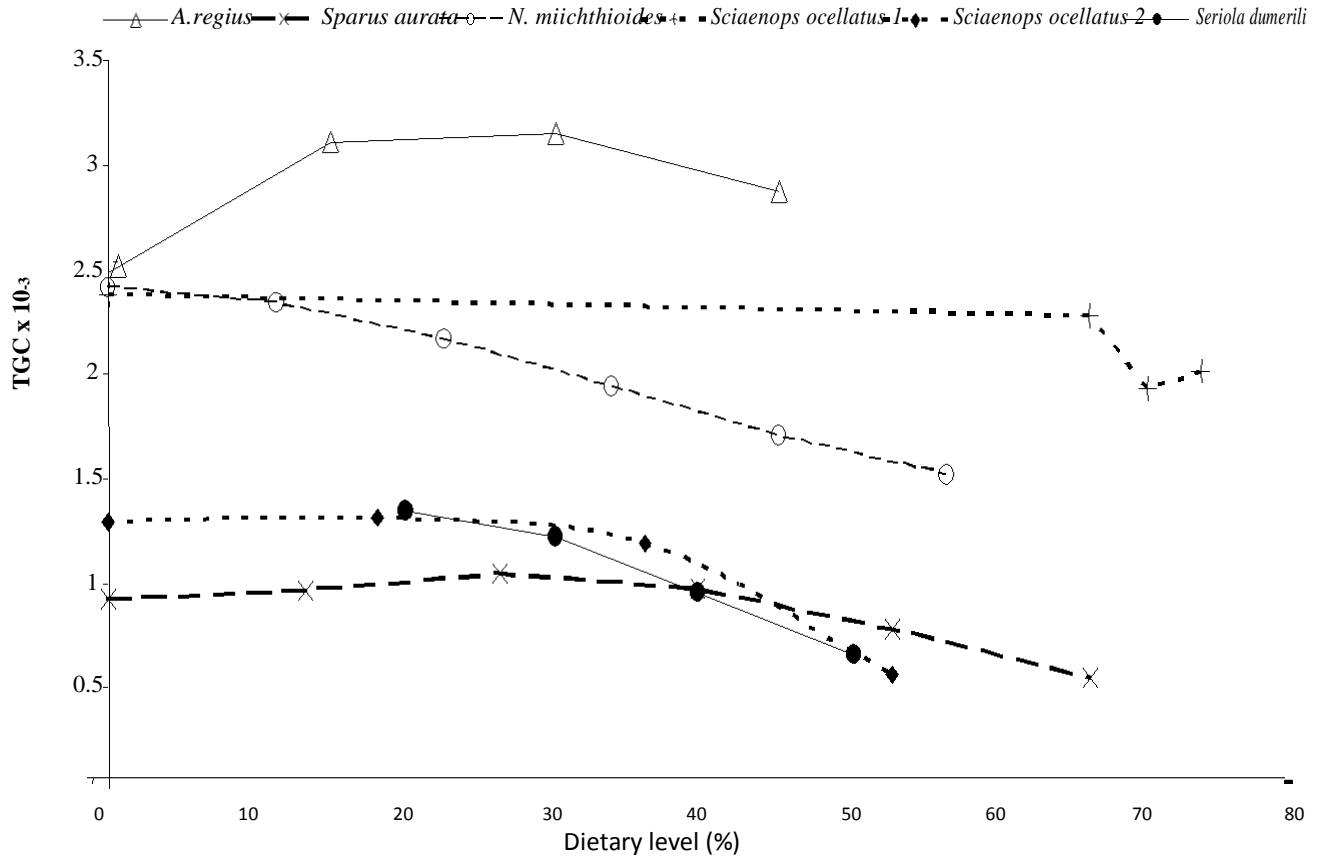
Parameter	Diet				SEM
	0	15	30	S45	
Cost of diet ( $\text{€ kg}^{-1}$ )*	1.18	1.10	1.03	0.95	
ECR ( $\text{€ kg}^{-1}$ ) <sup>†</sup>	2.31 <sup>b</sup>	1.50 <sup>a</sup>	1.55 <sup>a</sup>	1.47 <sup>a</sup>	0.06

Data in the same row with different superscripts differ at  $P < 0.05$ . \*Calculated from price of ingredients: Fish meal=1.38  $\text{€ kg}^{-1}$ ; Soybean meal=0.32  $\text{€ kg}^{-1}$ ; Wheat= 0.154  $\text{€ kg}^{-1}$ ; Fish oil=0.78  $\text{€ kg}^{-1}$ ; Vit-Min-AA Mix=7.5  $\text{€ kg}^{-1}$ .<sup>†</sup> ECR ( $\text{€ kg}^{-1}$  fish) = feed conversion ratio (kg diet  $\text{kg}^{-1}$  fish)\*price of diet ( $\text{€ kg}^{-1}$  diet).

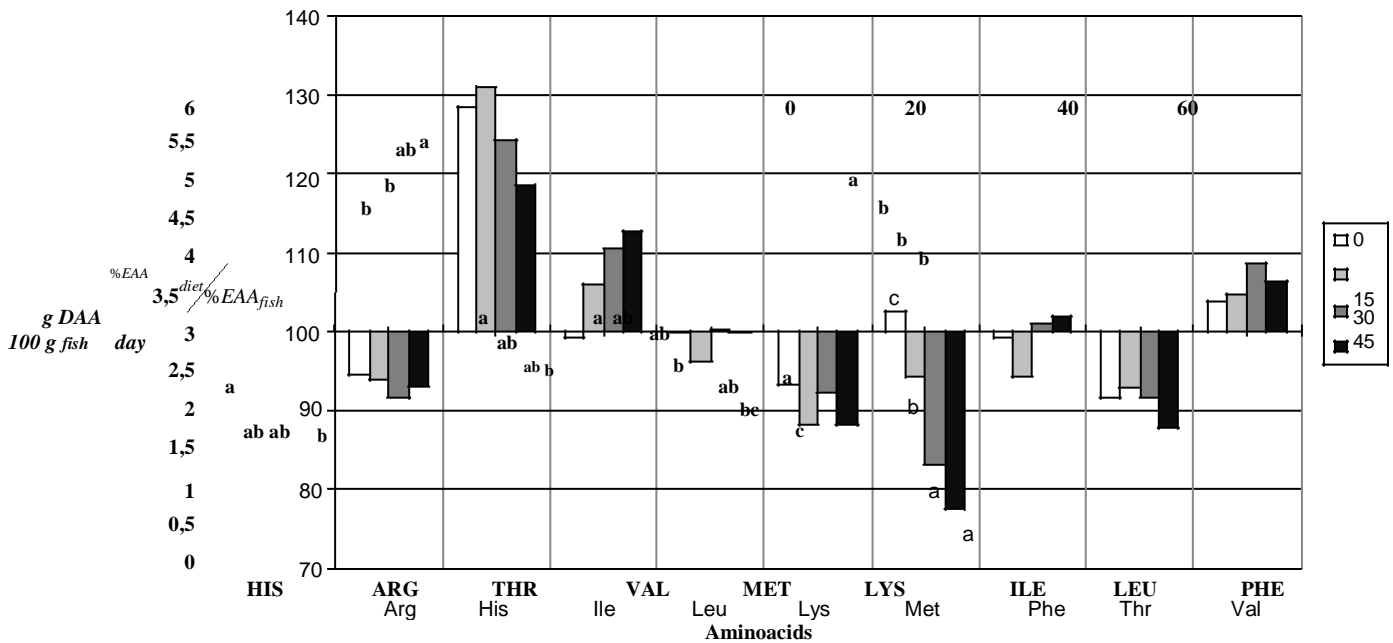
approximately content was 72% of humidity, 17% of CP and 7% of lipids. These results demonstrate the excellent meat quality that presents the meagre, being the main characteristic its lower fat content, representing an important parameter of quality for the consumer (Poli et al., 2003).

The detriment of growth in 45 diet could be to several factors as the presence of anti nutritional factors in plant proteins (Francis et al., 2001; Gatlin III et al., 2007), that various effects can also caused the activities reduces of alkaline phosphatase and aminopeptidase in meagre (Estevez et al., 2010). In addition, the growth could be affected by the amino acid deficiencies of diets (Gomez-Requeni et al., 2004; Peres et al., 2003; Refstie et al., 2006; Wang et al., 2006b). Following this reasoning, the information about amino acids requirements for meagre is not available. Nevertheless, a first approach about the

excess or defect of EAA could be done by estimating amino acid retention, which has been carried out in other fish species (Peres and Oliva-Teles 2009; Sánchez-Lozano et al., 2010). In this present experiment, no significant effect in amino acid retentions was observed with the different diets, but great differences can be observed among amino acids, Arg, Lys, Met and Thr presented the highest retention and the His the lowest. The amino acid efficiency retention and amino acid intake is closely related and the reason of the high retention was due to the low amino acid intake, if this one is made below its requirements. Ratio between EAA profile of diets and whole body (Figure 5) could be a good tool to estimate the deficiencies as Sánchez-Lozano et al. (2009, 2010) have shown in the sea bream, so, that if this relation is less than 100% this amino acid would be deficient and if it is greater than 100% it would be in



**Figure 4.** TGC of *A. regius* in the present trial, compared, *Sparus aurata* (Martínez-Llorens et al., 2009), *N. miichthioides* (Wanget al., 2006), *Sciaenops ocellatus 1* (McGoogan and Gatlin III, 1997), *Sciaenops ocellatus 2* (Reigh and Ellis, 1992) and *Seriola dumerili* (Tomás et al., 2005).



**Figure 5.** Ratio between essential amino acids profile of experimental diets and whole body fish expressed as g per 100 g<sup>-1</sup> of protein. Each value is the mean of duplicate groups. Different superscripts indicated differ at P < 0.05.



excess. Arg, Lys and Thr were deficient in diets and therefore the efficiency retention of these amino acids was high. Met was significant different among diets and fish fed diet 0 (100% of fish meal) presented the highest relation between  $\%EAA_{\text{diet}}/\%EAA_{\text{fish}}$  and for reason Met efficiency retention increased with soybean meal dietary level. In summary, it is necessary to determine the amino acids requirement of meagre because, even in the diet with the 100% of fish meal, there are amino acids that could be deficient, such as Arg, Lys and Thr. From an economic point of view, it was clearly improved the ECR when fish meal was substituted for soybean meal in the diet.

Then, if the growth and body indices show that diets 15 and 30% were quite similar, although the cost of the diets is not significantly different, the best diet must be considered the diet 30 Reigh and Ellis (1992) recommended up to 70% of dietary soybean meal for a growth of *Sciaenops ocellatus*, but observed that the 35.5% soybean level (50% of dietary protein from soybean meal) was the most cost-effective diet. The soybean meal level for minimum ECR (optimum ECR) in diets for *Seriola dumerili* (Tomás et al., 2005) resulted around 20.5% and similar results (Martínez-Llorens et al., 2007) were obtained for gilthead sea bream (22%).

## Conclusion

The results obtained in this present trial showed that 30% SB inclusion could be an excellent plant meal to substitute 25% of fish meal dietary, because no effects on growth and feed efficiency parameters were detected and in addition improve the profitability of diets.

## REFERENCES

- Association of official Analytical Chemists, AOAC. (1990). Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, VA, USA. pp. 1298.
- Bosch L, Alegria A, Farré R (2006). Application of the 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC), reagent to the RP-HPLC determination of amino acids in infant foods. *J. Chromatogr. B* 831:176–183.
- Calderón JA, Esteban JC., Carrascosa MA, Ruiz PL, Valera F (1997). Estabulación y crecimiento en cautividad de un lote de reproductores de corvina (*Argyrosomus regius*). *Actas del VI Congreso Nacional de Acuicultura*, Cartagena, Spain:365–370.
- Chatzifotis S, Panagiotidou M, Papaioannou N, Pavlidis M, Nengas I, Mylonas C (2010). Effect of dietary lipid levels on growth, feed utilization, body composition and serum metabolites of meagre (*Argyrosomus regius*) juveniles. *Aquaculture* 307:65–70.
- Chou RL, Her BY, Su MS, Hwang G, Wu YH, Chen HY (2004). Substituting fish meal with soybean meal in diets of juvenile cobia *Rachycentron canadum*. *Aquaculture* 229:325–333.
- El-Shebly A, El-Kady MAH, Hussin A, Yeamin Hossain MD (2007). Preliminary observations on the pond culture of meagre *Argyrosomus regius* (Asso, 1801) (Sciaenidae) in Egypt. *J. Fisheries. Aquatic. Sci.* 2(5):345–352.
- Estévez A, Treviño L, Kotzamanis Y, Karacostas I, Tort L, Gisbert E (2010). Effects of different levels of plant proteins on the on-growing of meagre (*Argyrosomus regius*) juveniles at low temperatures. *Aquacult. Nutr.* 17:e572–e582.
- Francis G, Makkar H, Becker K (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture* 199:197–227.
- Gatlin III, D, Barrows F, Bronwn P, Dabrowski K, Gaylord T, Hardy R, Herman E, Hu G, Krogdahl A, Nelson R, Overturf K, Rust M, Sealey W, Skonberg D, Souza E, Stone D, Wilson R, Wurtele E (2007). Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquacult. Res.* 38:551–579.
- Gómez-Requeni P, Mingarro M, Calduch-Giner J, Médale F, Martin S, Houlihan DF, Kaushik S, Pérez-Sánchez J (2004). Protein growth performance, amino acid utilisation and somatotropic axis responsiveness to fish meal replacement by plant protein sources in gilthead sea bream (*Sparus aurata*). *Aquaculture* 232:493–510.
- Hernández M, Martínez F, Jover M, García B (2007). Effects of partial replacement of fish meal by soybean meal in sharpsnout seabream (*Diplodus puntazzo*) diet. *Aquaculture* 263:159–167.
- Jiménez MT, Pastor E, Grau A, Alconchel JI, Sánchez R, Cárdenas S (2005). Revisión del cultivo de esciéndidos en el mundo, con especial atención a la corvina *Argyrosomus regius* (Asso, 1801). *Boletín Instituto Español de Oceanografía*. 21(1-4):169–175.
- Martínez-Llorens S, Moñino AV, Tomás A, Pla M, Jover M (2007). Soybean meal as partial dietary replacement for fish meal in gilthead sea bream (*Sparus aurata*) diets: effects on growth, nutritive efficiency and body composition. *Aquacult. Res.* 38:82–90.
- Martínez-Llorens S, Tomás A, Jauralde I, Pla M, Jover M (2009). Optimum dietary soybean meal level for maximizing growth and nutrient utilization of on-growing gilthead sea bream (*Sparus aurata*). *Aquacult. Nutr.* 15:320–328.
- Mayer P, Estruch V, Blasco J, Jover M (2008). Predicting the growth of gilthead sea bream (*Sparus aurata* L.) farmed in marine cages under real production conditions using temperature- and time-dependent models. *Aquacult. Res.* 39:1046–1052.
- McGoogan B, Gatlin III D (1997). Effects of replacing fish meal with soybean meal in diets for red drum *Sciaenops ocellatus* and potential for palatability enhancement. *J. World Aquacult. Soc.* 28:374–385.
- Murray H, Lall S, Rajaselvam R, Boutilier L, Blanchard B, Flight R, Colombo S, Mohindra S, Douglas S (2010). A nutrigenomic analysis of intestinal response to partial soybean meal replacement in diets for juvenile Atlantic halibut, *Hippoglossus hippoglossus*, L. *Aquacult.* 298: 282–293.
- Peres H, Lim C, Klesius P (2003). Nutritional value of heattreated soybean meal for channel catfish (*Ictalurus punctatus*). *Aquacult.* 225: 67–82.
- Peres H, Oliva-Teles A (2009). The optimum dietary essential amino acids profile for gilthead seabream (*Sparus aurata*) juveniles. *Aquaculture* 296:81–86.
- Pirozzi I, Booth M, Pankhurst P (2009). The effect of stocking density and repeated handling on the growth of juvenile mullet, *Argyrosomus japonicus* (Temminck, Schlegel 1843). *Aquacult. Int.* 17:199–205.
- Poli B, Parisi G, Zampacavallo G, Iurzan F, Mecatti M, Lupi P, Bonelli A (2003). Preliminary results on quality changes in reared meagre (*Argyrosomus regius*): body and fillet traits and freshness changes in refrigerated commercial-size fish. *Aquacult. Int.* 11:301–311.
- Refstie S, Landsverk T, Bakke-McKellep A, Ringo E, Sundby A, Shearer K, Krogdahl A (2006). Digestive capacity, intestinal morphology, and microflora of 1-year and 2-year old Atlantic cod (*Gadus morhua*) fed standard or bioprocessed soybean meal. *Aquaculture* 261:269–284.
- Reigh R, Ellis S (1992). Effects of dietary soybean and fish-protein ratios on growth and body composition of red drum (*Sciaenops ocellatus*) fed isonitrogenous diets. *Aquaculture* 104:279–292.
- RoóJ, Hernández-Cruz C, Borrero C, Schuchardt D, Fernández-Palacios H (2010). Effect of larval density and feeding

- sequence on meagre (*Argyrosomus regius*; Asso, 1801) larval rearing. *Aquaculture* 302:82–88.
- Sánchez N, Tomás A, Martínez-Llorens S, Nogales S, Blanco J., Moñino A, Pla M, Jover M (2007). Growth and economic profit of gilthead sea bream (*Sparus aurata*, L.) fed sunflower meal. *Aquaculture* 272:528–534.
- Sánchez-Lozano N, Martínez-Llorens S, Tomás-Vidal A, Jover Cerdá M (2009). Effect of high-level fish meal replacement by pea and rice concentrate protein on growth, nutrient utilization and fillet quality in gilthead seabream (*Sparus aurata*, L.). *Aquaculture* 298:83–89.
- Sánchez-Lozano N, Martínez-Llorens S, Tomás-Vidal A, Jover Cerdá M (2010). Amino acid retention of gilthead sea bream (*Sparus aurata*, L.) fed with pea protein concentrate. *Aquacult. Nutr.* 17:e604–e614.
- Shearer K (2000). Experimental design, statistical analysis and modelling of dietary nutrient requirement studies for fish: a critical review. *Aquacult. Nutr.* 6:91–102.
- Snedecor G, Cochran W (1971). *Statistical methods*. The Iowa State University Press Ames, Iowa, USA. p. 593.
- Tibaldi E, Hakim Y, Uni Z, Tulli F, De Francesco M, Luzzana U, Harpaz S (2006). Effects of the partial substitution of dietary fish meal by differently processed soybean meals on growth performance, nutrient digestibility and activity of intestinal brush border enzymes in the European sea bass (*Dicentrarchus labrax*). *Aquaculture* 261:182–193.
- Tomás A, De La Gándara F, García-Gomez A, Pérez L, Jover M (2005). Utilization of soybean meal as an alternative protein source in the Mediterranean yellowtail, *Seriola dumerili*. *Aquacult. Nutr.* 11:333–340.
- Wang Y, Guo J, Bureau D, Cui Z (2006a). Replacement of fish meal by rendered animal protein ingredients in feeds for cuneate drum (*Nibeamichthioides*). *Aquaculture* 252:476–483.
- Wang Y, Kong L, Li C, Bureau D (2006b). Effect of replacing fish meal with soybean meal on growth, feed utilization and carcass composition of cuneate drum (*Nibea miichthioides*). *Aquaculture* 261:1307–1313.
- Zhou Q, Mai K, Tan B, Liu Y (2005). Partial replacement of fishmeal by soybean meal in diets for juvenile cobia (*Rachycentron canadum*). *Aquacult. Nutr.* 11:175–182.