MÁSTER INTERUNIVERSITARIO EN ACUICULTURA

Potential of vegetable proteins in diets of Gilthead Sea bream (*Sparus aurata*)

TESINA DE MÁSTER

Alumna:
Ana Isabel Navarro Ramírez

Directora Académica:
Dra. Ana Tomás Vidal

Valencia, Septiembre 2013

CONTENTS

Potential of vegetable proteins in diets of Gilthead Sea bream (*Sparus* aurata)

Navarro A.[1]

[1] Research Group of Aquaculture and Biodiversity. Institute of Animal Science and Technology. Universitat Politècnica de València. Camino de Vera, 14. 46071-Valencia (Spain).

Abstract

Effect of partial or total replacement of fish meal (FM) by plant protein sources on growth, nutritional parameters, nutritional value and sensory quality of gilthead sea bream (*Sparus aurata*) was evaluated. The initial weight was 130g to commercial weight. Vegetable protein diets were supplements with synthetic amino acids and also contained 50 g kg^{-1} of krill meal and 100 g kg^{-1} of squid meal in order to improve palatability and promote acceptance of the fish.
At the end of the experiment (154 days) survival of the fish was 86-88%. No significant differences in growth and nutritional parameters with the different diets, or biometric parameters, except hsi that showed significant differences in the control diet compared to the other two, with a higher value (1.55%). The values of moisture, protein, lipid and ash content were not affected by the fish meal substitution.
Regarding fish fillets, differences were detected between the different diets (FM0, FM25 and FM100) for physicochemical parameters (pH, lightness, hardness, chewiness, cohesiveness, gumminess, springiness and force), for mechanical properties, and in optical properties, the Colour difference (ΔE).
Regarding the sensory test raw and cooked fillets, judges do not discriminate between the two diets tested (FM0 and FM100).

Keywords: Sparus aurata, fish meal, vegetable protein, fish quality, sensory test

Resumen

En el presente trabajo se ha estudiado el efecto de la sustitución total o parcial de la harina de pescado (FM) por fuentes de proteína vegetal sobre el crecimiento, los parámetros nutricionales, el valor nutricional y la calidad sensorial de una producción de dorada (*Sparus aurata*). El peso inicial fue de 130g hasta peso comercial. Las dietas de proteína vegetal fueron suplementadas con amino ácidos sintétitcos y también contenían contenían 50 g Kg^{-1} de harina de krill y 100 g Kg^{-1} de harina de calamar con el fin de mejorar la palatabilidad y favorecer la aceptación por parte de los peces.
Al final del experimento (154 días), la supervivencia de los peces fue de 86-88%. No se encontraron diferencias significativas en el crecimiento y los parámetros nutricionales con las diferentes dietas, ni en los parámetros biométricos, excepto que el his mostró diferencias significativas en la dieta control comparada con las otras dos, con un valor mayor (1.55%). Los valores de humedad, proteína, lípidos y contenido en ceniza no se vieron afectados por la sustitución de harina de pescado.
En lo referente a los filetes de pescado, se detectaron diferencias entre las distintas dietas (FM0, FM25 y FM100) para los parámetros fisicoquímicos (pH, luminosidad, dureza, masticabilidad, cohesividad, gomosidad, elasticidad y fuerza), para las propiedades mecánicas y en las propiedades ópticas, la diferencia de color (ΔE).
En cuanto al test sensorial de los filetes crudos y cocinados, los jueces no discriminaron entre las dos dietas probadas (FM0 y FM100).

Palabras clave: Sparus aurata, harina de pescado, proteína vegetal, calidad del pescado, prueba sensorial

1- INTRODUCTION

From a nutritional stand point, the protein in the diet is the most important ingredient in fish feed (30-60% dry weight), using fish meal in greater proportion in the same. Decline in its production is performed to replace it by animal or vegetable meals in order to reduce costs in diets and ensure the supply of high quality feed and relatively stable, not dependent on supply issues, quality and price fluctuation fish meal (Robaina *et al*., 1995).

During last years, different European researchers have studied the way to develop efficient sustainable and economic aqua feeds using alternative ingredients, mainly in the four carnivorous species, Atlantic salmon (*Salmo salar*), Rainbow trout (*Oncorhynchus mikiss*), Gilthead sea bream (*Sparus aurata*) and Mediterranean sea bass (*Dicentrarchus labrax*) farmed under intensive production system, but common carp (*Cyprinus carpio*) has been produced in extensive pond with natural feed.

In salmon, total substitution of fish meal has not reaching, but good results of growth were obtained with only 10% fish meal in diets (Johnsen *et al*., 2011), but in other trials, this level gave a poorer growth (Tortensen *et al*., 2008; Pratoomyout *et al*., 2010). Although these works have not show the dietary levels of EAA, it seems that mixture of plants used by Johnsen *et al*. (2011) were more effective.

In trout, mixtures of several ingredients gave the best results (Gomes *et al*., 1995; Adelizi *et al*., 1998; Watanabe *et al*., 1998; Yamamoto *et al*., 2002; Gaylord *et a*l., 2006; Oo *et al*., 2007; Santiagosa *et al*., 2008; Panserat *et al*., 2008, 2009; Lee *et al*., 2010). Some successes has been obtained in trout of ration size (250 g) in diets without fish meal using taurine as suplementation (Gaylord *et al*., 2006; Lee *et al*., 2010), but in other trials the only suplementation with EAA was not enough (Gomes *et al*., 1995; Santiagosa *et al*., 2008). Total replacing of fish meal origined a lower growth (Panserat *et al*., 2008, 2009).

In sea bass different single materials has been considered, as dehulled pea seed meal (Gouveia & Davies, 1998, 2000), brewers yeast (Oliva-Teles & Goncalves, 2001), soy bean meal (Tibaldi *et al*., 2006), hazelnut meal (Emre *et al*., 2008), but few trials exit using mixtures (Kaushik *et al*., 2004; Adamidou *et al*., 2009).

The main plant protein source rehearsed in the sea bream feed has been soy bean, both integral flour, cake and protein concentrates ((Robaina *et al*., 1995, 1997), Nengas *et al*., 1996; Kissil *et al*., 2000; Kissil & Lupatsch 2002; Ceulemans *et al*., 2003; Martínez-Llorens *et al*., 2007; Bonaldo *et al*., 2008; Martínez-Llorens *et al*., 2009), due to its high availability in world markets. Other individual plant sources such as lupin, gluten, peas, canola (Robaina *et al*., 1995, 1997; Kissil *et al*., 2000, Kissil & Lupatsch., 2002; Pereira & Oliva-Teles, 2002, 2003, 2004; Sánchez-Lozano *et al*., 2007, 2010) have been assayed. In general, results indicate that up to 30-40% of different protein sources such as single ingredient, inclusions give good growth. However, published works have been performed with small fish and for short periods, and generally fish did not achieve commercial weight, by which the economic analysis of replacement levels and analyze sensory quality of the meat were not made.

In other works have been studied corn gluten (Robaina *et a*l., 1997, Pereira and Oliva-Teles, 2003), lupine flour (Pereira and Oliva-Teles, 2004), pea flour (Pereira and Oliva-

Teles, 2002), rapeseed meal (Gómez-Requeni *et al*., 2004), rapeseed protein concentrate (Kissil *et al*., 2000), soybean (Lupatsch *et al*., 1997; Robaina *et al*., 1995; Ceulemans *et al*., 2003; Martinez-Llorens *et al*., 2007) and soy protein concentrate (Kissil *et al*., 2000) which has been the most studied protein source in this species, but has not achieved a similar growth as obtained with a control diet, prepared with fish meal as the sole protein source.

However, best results were obtained with mixtures of different ingredients, 50-75% with only 130 - 200 g/kg of fish meal (Gómez-Requeni *et al*., 2004; De Francesco *et al*., 2007; Dias *et al*., 2009; Sánchez-Lozano *et al*., 2009), and exceptionally reaching 95-100% in some cases (Kissil & Lupastch *et al*., 2004, Tomás *et al*., 2011).

In general, 50% inclussion of alternative protein mixtures, with 18-20 % fish meal, gave goods results (De Francesco *et al*., 2007; Dias *et al*., 2009). It seems reasonable to think that synergy obtained with the mixture of different protein sources is due to the complementary of amino acids present in several ingredients. In this sense, in the future must be made greater efforts to determine quantitative amino for formulating diets correctly.

Furthermore, it is described that higher levels of plant proteins inclusion has a negative effect on feed intake. This reduction in feed intake is generally associated with the lower biological value and palatability properties of vegetal sources, thus in some studies, palatability was potentiated by the addition of different substances, such nucleotides or nucleosides, free amino acids, organic acids and bile salts (Carr *et al*., 1996). On the contrary, Fournier *et al*. (2004) reported that even though the amino acids were balanced with crystalline amino acids, feed intake was significantly decreased by incorporation of plant protein to the diet for juvenile turbot and reduced growth was obtained when less than 10% FM was included. Other products such as oil krill (*Euphausia pacifica*) and squid also have been considered for their high nutritional quality, as they are rich sources of essential fatty acids, phospholipids, and carotenoids (Kang *et al*., 2005; Maki *et al*., 2009). Krill and squid and another feed attractants have been characterised and isolated from different marine organisms such as mussels, clam and brine shrimp or fish protein hidrolysates, FPH (Refstie *et al*., 2004; Espe *et al*., 2006; Tomás *et al*., 2011). Microorganisms as *Schizochytrium spp*., *Crypthecodinium cohnii* and *Phaeodactylum tricornutum* have been used as food component to gilthead sea bream (Atalah *et al*., 2007; Ganuza *et al*., 2008). Other microorganisms and copepods also appear to provide good results in feeds for salmon farming (Olsen *et al*., 2004; Miller *et al*., 2007). However, the production cost of such oils is extremely high, reducing its commercial potential and difficulty in large-scale use. Authors as Espe *et al*. (2006) and Torstensen *et al*. (2008) recommended the use of attractants, since in many studies in which diets used replacing fish meal, produced lower growth rates and worse conversion rates because in most cases there is a smaller feeding by the fish. Therefore commented attractants are used as they are efficient in improving the palatability.
Also, the maximum benefit from feeding can only be achieved if the food provided is ingested. The use of dietary feeding stimulants is therefore essential to elicit an acceptable feeding response.

Equally important is that these protein sources do not change physicochemical properties and sensory quality of fish in order to evaluate the meat quality of

aquaculture products, is very important to use a multidisciplinary approach, including physicochemical and sensory properties, which depends mainly on the chemical composition of the fish, not to mention their quality health.

Despite being the sea bream one of the most consumed fish in the Mediterranean, there are few studies evaluating the effect of the fish meal replacement by plant protein sources on the sensory quality of fillet (De Francesco *et al*., 2007; Martínez-Llorens *et al*., 2009; Sánchez -Lozano *et al*., 2007 and 2010).

The aim of this work was to study the possibility of total replacing fish meal by a plant protein mixture in gilthead sea bream extruded diets to optimize the growth, feed profitability and to evaluate fillet quality.

2- MATERIALS AND METHODS

2.1. Production system

The trial was conducted in 9 cylindrical fibreglass tanks (1750 L) within a recirculating saltwater system (65 m^3 capacity) with a rotary mechanical filter and a gravity biofilter (approximately 6 m^3). All tanks were equipped with aeration. The water was heated by a heat pump installed in the system.

During the experiment, the temperature was 23 ±1ºC, dissolved oxygen was 6 mg L^{-1}, salinity was 37-38 g L^{-1}, pH was 7.5 and ammonium value was 0.0 mg L^{-1}. Photoperiod was natural throughout the experimental period and all tanks had similar lighting conditions.

2.2. Fish and experimental design

The test lasted 154 days (from November 2012 to April 2013). The experiment ended when the fish reached market weight. The gilthead sea bream (*Sparus aurata*) were brought from a local marine fish farm (Piscimar S.A., Burriana (Castellón), Spain), transported alive to the Fish Nutrition Laboratory of the Polytechnic University of Valencia (Spain) and randomized into experimental tanks (20 per tank). All fish were weighed every 30 days. Previously, fish were anesthetized with 30 mg L^{-1} clove oil (Guinama ®, Valencia, Spain), containing 87% of eugenol.

Before the feeding trial, all fish were acclimated to the indoor rearing conditions for 2 weeks and fed a standard diet sea bream (48% crude protein, CP, 23% crude lipid, CL, 11% of ash, 2.2% crude fiber, CF, and 14% nitrogen free extract, NFE).

At the end of the growth trial, all fish were individually weighed and five fish from each tank were used for the determination of the biometric parameters and for immediate analysis and other five for fillet fish evaluation.

2.3. Diets and feeding

Three isonitrogenous (45% crude protein) and isolipidic diets (20% crude lipid) were formulated with fish meal, fish oil and soybean oil, wheat and wheat gluten, faba bean meal, soybean meal, pea meal, sunflower meal (plant sources), krill and squid meal, as attractants, and calcium phosphate, L-methionine, lysine, minerals and vitamins. Also added 2% Taurine because fish meal contains large amounts and can be detrimental (Table 1). The amino acid composition is shown in table 2.

Table 1. Formulation and proximate composition of the experimental diets.

	Diets		
Ingredients (g kg $^{-1}$)			
	FM100	**FM 25**	**FM 0**
Fish meal herring	589	150	
Wheat	260	60	
Wheat gluten		105	202
Faba bean meal		25	40
Soybean meal		132	160
Krill meal		50	50
Pea meal		25	25
Sunflower meal		132	160
Fish oil	65	78	90
Soybean oil	66	66	65
Squid meal		100	100
Mono calcium phosphate		27	38
Soybean Lecithin	10	10	10
Taurine		20	20
L-Methionine		5	5
Lysine		5	10
Multivitamin and minerals mix [1]	10	10	10
Proximate composition (g kg $^{-1}$ dry weight matter)			
Dry matter DM	881	902	928
Crude protein CP	442	445	446
Crude lipid CL	185	201	200
Ash	101	101	88
Carbohydrates[2]	271	252	265

[1]Vitamin and mineral mix (values are g kg^{-1} except those in parenthesis): Premix: 25; Choline, 10; DL-a-tocopherol, 5; ascorbicacid, 5; $(PO_4)_2Ca_3$, 5. Premix composition: retinol acetate, 1 000 000 IU kg^{-1}; calcipherol, 500 IU kg^{-1}; DL-a-tocopherol, 10;menadione sodium bisulphite, 0.8; thiamine hydrochloride, 2.3;riboflavin, 2.3; pyridoxine hydrochloride, 15; cyanocobalamine, 25;nicotinamide, 15; pantothenic acid, 6; folic acid, 0.65; biotin, 0.07;ascorbic acid, 75; inositol, 15; betaine, 100; polypeptides 12.

[2] Carbohydrates, CHO (%) =100-%CP-%CL-%Ash

Table 2. Amino acid composition of experimental diets in dry matter.

	Experimental diets		
	FM 100	**FM 25**	**FM 0**
EAA (g 100g $^{-1}$)			
Arginine	3.39	3.86	3.58
Histidine	1	0.81	0.81
Isoleucine	1.47	1.24	1.08
Leucine	3.24	3.11	2.45
Lysine	3.68	2.78	2.38
Methionine	1.16	1.06	1.05
Phenylalanine	1.8	1.78	1.76
Threonine	1.98	1.5	1.28
Valine	2.01	1.6	1.32
NEAA (g 100g $^{-1}$)			
Alanine	2.74	2.14	1.66
Aspartate	4.29	3.68	3.09
Cystine	0.61	0.62	0.67
Glutamine	7.64	9.66	10.86
Glycine	2.44	2.28	2.09
Proline	2.3	2.72	3.49
Serine	2.11	1.96	1.87
Tyrosine	1.5	1.02	1.02

EAA: Essential amino acids.
NEAA: Non-essential amino acids.

Diets were prepared by cooking extrusion processing with a semi-industrial twin-screw extruder (CLEXTRAL BC-45, St. Etienne, France). The processing conditions were as follows: 100 rpm speed screw, 110 ºC temperatures, and 40-50 atm pressure and 3-5 mm diameter pellets. Experimental diets were assayed in triplicate groups.
Fish were fed by hand twice a day to apparent satiation. Pellets were distributed slowly, allowing all fish to eat.

2.4. Biochemical analysis

Composition of diets and fish body composition were analysed following AOAC (1990) procedures: dry matter (105 ºC to constant weight), ash (combusted at 550 ºC to constant weight), crude protein (N x 6.25) by the Kjeldahl method after an acid digestion (Kjeltec 2300 Auto Analyser, Tecator Höganas, Sweden) and crude lipid extracted with dichloromethane-methanol (Soxtec 1043 extraction unit, Tecator). All analyses were performed in triplicate.

2.5. Fillet fish evaluation

The physicochemical properties analyzed in crude fillets were colour, pH, texture and moisture. Three points were chosen to measure the colour and at each of these points two measurement was taken (total of 6 measurements per fillet) with. Colour parameters

were measured using a Minolta Spectrophotometer model CM-700d (Minolta Camera Co, Osaka, Japan). Colour system employed was CIE L*a*b* (1976) with 10 ° observer and illuminant D65 (UNE 40-080, 1984). Colour parameters measured were lightness (L*), redness (a*) indicates the chromaticity from green (-) to red (+), yellowness (b*) indicates the chromaticity from blue (-) to yellow (+). Colour difference (ΔE) was determined with respect to a pattern, in this case FM100, according to equation 1. The Chroma (C*) of the fillets was calculated according to equation 2 (Schubring, 2009). The colorimeter was standardized using a white calibration plate.

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

$$C^{*} = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

The pH was measured with a portable pHmeter micropH 2001 (Crison Instruments, S.A., Barcelona, Spain) with a puncture electrode (Crison 5231) in six steak, each in a different area.

Textural properties were measured by simple and double compression (TPA) using a Texture Analyzer RT. XT. Plus (Stable MicroSystems, UK) with a load cell of 50 kg. Top´s fillets (area nearest to the head) were used to perform the texture analysis. The samples of 50mm x 50mm and thickness from 10 to 15 mm, with skin left on, were placed with se skin underneath on platform (Stable Micro Systems). In simple compression test, the samples were compressed with a 45 mm diameter cylindrical probe. The test conditions were prestest speed 1 mm/s, test speed 2 mm/s, posttest speed 10 mm/s and 90% of compression. The measured parameter was force (N). In the TPA test, the instrument was equipped with a 6 mm diameter cylindrical probe. The test conditions involved two consecutive cycles of 30% compression with 5 seconds between cycles. The pretest speed, test speed and posttest were 1, 1 and 5 mm/s, respectively. The measured parameters were hardness (N), adhesiveness (N.s), springiness, cohesiveness, chewiness and gumminess. Measurements were performed on each fillet in a position corresponding to the highest thickness.

The moisture content was determined by oven drying at 105 ºC for 20-24 h or until constant weight (AOAC, 1997). Determinations were performed in triplicate.

The effect of diet on the sensory properties of the fish fillets was studied by comparing fish fed the diet without growth differences FM100 and FM0. For sensory analysis, a hedonic scale was employed for raw and cooked samples. Attributes were rated from 1 to 5.

In raw fillets, the attributes analyzed were: smell, sea smell where 1 be very intense and five non-existent smell), smell degradation (non-existent be 5 and 1 very intense), strange smells (very intense be 5 to 1 non-existent unusual odor), within the colour, lightness (1 be very bright and five opaque) and whiteness (1 be very intense and 5 off). Texture were scored compactness (1 be very compact and 5 crumbled), water retention (1 be very wet and 5 dry), surface silt (1 be without sliminess and 5 evident presence of silt), separation chips (1 be no separation and 5 very apparent separation), elasticity (1 be very soft and 5 hard).

The cooked samples were prepared in a microwave oven to an internal temperature of 65 – 70ºC (CAC-GL 31-1999). The attributes of colour in cooked samples were lightness (translucent to 5 with 1 be opaque) and whiteness (1 be very intense and 5

off). Taste attributes analyzed were marine flavor (1 be very intense and 5 non-existent), degradation flavor (1 be non-existent and 5 intense) and strange flavor (1 be non-existent and 5 intense). In mouth feel, compactness (1 be very compact and 5 crumbled), water retention (1 be juicy and 5 dry), unctuous (1 be very fatty and 5 little fatty) and adhesiveness (1 be none adherent and 5 very adherent) were evaluated. In this study, a panel of 15 experts was used.

2.6. *Statistical analysis*

Growth data and physicochemical properties were treated using multifactor analysis of variance (ANOVA), introducing the initial live weight as covariate (Snedecor & Cochran 1971). Newman-Keuls test and LSD test was used to assess specific differences among individual's diets at 0.05 significant levels (Stat graphics, Statistical Graphics System, Version Plus 5.1, Herndon, Virginia, USA).

3- RESULTS

Figure 1 shows the weight evolution of gilthead sea bream (*Sparus aurata*) fed the three experimental diets. Animals fed the diet FM25 had a slightly high growth than the fish fed the other two diets.

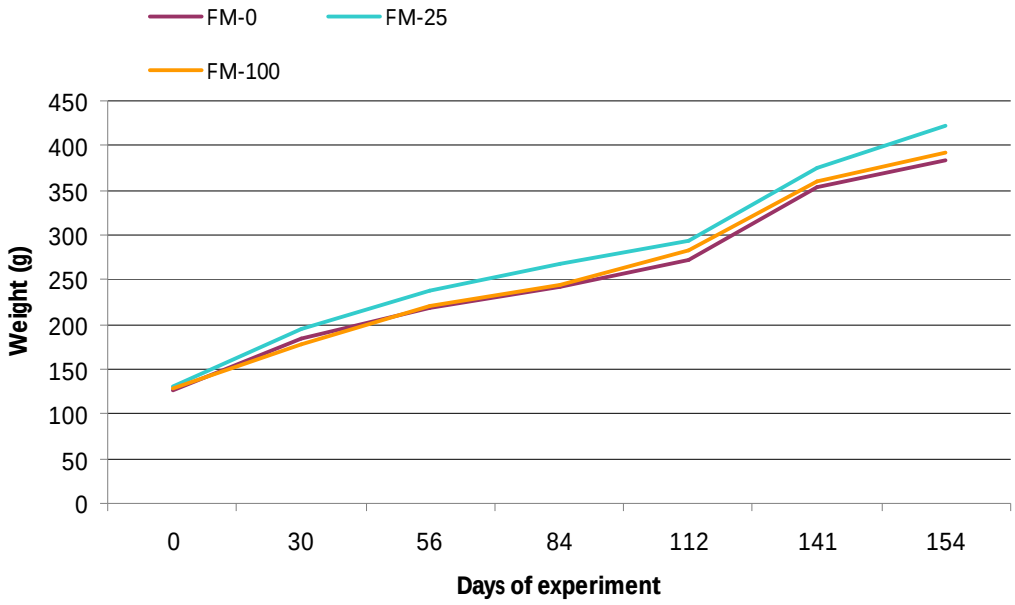

Figure 1. Evolution of weigth of sea bream with the experimental diets.

Survival rates were 86-88% and did not differ significantly between treatments. At the end of the growth period, weight and specific growth rate (SGR) showed no significant differences (Table 3). There were no differences in the feed intake (FI), feed conversion ratio (FCR) and protein efficiency ratio (PER).

Table 3. Main performances of gilthead sea bream fed experimental diets.

	FM 100	**FM 25**	**FM 0**	**SEM**
Initial weight (g)	129.5	130	125.5	3.54
Final weight (g)	393.7	423.7	383	10.86
Survival (%)	88.33	88.33	86.66	5.53
SGR (% day^{-1}) [1]	0.73	0.77	0.71	0.02
FI (g 100 g $fish^{-1}day^{-1}$)[2]	1.35	1.26	1.33	0.02
FCR[3]	2.14	1.90	2.08	0.09
PER[4]	1.07	1.19	1.082	0.039

Means of triplicate groups. Data in the same row with different superscripts differ at $P < 0.05$. SME: pooled standard error of the mean. Initial weight was considerer as covariable for final weight and SGR. Newman-Keuls test.

[1] Specific growth rate (%day^{-1}) SGR=100×ln(final weight/initial weight)/days.

[2] Feed Intake ratio (g 100 g $fish^{-1}day^{-1}$). FI=100×feed consumption (g)/average biomass (g)×days.

[3] Feed Conversion Ratio FCR=feed intake (g)/ weight gain (g).

[4] Protein Efficiency ratio PER=weight gain (Kg)/ ingested protein (Kg)

Respect biometric parameters, significant differences in hepatosomatic index were found; being HSI of fish fed FM100 the highest, with a value of 1.55%. There were no statistical differences in the other parameters (Table 4).
Regarding the composition of the whole body (Table 4), the parameters of moisture, protein, lipid, and ash content of the fish at the end of the growth trial were unaffected by the diet. Retention of ingested protein was similar in the three diets (Table 4).

Table 4. Biometric parameters, proximate composition and efficiency retention (expressed as percentage of wet weight, ww) of gilthead sea bream fed experimental diets.

	FM 100	**FM 25**	**FM 0**	**SEM**
CF [r]	1.92	1.89	1.77	0.05
N=24				
VSI (%) [s]	9.16	10.01	9.19	0.6
N=24				
HSI (%) [t]	1.55[a]	1.27[b]	1.31[b]	0.06
N=15				
VFI (%)[u]	1.5	1.24	1.76	0.21
N=15				
Moisture (%)	62.3	64	63	0.83
Crude protein (% *ww*)	17.6	17.8	18.3	0.4
Crude lipid (% *ww*)	16.1	14.9	14.9	0.45
Ash (% *ww*)	2.7	2.76	3.2	0.35
PIR (%) [w]	19.74	20.9	20.06	0.66

Means of triplicate groups. Data in the same row with different superscripts differ at $P < 0.05$. SME: pooled standard error of the mean.
Test de Newman-Keuls.
[r]Condition factor. CF=100×total weight (g)/total length (cm^3).
[s]Viscerosomatic index (%) VSI = 100×Visceral weight (g) / fish weight (g)
[t]Hepatosomatic index (%) HSI=100×liver weight (g)/ fish weight (g).
[u]Visceral fat index (%) VFI= 100 x Visceral fat (g) / fish weight (g)
[w] Efficiency retention of protein intake PIR=Fish Protein gain (g)/Protein intake (g)×100.

After obtaining the above results, we proceeded to analyze the effect of the diets on the physicochemical parameters of raw fillet fish by treatments. The selected parameters were: pH, colour, texture and moisture.

Respect pH measurements (Table 5) resulted significant differences between three diets, being fillet of fish fed FM25 diet with the highest value (6.36), followed fish fed FM100 (6.02) and FM0 (5.97).

The lightness (L*) was statistical different in fillet fish fed FM0 respect to the other two treatments, with a higher value (50.13).

The redness (a*), yellowness (b*) and Chroma (C*) was not significantly different ($p < 0.05$) between the different treatments.

Colour difference (ΔE) was statistical different in fillet fish fed FM0, with a higher value (4.005).

Table 5. pH and colour parameters of fillet fish.

	FM 100	FM 25	FM 0	SEM
pH	6.02[b]	6.36[a]	5.97[c]	0.014
L*	47.59[b]	48.22[b]	50.137[a]	0.455
a*	-3.277	-3.41	-3.573	0.135
b*	2.051	1.882	0.9429	0.545
Delta E	2.94[b]	3.48[ab]	4.005[a]	0.313
C*	11.293	10.946	10.626	1.627

Data in the same row with different superscripts differ at $P < 0.05$. SME: pooled standard error of the mean. (n = 24). LSD test.

Texture analysis (TPA) (Table 6): adhesiveness showed no significant differences among treatments. The chewiness showed significant differences among all treatments, with the highest value on the FM 0. As for the cohesiveness, FM 100 treatment showed significant differences compared to the other two treatment given, with a lower value.
Regarding the gumminess, significant differences among all treatments.
The hardness, FM 100 treatment showed significant differences compared to the other treatments, with a higher value (1.09).
With respect to the springiness, the FM 100 showed significant differences with respect to the other two treatments, with a lower value (0.25).

Table 6. Texture parameters of fillet gilthead sea bream fed the experimental diets.

	FM 100	FM 25	FM 0	SEM
Texture				
Adhesiveness (N.s)	- 0.022	- 0.021	- 0.022	0.0024
Chewiness	- 0.004[c]	0.306[b]	0.348[a]	0.01
Cohesiveness	- 0.015[b]	0.577[a]	0.585[a]	0.006
Gumminess	- 0.015[c]	0.458[b]	0.518[a]	0.014
Hardness (N)	1.094[a]	0.806[b]	0.89[b]	0.044
Springiness	0.257[b]	0.669[a]	0.67[a]	0.008

Data in the same row with different superscripts differ at $P < 0.05$. SME: pooled standard error of the mean. (n=24). LSD test.

Force (N) (Figure 2) with a 90% compression showed that treatment FM 100 had significant differences with respect to the other two treatments, taking a higher value (197).

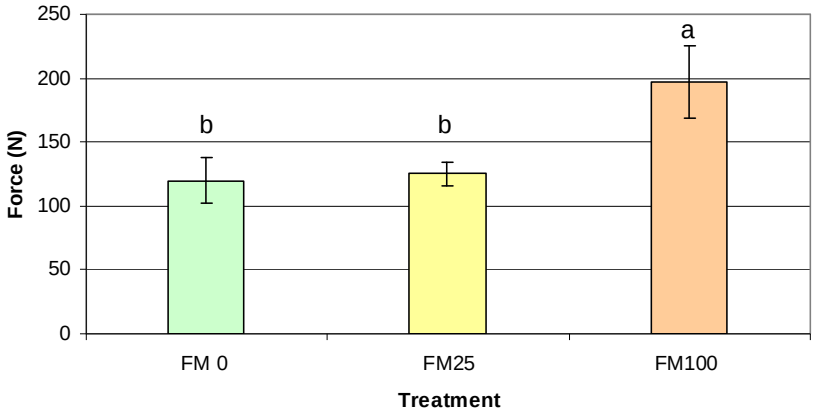

Figure 2. Results of force of the fish fillets. Test LSD. (n = 24). Significant differences: [a]High value. [b]Intermediate value.

Moisture (%) (Figure 3) showed no significant differences among the three treatments.

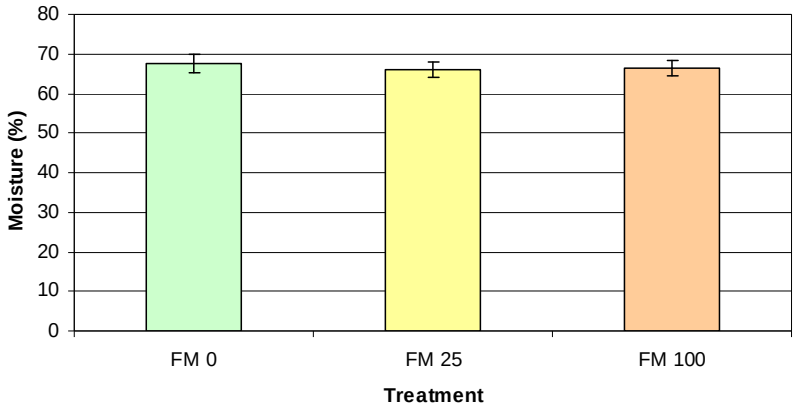

Figure 3. Moisture results of the fish fillets. Test LSD. (n = 24).

Regarding the sensory test (Figure 4 and 5), tasters found no significant differences ($p < 0.05$) between treatments FM 100 and FM 0, both raw and cooked samples, taking into account the attributes already set.

The attributes evaluated for raw samples were: sea smell, smell degradation, strange smells, lightness, whiteness, compactness, water retention, surface silt, separation chips and elasticity (Figure 4).

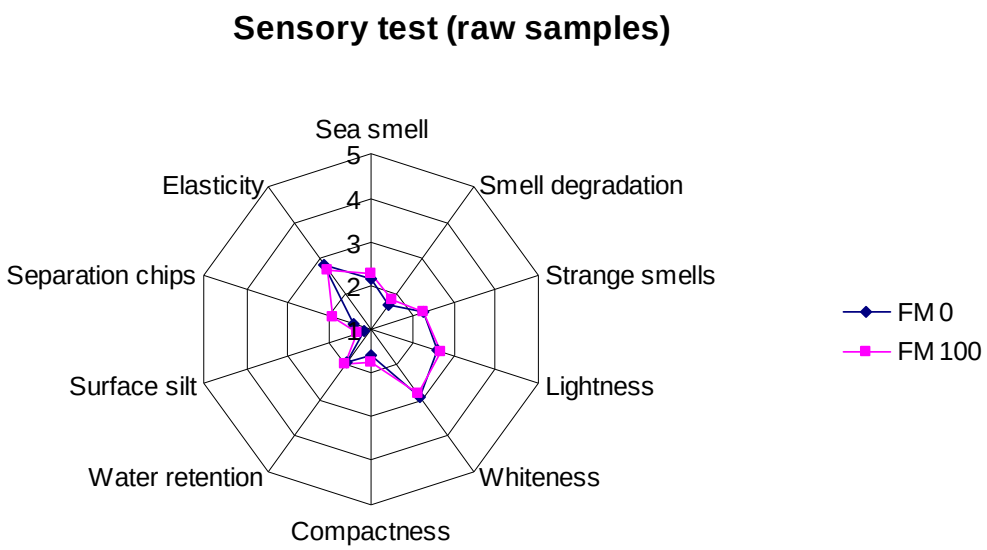

Figure 4. Sensory test of raw fish fillets. Test LSD.

The attributes evaluated for cooked samples were: lightness, whiteness, marine flavor, degradation flavor, strange flavor, compactness, water retention, unctuous and adhesiveness (Figure 5).

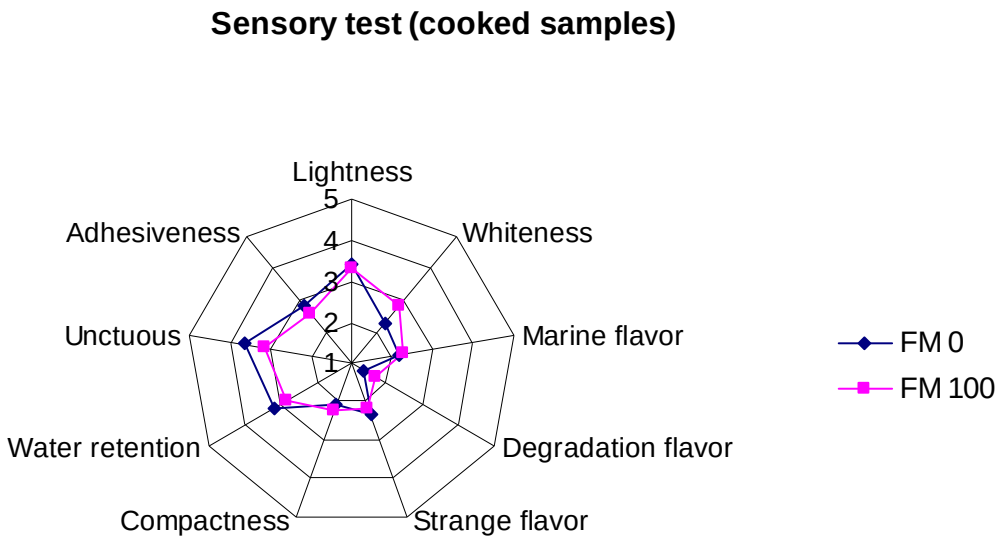

Figure 5. Sensory test of cooked fish fillets of gilthead sea bream. Test LSD.

4- DISCUSSION

Fish diets composition was made from a variety of feed ingredients to satisfy the nutrient requirements of the sea bream. The ingredients were incorporated as nutrients that contribute to improve the fish growth and health without the fishmeal utilization. For this reason, it was chosen the present plant proteins mixture, supplemented with synthetic amino acids (Peres and Oliva-Teles, 2009), as a substitute for fish meal in gilthead sea bream diets.

The most common alternatives for FM as protein source are different kinds of plant protein meals that vary in content of available nutrients, but by using a mixture of different ingredients (Burr *et al*., 2012; Slawski *et al*., 2012) and supplementing the diet with indispensable amino acids (Rodehutscord *et al*., 1995; Kaushik *et al*., 2004; Espe *et al*., 2007). What allows formulation of nutritionally complete diets with an inclusion level of FM as low as 10%. In the present study, gilthead sea bream fed a diet in which the vegetal mix replaced 100% of fish meal in the formulation grew the same than fish fed fish meal control diet; these results are in agreement with that conclusion.

The survival rate was 86-88% and found no significant difference between the three treatments, as in the studies by Tomás *et al*. (2011), although their survival was greater 91%. Like this, Kissil and Lupatsch (2004) with 100% of fish meal substitution in gilthead sea bream.

Final body weight of gilthead sea bream fed FM100, FM25 and FM0 were not significantly different among each other. Supplementation of EAA in the non-FM diets to match the amino acid profiles with that of the control FM diet, prove to be beneficial. FM based diets usually contain sufficient amounts of EAA at a level above the requirement of the fish; therefore, the need to balance the EAA profile rarely arises. Similar results were obtained by Kissil and Lupatch (2004) in younger fishes or Tomás *et al*. (2011), with the total fish meal replacement, with a vegetal mixture more deficient en amino acids (higher levels of amino acids synthetics).

About nutritional parameters, FI and FCR obtained in the test, no significant differences among the three treatments. Similar to the experiment of Tomás *et al*. (2011), in which diets a attractant natural (Krill meal) was added in the diets like in present work.
The lower growth rates and reduced feed conversion in fish fed diets FM-free diets is in most cases caused for a reduced feed intake. For prospective, it appears recommendable to use feed attractants. Fish protein hydrolysate, blood meal, squid hydrolysate, stick water or krill meal at dietary levels from 30 to 50 g kg-1, have shown to be effective feed attractants (potentially contributed to maintaining appetence) and sources of amino acids and minerals when diets low in FM were fed to carnivorous fish (Espe *et al*., 2006; Torstensen *et al*., 2008). In present study a minor inclusion of krill meal was added to all replacement diets to increase acceptability of the plant protein diets and no significant difference was found in nutrient utilisation when sea bream were fed increasing inclusions vegetal diets.

In the biometric parameters, differences were found in the hepatosomatic index that was higher in sea bream fed FM100 (1.55%). Also, it has been demonstrated that some changes in the diet may lead to accumulation of glycogen and fat in the liver, causing them to increase in size and weight with respect to what is considered a normal size.

Similar results were obtained by De Francesco *et al*. (2004). In this case, the hepatosomatic index was higher in trout fed the 100% FM diet (1.01%) that in the fish fed PP diet (plant protein mixture) (0.92%).

In terms of whole-body composition, moisture, protein, lipid, and ash contents of fish at the end of the growth trial were not affected by FM substitution like Tomás *et al*. (2011) with similar diets in commercial weight sea bream. In the study of Kissil and Lupatch (2004), lipid content increased while protein and ash showed little change. Differences in body composition did not significantly differ at the end of the trial.

The Retention efficiency of protein intake was unaffected by the three diets used, like in study of Tomás *et al*. (2011). This could confirm the correct design of the diets, respect amino acid composition.

In conclusion, thiese results show that the gilthead sea bream fed diets with and without fish meal has the similar growth. And confirm results obtained by Kissil and Lupatch (2004) and Tomás *et al*. (2011).

Regard to physicochemical fillet properties, pH values showed significant differences among the three treatments with values between 6-6.3. Casas Moreno (2009) shows that these values are within a normal range.

Found significant differences in diet FM0 lightness over the other two, in contrast to a similar study Matos *et al.* (2012) in a study of plant proteins and vegetable oil do not have detrimental effects on post-mortem muscle instrumental texture, sensory properties and nutritional value of gilthead sea bream, found no differences in either ventral or dorsal muscle lightness.
Izquierdo *et al*. (2005) in his study of alterations in fillet fatty acid profile and flesh quality in gilthead sea bream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding found a significant decrease in redness of the fillets, which is not in accordance with the present results, since no significant differences between the three treatments.
In this study, found no significant differences in yellowness and chroma between the three treatments, like in the study of Matos *et al.* (2012). However, in this case exhibit higher values.

Present work shows that high replacement of fish meal in diets affect instrumental texture parameters.
Moreover, Izquierdo *et al.* (2005) have reported a slight reduction in hardness of sea bream fed vegetable oils (60% replacement of fish oil), this effect was attributed to the higher lipid content in the fillet compared to sea bream fed the control diet. As in our case with FM25 and FM0, but in the present study, no such differences were found in lipid content of the fillets.
Hardness, chewiness and cohesiveness are technologically important characteristics of fish fillets. In this study, raw fillets adhesiveness showed no significant difference, while in the chewiness and cohesiveness in fillet of fish fed diet FM0 were the highest values. Hu *et al*. (2013) in a study of effects of fish meal quality and fish meal substitution by animal protein blend on growth performance, flesh quality and liver histology of Japanese sea bass (*Lateolabrax japonicus*) obtained the same results but for cooked fillets.

Also, gumminess and springiness for FM0 diet showed higher values than for the other two treatments.
In the study of Hu *et al*. (2013), the differences were attributed for flesh quality might be due to different experimental periods, feed composition or the species size used in the trials. But, in our case, the differences are only possible by the composition of the diet.
Moisture values showed no significant differences between all treatments, with values between 66-67%. These results are similar those obtained by Casas Moreno (2009), in one of the studies conducted that involves evaluating the effect of the incorporation of active ingredients by impregnation or spraying, on the physicochemical parameters relevant bream fillets stored in refrigeration, moisture patterns gave values around 75%. Moisture is related to the water holding capacity. In this case, gilthead sea bream fillets fed FM0 diet showed a higher value than those fed with the other two diets, like lightness, colour and some texture parameters. These parameters can be related to the water holding capacity, which in turn influences the diets. Also, diets influence the physicochemical properties of gilthead sea bream.

In fact, Sea bream fed with plant proteins could have a notable impact on parameters directly influencing the quality of fish such as colour and appearance.

In this trial, judges no detected sensory differences in FMO and FM100 diets. Similar results were obtained by De Francesco *et al*. (2007), who replaced 75% vegetable mixture and Sanchez-Lozano *et al*. (2007), who replaced 24% sunflower meal.
Sanchez-Lozano *et al*. (2009) with 60% of substitution with a vegetal mixture, while Martínez-Llorens *et al*. (2008) obtained statistical differences at first instance with 39% inclusion of soybean meal, although after re-feeding no differences were found.

5- ACKNOWLEDGEMENTS

Thanks to the teachers Ana Tomás y Mª Jesús Pagán, for the great help, information and advice provided in the experiment and project. Without them I could not have done.

Thanks to Raquel and Amparo for teaching me the techniques to analyze the parameters, for their patience and support. For caring and worrying about my.

Thanks to Andrés for all your help.

Thanks to all.

6- REFERENCES

Adamidou, S., Nengas, I., Alexis, M., Foundoulaki, E., Nikolopoulou, D., Campbell, P., Karacostas, I., Rigos, G., J. Bell, G., Jauncey, K., (2009). Apparent nutrient digestibility and gastrointestinal evacuation time in European sea bass (*Dicentrarchus labrax*) fed diets containing different levels of legumes. Aquaculture, 289, 106-112.

Adamidou, S., Nengas, I., Henry, M., Grigorakis, K., Rigos, G., Nikolopoulou, D., Kotzamanis, Y., J. Bell, G., Jauncey, K., (2009). Growth, feed utilization, health and organoleptic characteristics of European sea bass (*Dicentrarchus labrax*) fed extruded diets including low and high levels of three different legumes. Aquaculture, 293, 263-271.

Adelizi, P.D., Rosati, R.R., Warner, K., Wu, Y.V., Muench, T.R., White, M.R. & Brown, P.B., (1998). Evaluation of fish-meal free diets for rainbow trout, *Oncorhynchus mykiss*. Aquaculture Nutrition, 4, 255–262.

A.O.A.C., Association of official Analytical Chemists (1990) Official Methods of Analysis, 15th end. Association of Official Analytical Chemists, Arlington, VA, USA. 1298. pp.

A.O.A.C. (1997). Official methods of analysis of the Association of Official Analytical Chemists. En: W. Horwitz (Ed.). Association of Official Analytical Chemist, Washington, DC.

Atalah, E., Cruz, C.M.H., Izquierdo, M.S., Rosenlund, G., Caballero, M.J., Valencia, A., Robaina, L., (2007). Two microalgae *Crypthecodinium cohnii* and *Phaeodactylum tricornutum* as alternative source of essential fatty acids in starter feeds for sea bream (*Sparus aurata*). Aquaculture 270: 178-185.

Bonaldo, A., Roem, A. J., Fagioli, P., Pecchini, A., Cipollini, I. & Gatta, P.P., (2008). Influence of dietary levels of soybean meal on the performance and gut histology of gilthead sea bream (*Sparus aurata L.)* and European sea bass (*Dicentrarchus labrax L.)*. Aquaculture Research, 39, 970-978.

Burr, G.S., Wolters, W.R., Barrows, F.T. & Hardy, R.W., (2012). Replacing fishmeal with blends of alternative proteins on growth performance of rainbow trout (*Oncorhynchus mykiss*), and early or late stage juvenile Atlantic salmon (*Salmo salar*). Aquaculture, 334-337, 110-116.

CAC-GL 31 (1999) CODEX GUIDELINES FOR THE SENSORY EVALUATION OF FISH AND SHELLFISH IN LABORATORIES.

Carr, W.E.S., Netherton III, J.C., Glesson, R.A. & Derby, D.C., (1996). Stimulants of feeding behavior in fish: analysis of tissues of diverse marine organisms. Biological Bulletin 190, 149–160.

Casas Moreno, M., (2009). Estudio del efecto bioconservante y/o probiótico del *Enterococcus faecium* y nisina en filetes de dorada. Proyecto Final de Carrera, Escuela Técnica Superior del Medio Rural y Enología, Universidad Politécnica de Valencia.

Ceulemans, S., Van Halteren, A., Robles, R. & Coutteau, P., (2003). Fish Meal and Fish oil Replacement in Practical Diets for Gilthead sea Bream (*Sparus aurata*) With Nutritional Compensation. IX Congreso Nacional de Acuicultura, Cádiz, Spain, Book of Abstracts, 173.

De Francesco, M., Parisi, G., Medale, F., Lupi, P., Kaushik, S.J. & Poli, B.M., (2004). Effect of long-term feeding with a plant protein mixture based diet on growth and body/fillet quality traits of large rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 236, 413–429.

De Francesco, M., Parisi, G., Perez-Sanchez, J., Gomez-Requeni, F., Medale, F., Kaushik, S., Mecatti, M. & Poli, B.M., (2007). Effect of high-level fish meal replacement by plant proteins in gilthead sea bream (*Sparus aurata*) on growth and body/fillet quality traits. Aquaculture Nutrition, 13, 361–372.

Dias, J., Conceição, L.E.C., Ramalho Ribeiro, A., Borges, P., Valente, L.M.P. & Dinis, M.T., (2009). Practical diet with low fish-derived protein is able to sustain growth performance in gilthead sea bream (*Sparus aurata*) during the grow-out phase. Aquaculture, 293, 255–262.

Emre, Y., Sevgill, H., & Sanil, M., (2008). Partial Replacement of Fishmeal with Hazelnut Meal in Diets for Juvenile Gilthead Sea bream (*Sparus aurata*). The Israeli Journal of Aquaculture - Bamidgeh, 60(3), 198-204.

Espe, M., Lemme, A., Petri, A. & El-Mowafi, A., (2006). Can Atlantic salmon (*Salmo salar*) grow on diets devoid of fish meal? Aquaculture, 255, 255–262.

Espe, M., Lemme, A., Petri, A. & El-Mowafi, A., (2007). Assessment of lysine requirement for maximal protein accretion in Atlantic salmon using plant protein diets. Aquaculture, 263, 168–178.

Fournier, V., Huelvan, C. & Desbruyeres, E., (2004). Incorporation of a mixture of plant feedstuffs as substitute for fish meal in diets of juvenile turbot (*Psetta maxima*). Aquaculture, 236, 451–465.

Ganuza, E., Benítez-Santana, T., Atalah, E., Vega-Orellana, O., Ganga, R., Hernández-Cruz, C.M., Izquierdo, M.S., (2008). *Crypthecodinium cohnii* and *Schizochytrium sp*. as potential substitutes to fisheries-derived oils in sea bream (*Sparus aurata*) microdiets. Aquaculture 277, 109-116.

Gaylord, G., Teague, A.M. & Barrows, F.T., (2006). Taurine supplementation of all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). Journal World Aquaculture Society, 37, 509–517.

Gomes, E.F., Rema, P. & Kaushik, S.J., (1995). Replacement of fish meal by plant proteins in the diet of rainbow trout (*Oncorhynchus mykiss*): digestibility and growth performance. Aquaculture, 130, 177–186.

Gómez-Requeni, P., Mingarro, M., Calduch-Giner, J.A., Médale, F., Martin, S.A.M., Houlihan, D.F., Kaushik, S. & Pérez-Sánchez, J., (2004). Protein growth performance, amino acid utilization and somatotropic axis responsiveness to fish meal replacement by plant protein sources in gilthead seabream (*Sparus aurata*). Aquaculture, 232, 493–510.

Gouveia, A., & Davies, S.J., (1998). Preliminary nutritional evaluation of pea seed meal (*Pisum sativum*) for juvenile European sea bass (*Dicentrarchus labrax*). Aquaculture 166, 311-320.

Gouveia, A., & Davies, S.J., (2000). Inclusion o fan extruded dehulled pea seed meal in diets for juvenile European sea bass (*Dicentrarchus labrax*). Aquaculture 182, 183-193.

Helrich, K. (Ed.)., (1997). Official Methods of Analysis of AOAC International. 15th ed. Arlington, VA: Association of Official Analytical Chemists International.

Hu, L., Yun, B., Xue, M., Wang, J., Wu, X., Zheng, Y., Han, F., (2013). Effects of fish meal quality and fish meal substitution by animal protein blend on growth performance, flesh quality and liver histology of Japanese sea bass (*Lateolabrax japonicus*). Aquaculture, 372-375, 52-61.

Izquierdo, M.S., Montero, D., Robaina, L., Caballero, M.J., Rosenlund, G., Gines, R., (2005). Alterations in fillet fatty acid profile and flesh quality in gilthead sea bream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. Aquaculture 250, 431–444.

Johnsen, C.A., Hagen, O., Bendiksen, E.A., (2011). Long-term effects of high-energy, low-fishmeal feeds on growth and flesh characteristics of Atlantic salmon (*Salmo salar L.*). Aquaculture, 312, 109-116.

Kang, K.Y., Ahn, D.H., Wilkinson, G.T., Chun, B.S., (2005). Extraction of lipids and cholesterol from squid oil with supercritical carbon dioxide. Korean Journal of Chemical Engineering 22: 399-405.

Kaushik, S.J., Covès, D., Dutto, G. & Blanc, D., (2004). Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchux labrax*. Aquaculture, 230, 391–404.

Kissil, G.W., Lupatsch, I., Higgs, D.A. & Hardy, R.W., (2000). Dietary substitution of soy and rapeseed protein concentrates for fish meal, and their utilization in gilthead seabream *Sparus aurata L.* Aquaculture. Res., 31, 595–601.

Kissil, G.W. & Lupatsch, I. (2002) Preliminary Results of Fish Meal Replacement in Gilthead sea Bream (Sparus Aurata) by Three Plant Proteins. 10 Symposium on Nutrition and Feeding of Fish, Rhodes, Grecian. Book of Abstracts, 127.

Kissil & Lupastch., (2004). Successful replacement of fishmeal by plant proteins in diets for the gilthead seabream, *Sparus aurata L.* Aquaculture, 56 (3), 188-199.

Lee, K-J., Madison S. Powell, Frederick T. Barrows, Scott Smiley, Peter Bechtel, Ronald W. Hardy, (2010). Evaluation of supplemental fish bone meal made from Alaska seafood processing by products and dicalcium phosphate in plant protein based diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 302, 248-255.

Lidia Esther Robaina Robaina (1995) Utilización nutritiva de fuentes de proteína alternativa a la harina de pescado en dietas de engorde para dorada (*Sparus aurata*). Universidad de las Palmas de Gran Canaria. Departamento de Biología.

Lupatsch, I., Kissil, G.W., Sklan, D., Pfeffer, E., (1997). Apparent digestibility coefficients of feed ingredients and their predictability in compound diets for gilthead sea bream, *Sparus aurata L.* Aquaculture Nutrition, 3: 81-89.

Maki, K.C., Reeves, M.S., Farmer, M., Griinari, M., Berge, K., Vik, H., Hubacher, R., Rains, T.M., (2009). Krill oil supplementation increases plasma concentrations of eicosapentaenoic and docosahexaenoic acids in overweight and obese men and women. Nutrition Research 29: 609-615.

Martínez-Llorens, S., Moñino, A.V., Tomás, A., Moya, V.J., Pla, M. & Jover, M., (2007). Soybean meal as a protein source in gilthead sea bream (*Sparus aurata L.*) diets: effects on growth and nutrient utilization. Aquaculture Research, 38, 82-90.

Martínez-Llorens, S., Tomas-Vidal, A., Jauralde, I., Pla, M. & Jover Cerdá M., (2009). Optimum dietary soybean meal level for maximizing growth and nutrient utilization of on-growing gilthead sea bream (*Sparus aurata*). Aquaculture Nutrition, 15, 320-328.

Matos, E., Gonçalves, A., Bandarra, N., Colen, R., Leonor Nunes, M., M.P. Valente, L., Teresa Dinis, M., Dias, J., (2012). Plant proteins and vegetable oil do not have detrimental effects on post-mortem muscle instrumental texture, sensory properties and nutritional value of gilthead seabream. Aquaculture, 358-359, 205-212.

Miller, M.R., Nichols, P.D., Carter, C.G., (2007). Replacement of fish oil with thraustochytrid Schizochytrium sp L oil in Atlantic salmon parr (*Salmo salar L.*) diets. Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology 148: 382-392.

Nengas, I., Alexis, M. & Davies, S.J., (1996). Partial substitution of fish meal with soybean meal products and derivatives in diets for the gilthead bream, *Sparus aurata* (L.). Aquaculture Research, 27, 147-156.

Oliva-Teles, A. and P. Goncalves, (2001). Partial replacement of fish meal by brewers yeast (*Saccaromyces cerevisae*) in the diets for sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture, 202: 269-278.

Olsen, R.E., Henderson, R.J., Sountama, J., Hemre, G.I., Ringø, E., Melle, W., Tocher, D.R., (2004). Atlantic salmon, *Salmo salar*, utilizes wax ester-rich oil from *Calanus finmarchicus* effectively. Aquaculture 240: 433-449.

Oo, A.N., Higgs, D.A., Huang. S.S.Y., Brauner, C.J., Satoh, S., (2007). Effect of dietary canola oil level on the growth performance and fatty acid composition of juvenile red sea bream, *Pagrus major*. Aquaculture, 271, 420-431.

Panserat, S., Ducasse-Cabanot, E. Plagnes-Juan, P.P. Srivastava, C. Kolditz, F. Piumi, D. Esquerré, S. Kaushik, (2008). Dietary fat level modifies the expression of hepatic genes in juvenile rainbow trout (*Oncorhynchus mykiss*) as revealed by microarray analysis. Aquaculture, 275, 235-241.

Panserat, S., G.A. Hortopan, E. Plagnes-Juan, C. Kolditz, M. Lansard, S. Skiba-Cassy, D. Esquerré, I. Geurden, F. Médale, S. Kaushik, G. Corraze, (2009). Differential gene expression after total replacement of dietary fish meal and fish oil by plant products in rainbow trout (*Oncorhynchus mykiss*) liver. Aquaculture 294, 123-131.

Pereira, T. G. & Oliva-Teles, A., (2002) Preliminary evaluation of pea seed meal in diets for gilthead sea bream (*Sparus aurata*) juveniles. Aquaculture Research, 33, 1173-1179.

Pereira, T.G. & Oliva-Teles, A., (2003). Evaluation of corn gluten meal as a protein source in diets for gilthead sea bream (*Sparus aurata L.*) juveniles. Aquaculture Research, 34, 1111–1117.

Pereira, T.G. & Oliva-Teles, A., (2004). Evaluation of micronized lupin seed meal as an alternative protein source in diets for gilthead sea bream (*Sparus aurata*) juveniles. Aquaculture Research, 35, 828–835.

Peres, H., Oliva-Teles, A., (2009). The optimum dietary Essentials amino acid profile for gilthead sea bream (*Sparus aurata*) juveniles. Aquaculture, 296, 81-86.

Pratoomyot, J., E.Å. Bendiksen, J.G. Bell, D.R. Tocher., (2010). Effects of increasing replacement of dietary fishmeal with plant protein sources on growth performance and body lipid composition of Atlantic salmon (*Salmo salar L.*). Aquaculture, 305 (2010), pp. 124–132.

Refstie, S.,Ollic, J.J. & Standald, H., (2004). Feed intake, growth, and protein utilisation by post-smolt Atlantic salmon (*Salmo salar*) in response to graded levels of fish protein hydrolysate in the diet. Aquaculture, 239, 331–349.

Robaina, L., Izquierdo, M.S., Moyano, F.J., Socorro J., Vergara, J.M., Montero, D. & Fernandez-Palacios, H., (1995). Soybean and lupin seed meals as protein sources in diets for gilthead seabream (*Sparus aurata*): nutritional and histological implications. Aquaculture, 130, 219-233.

Robaina, L., Moyano, F.J., Izquierdo, M.S., Socorro, J., Vergara, J.M. & Montero, D., (1997). Corn gluten and meat and bone meals as protein sources in diets for gilthead sea bream (*Sparus aurata*). Nutritional and histological implications. Aquaculture, 157, 347-359.

Rodehutscord M., Mandel S., Pack M., Jacobs S. and E. Pfeffer, (1995). Free amino acids can replace protein-bound amino acids in test diets for studies in rainbow trout (*Oncorhynchus mykiss*). J. Nutr., 125:956- 963.

Sánchez-Lozano, 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.B., Martinez-Llorens, S., Tomas-Vidal, A., Cerda, M.J., (2010). 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.

Santigosa, E., Sánchez, J., Médale, F., Pérez-Sánchez, J., Gallardo, M.A., (2008). Modifications of digestive enzymes in trout (*Onchorynchus mykiss*) and sea bream (*Sparus aurata*) in response to dietary fish meal replacement by plant protein sources. Aquaculture 252, 68–74.

Schubring, R., (2009). Differential scanning calorimetry. In: Rehbein, H., Oehlenschlager, J. (Eds.), Fishery Products — Quality, Safety and Authenticity.Wiley Blackwell, Oxford, pp. 173–213.

Slawski, H., Adem, H., Tressel, R.P., Wysujack, K., Koops, U., Kotzamanis, Y., Wuertz, S. & Schulz, C., (2012) Total fish meal replacement with rapeseed protein concentrate in diets fed to rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquaculture International DOI 10.1007/s10499-011-9476-2.

Snedecor, G. & Cochran, W. (1971) Statistical methods. The lowa State University Press Ames. lowa, USA. 593pp.

Tibaldi, E., Hakim Y., Uni Z., Tulli F., de Francesco M., Luzzana U., and 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, 2006/11/16, Volume 261, Issue 1, p.182-193.

Tomas, A., Martínez-Llorens, S., Jesus-Moriñigo, J.V., Planas B. y M. Jover. (2011). ¿Piensos sin harina de pescado? Sí es posible. Libro Resúmenes XIII CNA. Castelldefels.

Torstensen, B.E., Espe, M., Sanden, M., Stubhaug, I., Waagbø, R., Hemre, G.I., Fontanillas, R., Nordgarden, U., Hevrøy, E.M., Olsvik, P. & Berntssen, M.H.G., (2008). Novel production of Atlantic salmon (*Salmo salar*) protein based on combined replacement of fish meal and fish oil with plant meal and vegetable oil blends. Aquaculture, 285, 193–200.

Watanabe, T. ; Verakunpiriya, V. ; Watanabe, K. ; Viswanath, K. ; Satoh, S., (1998). Feeding of rainbow trout with non-fish meal diets. Fish Sci., 63 (2): 258-266

Yamamoto, T., Shima, T., Furuita, H. & Suzuki, N., (2002). Influence of feeding diets with and without fish meal by hand and by selffeeders on feed intake, growth and nutrient utilization of juvenile rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 214, 289–305.