Brewery by-products (yeast and spent grain) as protein sources in gilthead seabream (*Sparus aurata*) feeds

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Abstract

Two trials were conducted to test the effect of partial replacement of fishmeal by two brewery industry by-products, yeast and spent grain, included in isoproteic and isolipidic diets for gilthead sea bream (*Sparus aurata*), having in mind the commercial availability of these by-products. According to the obtained results, the inclusion of up to 30 % brewers' spent yeast and 15 % spent grain in the feed for gilthead seabream gave similar results in terms of growth, food conversion and fillet final composition to a feed with fish meal as the main protein source and show a protein digestibility of 89-95 %. Taking into account that these by-products are produced in large quantities in Europe, they can be a potential source of protein to reduce the use of plant proteins or fish/animal by-products (trimmings) and increase the sustainability of both sectors, brewery industry and aquaculture.

Introduction

According to the Food and Agriculture Organization (FAO) of the United Nations 2020, aquaculture accounted for around 46 % of total food production and 52 % of fish for human consumption. Besides, aquatic species farming is one of the fastest-growing food sectors with 82.1 million tonnes of finfish produced in 2018 (47 million of inland fish aquaculture and 7.3 million of marine and coastal farming).

According to Gatlin et al. (2007) the economic and environmental sustainability of aquaculture depends on the identification and application of alternative raw materials to fishmeal, with highly digestible nutrients that improve fish performance, less waste production, available in the market in large regular quantities and at a competitive price. Alternative protein sources to fishmeal have been used in diets, although some of them such as vegetable proteins derived from grains, legumes or vegetable oils, have anti-

nutritional factors, fibre, insoluble carbohydrates, amino acid imbalances and low palatability that limit their use and increase waste produced in the fish farms increasing the environmental impact of aquaculture (Muzquiz & Wood, 2009; Naylor et al., 2009). Other ingredients such as microalgae (Sarker et al., 2020) or insect meal (Cardinaletti et al., 2019; Randazzo et al., 2021a; Randazzo et al., 2021b) have been also used showing a high potential in aquafeed industry.

On the other hand, terrestrial animal by-products have a better nutritional composition and are available at low cost in markets but are not considered acceptable by consumers (Naylor et al., 2009). Thus, the economic and environmental interest in industrial by-products recovery to be used as alternative ingredients in feed and food, has increased significantly in recent years due to its high and continuous production and its future perspective with great availability at a reduced cost (Barrows et al. al., 2008).

The brewer industry produces more than 1.95 billion hectolitres of beer worldwide (Statista, 2019) and generates large amounts of waste that can be used as feed ingredients for their high nutritional value (Aliyu & Bala, 2011). Both brewer spent yeast (BSY) and grain (BSG) have been categorized as high quality ingredients (Mussatto, et al., 2006; Thomas & Rahman, 2006; Mussatto, 2009; Levic et al., 2010; Robertson et al., 2010; Zhou et al., 2018) and breweries have been seeking different ways to minimize losses and optimize production.

In Europe 40 billion litres of beer were produced in 2018 (Eurostat, 2019) generating 7 million tons of BSG and 0.9 million tons of BSY that are mostly reused as animal feed and bioethanol production (Djuragic et al, 2010; Buffington, 2014). During the last decade, the efforts in Europe to look for alternative ingredients have been focused on brewer's by-products recovery through its inclusion in aqua feeds (Oliva-Teles & Gonçalves, 2001; Kaur & Saxena, 2004; Cheng et al., 2004; Ozório et al., 2012; Castro et al., 2013; Sealey et al., 2014; Campos et al., 2018; Zhou et al., 2018; Zhang et al., 2018).

Brewer's spent grain (BSG) is the major by-product of beer production being around 85 % of waste generated by this industry (Mussatto et al 2006). This by-product is rich in cellulose, hemicellulose, lignin and proteins and breweries use them nearby due to the high cost of transport. It has been used in human and livestock food (Faccenda et al., 2017, Murdock et al., 1981), crustaceans (Muzinic et al., 2004) and some fish species (Yamamoto et al., 1994; Kaur & Saxena, 2004; Cheng et al., 2004; Campos et al., 2018; Jayant et al., 2018) for being a raw material rich in fibre and proteins and containing lipids, minerals and vitamins (Mussatto et al., 2006). However, due to its high-water content (70-85 %) and easily fermenting components it is considered an extremely perishable ingredient/feed that can be used only 2-3 days if stored at 5 ºC (Ben-Hamed

et al., 2011). The chemical composition of BSG can also vary depending on the variety of barley grain, harvest time and malting and maceration conditions during the brewing process (Robertson et al., 2010).

Brewer's *Saccharomyces* spent yeast (BSY) is the second major by-product of the brewing industry and its disposal is often an environmental problem. Once it is dried and inactived (dead yeast cells), it has been identified as an inexpensive nitrogen source with good nutritional characteristics. Brewer's yeast should not be confused with brewer's type yeast or nutritional yeast that are pure yeasts usually grown under controlled production conditions and cultivated specifically for use as a nutritional supplement and not a by-product of the brewing industrial process (Bekatorou et al., 2006). It has been considered as a potential alternative to fishmeal in aqua-feeds (Oliva-Teles and Gonçalves., 2001; Ebrahim & Abou-Seif, 2008; Ozório et al., 2012; Sealey et al., 2014), as well as in feeds for porcine and ruminants (Huige, 2006). BSY has been used in the aquaculture industry not only because its high content of cheap protein and excellent amino acid profile (Ovie & Eze, 2014), but also because of its rich content in other bioactive compounds such as β-glucans, mannan oligosaccharides, vitamins, minerals and nucleic acids (Ferreira et al., 2010). In brewer's yeast, nitrogen from nucleic acids is mostly in the form of RNA, representing between 20-25 %, fact that makes it toxic in humans and most of mono-gastric, due to the inability to excrete uric acid that is formed during its metabolic process. However, no negative effects have been found in fish, due to its high liver uricase activity (Rumsey et al., 1991).

Digestibility of these two ingredients have already been assessed (Nazzaro et al., 2021) and, having in mind their values, the main objective of this study was to assess the inclusion rate of these by-products and validate their use as aquafeed ingredients using gilthead seabream as a model for carnivorous marine fish.

Materials and Methods

The experiments were designed to re-evaluate *in vivo* apparent digestibility coefficients (ADCs) of crude protein, in hydrolysed and non-hydrolysed dried brewer spent grain (BSG) and brewer spent yeast (BSY) once they were included in feeds for on growing and to assess their effects on growth and food conversion in gilthead seabream (*Sparus aurata*). Two trials were conducted, the first one –Inclusion- to assess the inclusion rates of BSY and BSG and compare the results of growth and conversion with a commercially available yeast (ABN, Madrid, Spain) and the second one –Validation- to use a higher inclusion rate of BSY and BSG in parallel to a reduction in fish meal content in feed formulation.

Ingredients and experimental diet preparation

Trial 1 - Inclusion

The two by-products evaluated were obtained from Mahou-San Miguel (Lérida, Spain) European brewery. They were treated and stabilized before its inclusion in aquafeeds as in Nazzaro et al (2021), although in this case the processes of dewatering and drying were improved reducing the manipulation of the ingredients. The hydrolysis process was optimized as it is shown in San Martin et al (2020). Four ingredients were obtained: (1) dried spent yeast (DSY), (2) hydrolysed spent yeast (HSY), (3) dried spent grain (DSG) and (4) hydrolysed spent grain (HSG) with a moisture lower than 10 %. Commercial dried and hydrolysed yeast was purchased to ABN (Madrid, Spain) and included in the feeds at the same rate as the BSY in order to compare the digestibility of this commercial yeast with the spent yeast obtained from breweries and evaluate its effects on fish growth.

Nine diets were formulated for trial 1 and extruded (4 and 4.5 mm diameter) at IRTA Mas Bové (Tarragona, Spain). A commercial-fish meal (Super Prime 70 LT, Corpesca, Spain) was used to meet the nutritional requirements of gilthead seabream (FAO, 2020b) (Table 1). Yttrium oxide (Y_2O_3, Sigma Aldrich, Spain) served as the inert marker (0.2 g Kg^{-1}) for the evaluation of digestibility. All the feeds were iso-proteic and iso-lipidic and were formulated including 10 and 20 % of DSY, HSY, DYABN and HYABN and 7.5 and 15 % of DSG and HSG to the basal mixture. Formulation of the reference and experimental diets is shown in Table 1. Feeds including the commercial yeast from ABN have exactly the same formulation and inclusion rate (10 and 20%) than the feeds with brewery spent yeast included.

Trial 2 – Validation

This trial was designed after carrying out trial 1 and taking into account the results obtained. In this case feeds were formulated using the same ingredients but increasing the amounts of spent yeast and spent grain to 30 and 20 % respectively. Furthermore, fish meal content was reduced from 15 to 10 % in the experimental diets (Table 2). All the feeds were iso-proteic and iso-lipidic (40 and 16 % DW content, respectively) and extruded at IRTA Mas Bové facilities.

Fish rearing and faecal collection

The trials were conducted at IRTA Sant Carles de la Ràpita (Tarragona, Spain). In trial 1 gilthead seabream specimens were obtained from Andromeda Group (Castellón, Spain), transported to IRTA and kept in quarantine for 14 days. Seabreams were randomly distributed in twenty-two 200-L fibre glass tanks, in groups of 22 fish (body

weight 94.49 ± 9.07 g). In trial 2 fish were obtained from Albadalejo fish farm (Murcia, Spain), transported to IRTA and kept in quarantine for 14 days. The fish were distributed in fifteen 200-L fibre tanks in groups of 15 fish (body weight 113.43 ± 17.71 g)

In both trials the tanks were supplied with filtered seawater in a recirculation system (IRTAMar™) and maintained at 20 ºC with natural light and photoperiod.

At the end of the trial, all the fish were weighted individually and the growth in weight was calculated using both relative growth rate (RGR, %) and specific growth rate (SGR) using the formulae:

RGR= (Wf-Wi)/Wi x 100

SGR= (LnWf-LnWi)/ t x 100

Where Wi and Wf are the fish weight at the beginning (Wi) and at the end (Wf) of the feeding period.

In trial 1 the experimental diets were randomly assigned to the tanks and fed in duplicates once daily during the whole assay (70 days) using automatic feeders (Arvotec, Finland) to satiation, controlling the feed given and collecting the uneaten feed daily in order to calculate feed conversion ratio (FCR) and protein efficiency ratio (PER).

FCR = Feed consumed / (Final – Initial weight)

PER = (Final – Initial weight) / Protein consumed

In the case of the fish fed ABN commercial yeast only one replicate per treatment was used due to the high number of tanks used in the trial and because the main purpose of including this treatment was to compare the digestibility of both types of yeast (commercial vs obtained from brewery). Faeces were collected by abdominal stripping in alternate days during 2 weeks before final sampling in all the tanks fed the control and brewery by-products ingredients. Faecal samples were stored at -20 ºC until chemical analyses.

In trial 2 the experimental diets were also randomly assigned to the tanks and fed in triplicates once daily for 60 days using the same system.

In the final sampling (day 70 for trial 1, and 60 for trial 2), 5 fish per tank were eviscerated, the weight of viscera and liver recorded and samples of liver and muscle collected and kept at -20 ºC for biochemical analyses.

The ADCs of the experimental diets were calculated according to Maynard et al. (1979) using only the feeds with the highest inclusion rate (20% for spent yeast and 15% for spent grain):

ADC (%) = 100 × (1 − (dietary Y_2O_3 level/faeces Y_2O_3 level) × (faeces nutrient or energy level / dietary nutrient or energy level).

The ADCs of the test ingredients were estimated according to NRC (2011):

$$ADC_{BSG}\ (\%) = ADC_{test} + [(ADC_{test} - ADC_{ref}) \times ((0.8 \times D_{ref})/(0.2 \times D_{ing}))]$$

$$ADC_{BSY}\ (\%) = ADC_{test} + [(ADC_{test} - ADC_{ref}) \times ((0.7 \times D_{ref})/(0.3 \times D_{ing}))]$$

Where:

ADC_{test} = ADC (%) of the experimental diet,

ADC_{ref} = ADC (%) of the reference diet,

D_{ref} = g/kg nutrient (or MJ/kg gross energy) of the reference diet (DM basis)

D_{ing} = g/kg nutrient (or MJ/kg gross energy) of the test ingredient (DM basis)

Chemical analyses

All the faeces samples were dehydrated by freeze-drying (LyoAlfa 6, Telstar, USA) before chemical analyses in order to avoid nutritional losses or alterations that occur by using heat. The biochemical analyses of the diets, ingredients, faeces, and muscle and liver of the fish were performed in duplicates according to standard methods of Association of Official Analytical Chemists (AOAC, 2006). All the samples were analysed for dry matter (105 ºC for 14 h, AOAC 925.09), ash by incineration in a muffle furnace (Nabertherm, Germany 500 ºC for 5 h, AOAC, 942.05); crude protein by Dumas's procedure (Nitrogen analyser FP-528 Leco, USA, AOAC 968.06), crude fat using a Büchi Extraction System B-811 (Büchi, Switzerland, AOAC 920.39), lipid content was quantified gravimetrically after evaporation of the solvent under a stream of nitrogen followed by vacuum desiccation overnight. Acid catalysed transmethylation was carried out using the method of Christie (1982). Methyl esters extracted, purified on TLC plates and analysed by gas-liquid chromatography on a Thermo TraceGC instrument fitted with a BPX70 capillary column as in Villalta et al (2005). Ytrium oxide content in diets and faeces was determined according to Garantun-Tjeldsto et al (2006) by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent Technologies 7700x)

Data analysis

All the biochemical analyses were carried out in duplicates using pooled samples of each tank. In the case of commercial yeast with only one replicate, the results were not considered in the comparisons among treatments.

Growth, food conversion, biochemical composition of fillet and liver and apparent digestibility coefficients data were tested for normality of variances using Levene's test before being submitted to a one-way analyses of variance (ANOVA) using Sigma Plot 12.0 program (Systat Software Inc. USA). The differences were considered statistically significant if $P < 0.05$ after using a Holm-Sidak *post hoc* test to perform pair wise comparisons of means.

Results

Trial 1.- Inclusion of SY and SG

Table 3 shows the results in growth of gilthead seabream at the end of the feeding period. Final weight, SGR and RGR were not significantly different between the fish fed brewery by-products (DSY, HSY, DSG and HSG) included at 2 different levels and those fed the control diet (CTRL). Feed conversion ratios (FCR) results were not significantly different among the groups and only protein efficiency ratio (PER) was found to be significantly higher for the group fed 20 % HSY and lower in CTRL group.

Table 4 shows the ADC results of the diets and ingredients used for seabream. Digestibility of protein was high in all the experimental diets, from 88.5 % to 95.5 % being lower in the case of the feeds formulated with the commercial ABN yeast. ADC of lipids was also very high, from 85.2 % to 92.7 % and, as in the case of protein, commercial yeast shows the lowest values.

Protein digestibility coefficients of experimental ingredients were higher than 85 % for spent yeast, whereas spent grain showed values between 62 and 78 % and commercial yeast presented the lowest values. As in the previous study (Nazzaro et al., 2021) hydrolysis of spent yeast and spent grain did not result in higher digestibility of the ingredients.

Table 5 shows the results of protein and lipid content of muscle and liver of the fish fed the CTRL and experimental diets. In the case of the muscle a significantly higher protein content was recorded in the fish fed ABN commercial yeast included at 10 % and spent grain included at 7.5 % with values around 80 % whereas the lowest values were obtained in the fish from DSY 10 group and CTRL. Lipids were higher in the muscle of

fish from the groups fed HSY 10 and HYABN 20 with 16-17 % DW, followed by DSY and HYABN included at 10 % (approx. 11-12 % lipids), whereas the lowest content was found in CTRL, DYABN and SG (8-9 % lipids). In the case of the liver the highest protein content (19 % DW) was recorded in the control group and the fish fed spent grain whereas it was lower in those fed ABN yeast. Fat content in the liver was higher in the fish fed DYABN included at 10 % and lower in those fed DSY at 10 % (39 % lipids) with the rest of the groups showing levels between 40-47 %.

The fatty acid composition of feeds, muscle and liver is presented in Tables 6, 7 and 8 showing that muscle and liver fatty acid profile reflects that of the feeds. Figure 1 summarizes the fatty acid profile of the fillet with the fish fed spent yeast showing a higher percentage of SAT and lower N-3 PUFA content and fish fed spent grain showing higher percentage of MUFA and N-6 PUFA and similar levels of SAT and N-3 PUFA to the CTRL group.

Trial 2.- Validation of the ingredients with higher inclusion levels

Table 9 shows the results of the growth and food conversion of the fish fed higher inclusion levels of brewery by-products. In this case significant differences were detected in SGR and RGR being higher for the fish fed 30 % DSY, followed by the fish fed the control diet and hydrolysed spent yeast. Fish fed spent grain showed a significantly lower growth, especially those in which hydrolysed spent grain was used. Conversion rate and protein efficiency ratios also showed significant differences being better for the fish fed dried yeast included at 30 %.

Table 10 shows the results of muscle and live protein and fat content. Protein content in both tissues did not show any significant difference among the groups fed the experimental feeds, however lipid content was significantly higher in the muscle of fish fed the control, DSY and HSG feeds compared to the other 2 diets. In the case of the liver fat content was higher for fish fed control and DSG diet and the lowest level was found in HSY fed fish.

Fatty acid composition of feeds used in the validation trial and muscle of liver of the fish is presented in tables 11, 12 and 13 and as in the case of trial 1 the tissues tended to show a fatty acid profile mimicking that of the feeds used. Figure 2 summarizes the fatty acid profile of the fillet showing a very similar composition for all the groups.

Discussion

Fish meal is becoming a limited source to be used as the main protein ingredient in aquafeeds and several new protein sources are being tested as alternatives, most of

them derived from plants with low digestibility in carnivorous animals as well as anti-nutritional elements that affect feed intake, feed efficiency and health (Gatlin et al., 2007). Other important sources of protein that have been considered as alternatives are fish trimmings (viscera, heads, skin, bones and blood, Stevens et al, 2018), insects (IPIFF, 2018), algae (Loveday, 2019), by-products derived from the processing industry, and microbial biomass (Hua et al., 2019). Brewery-derived by-products are included in the last group of products (industrial by-products and microbial biomass) and considering their content of protein (around 47 % for spent yeast) and other nutrients (lipids, vitamin B2, β-glucans, mannan-oligosaccharides and nucleic acids) can be considered good ingredients for aquaculture. In a recent review about the use of yeast in aquafeeds, Agboola et al (2020) indicated that an alternative protein source must be not only nutritionally adequate but also commercially available with consistent supply to end users. One of their conclusions was that to become competitive with FM and soy protein in aquafeeds, investment in large scale production of yeast at affordable cost for feed producers and fish farmers is a need. Breweries spent yeast and spent grain are already produced at very high scale and considered valuable by-products that need to be recovered and recycled. Once the mechanical dewatering and drying processes are totally developed with a reduction of the energy consumption, making the process more economical and environmentally sustainable (San Martin et al., 2020), the quantities of dried spent yeast and grain will satisfy the demand of aquafeed industry. This is one of the objectives of this project and these 2 trials, to show that once the breweries by-products are dewatered and dried can be used as ingredients in aquafeeds to produce high-value fish such as salmon, rainbow trout or European sea bass and gilthead seabream.

In the present study two trials were designed to evaluate the inclusion of these products and re-evaluate the digestibility of ingredients and feeds. Thus, the ADC values obtained, both for the feeds and the ingredients, were higher than those published in a previous study by Nazzaro et al. (2021) due to the improvements introduced in the mechanical dewatering and drying process, reducing the manipulation of spent yeast during the process. They were also better than the apparent digestibility coefficients obtained using a commercial yeast from ABN.

No differences in growth or conversion were observed using 2 inclusion levels of breweries' by-products in the first trial carried out (see tables 3 and 4) and a second trial was designed in order to increase the level of the ingredients (spent yeast included at 30% and spent grain at 20%) and also reduce the level of fish meal in the feed from 15% (control group) to 10%. Similar results were obtained by Oliva-Teles and Gonçalves

(2001) in a trial conducted with sea bass and brewer's yeast included in the feeds up to 50%, although in their study growth was not affected, conversion rate was significantly improved until a fishmeal protein replacement of 30% with digestibility values similar to those obtained in the present study.

Regarding FCR and PER differences between the 2 trials were detected, being the FCR values higher than 2 in the second trial. The reason for this difference can be the origin of the fish, being the second group collected from sea cages far away from IRTA facilities and showing a lower growth than the fish used in trial 1, including the control group of fish fed the same formulation than the control group of trial 1.

Thus, according to the results obtained in the present study, considering both trials, in the case of spent yeast inclusion levels up to 30 % have shown very good results in gilthead seabream growth and food conversion, even when fish meal content was reduced to 10% in the formulation. Dried yeast fed fish showed higher growth and better conversion rate than fish fed the control diet, without affecting fish quality with fillet showing good protein and lipid levels, and omega-3 fatty acid content similar to those found in the control fed fish. In a previous study carried out with gilthead seabream by Fronte et al (2019) using 4.6 % inclusion level of an autolyzed yeast, and a higher FM content (18-22 %) than in the present study, positive effects were also observed in the growth performance of the fish and in gut morphology.

In the case of spent grain, it is also produced in large breweries daily but due to its high moisture content and transport cost it is recommended to be used in the neighbourhood of the breweries as animal feed or as a land fill. Another option is to dry it to get a desirable raw material for feed and food. Not much information is available about the use of this raw material in aquafeeds for marine carnivore fish because this ingredient has only been used for the on growing of freshwater fish (carp and Nile tilapia, Kaur & Saxena, 2004) with good results in growth and conversion but without any previous digestibility study. Cheng et al (2004) evaluated the incorporation of raw spent grain in the diet for rainbow trout and found similar results as in the present validation trial. The inclusion of DSG or HSG at 20-30 % reduced the growth of seabream as well as feed digestibility due to its high-fibre content. In another recent study, He et al (2020), using an enzymatic-based fractionation process to remove fibre and concentrate protein, produced a protein rich product derived from spent grain and used it for Pacific white shrimp on growing included in the feed at different levels (0, 5, 15, 25 and 35 %) with relatively good growth results although the highest inclusion level produced the lowest growth. In the present study, one of the few studies using spent grain as an ingredient in

aquaculture feeds for marine fish, BSG dried or hydrolysed can be included up to 15 % in the feeds giving good results in terms of growth performance and feed conversion (inclusion trial), although once it is included at 20 % together with a reduction in fish meal content in the feed we obtained the lowest growth of sea bream, compared with the fish fed the control diet and spent yeast fed fish. Food conversion was also affected giving the highest values, probably due to the high fibre content of spent grain and being more evident when FM was reduced to only 10 % in feed formulation. However, inclusion of this high-fibre content ingredient did not cause any problem in terms of fish quality with fillet showing good protein and lipid levels and a content of omega-3 fatty acids similar to those obtained in the control diet with a higher level of fish meal.

As a conclusion, the inclusion of 30 % of brewers spent yeast in the feed for carnivorous marine fish (gilthead seabream) resulted in a better growth than that obtained using fish meal as the main protein ingredient, and show a good protein digestibility and food conversion. However, the inclusion of 20 % spent grain, either dried or hydrolysed together with the reduction of fish meal content produced a lower growth of the fish and a worse feed utilization. Thus, inclusion of spent grain should not be higher than 15 %. Taking into account that these by-products are produced in large quantities in Europe, they can be a potential source of protein to reduce the use of plant proteins or fish/animal by-products (trimmings) and increase the sustainability of both sectors, brewery industry and aquaculture.

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Ethical statement

All the personnel involved in the experiments were accredited for experimental work with livestock according to national and EU legislations, and experiments were performed with authorization from the Direcció General de Polítiques Ambientals i Medi Natural of the Generalitat de Cataluña

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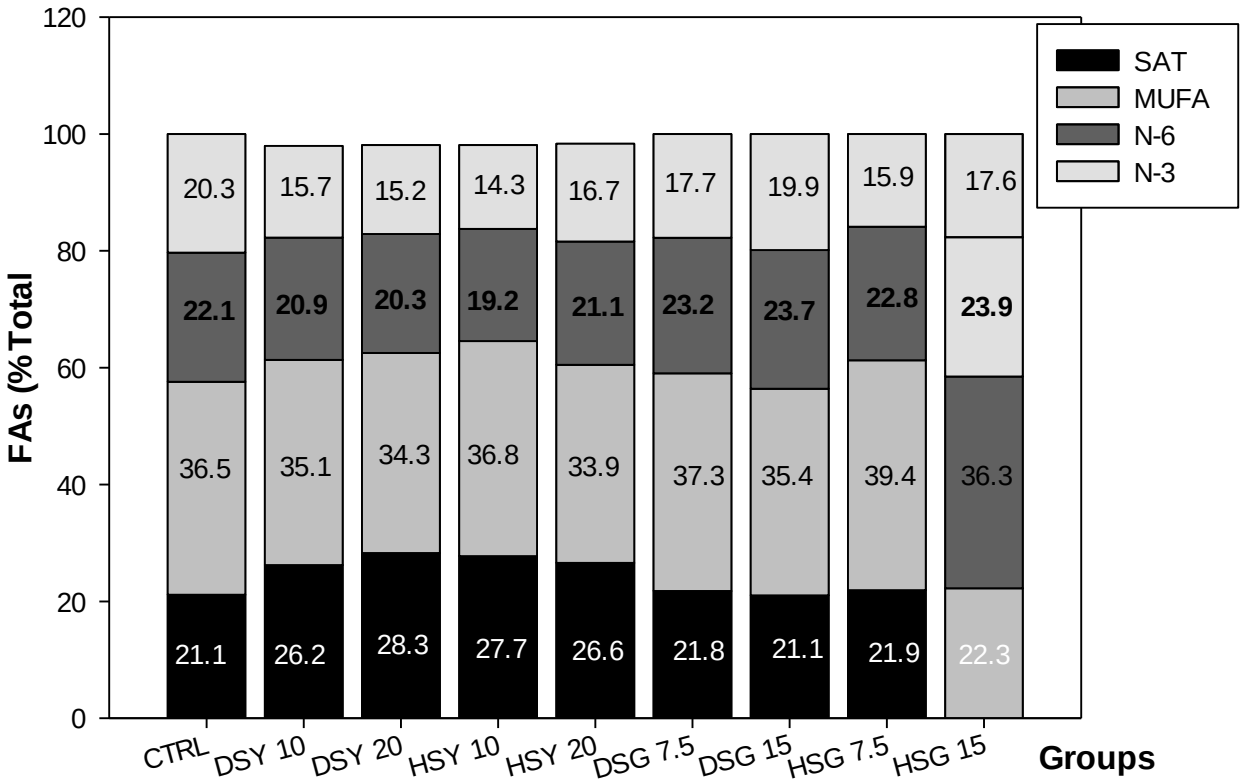

Fig. 1.- Fatty acid composition of the muscle of the fish used in the inclusion trial (Trial 1). SAT: Saturated, MUFA: Monounsaturated, N-3: Omega-3 and N-6: Omega 6 fatty acids

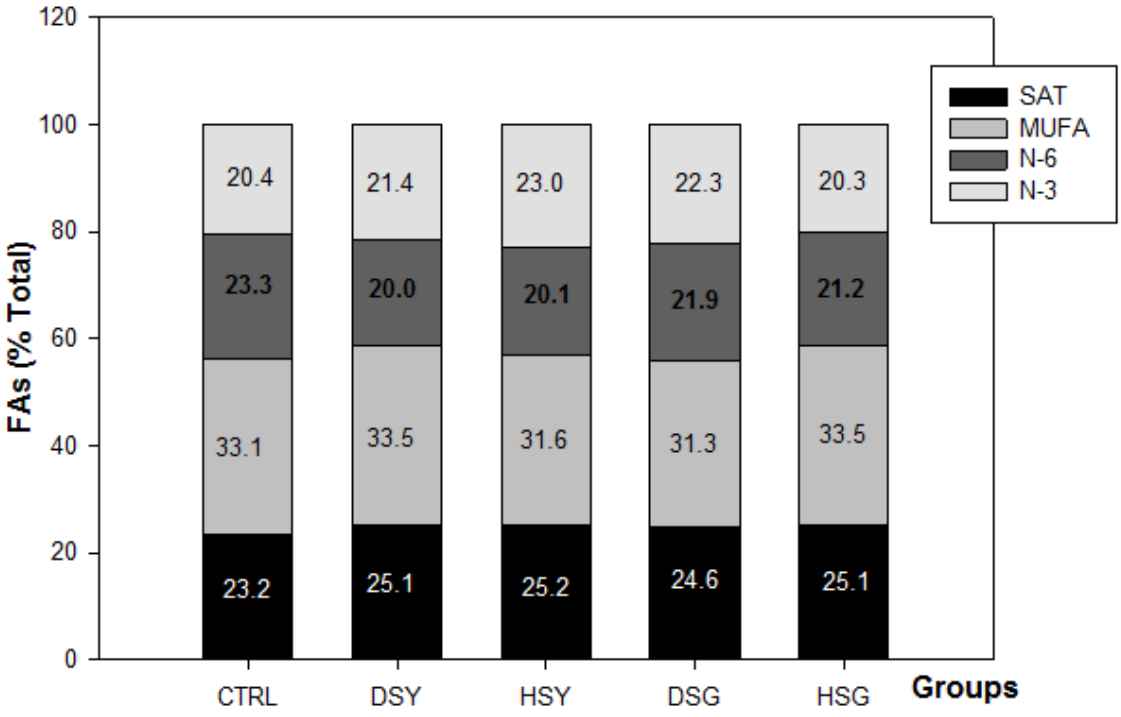

Fig. 2.- Fatty acid composition of the muscle of the fish used in the validation trial (Trial 2). SAT: Saturated, MUFA: Monounsaturated, N-3: Omega-3 and N-6: Omega 6 fatty acids

Ingredient (g)	CTRL	SY 10		SY 20		SG 7.5		SG 15	
Soy bean meal	6.00	4.06		1.00		6.00		2.52	
Wheat gluten	21.78	19.52		16.78		20.40		18.40	
Soycomeal	17.00	17.00		17.00		17.00		17.00	
Fish meal[a]	15.00	15.00		15.00		15.00		15.00	
Fish oil[c]	7.75	7.50		7.38		7.74		7.72	
Soya oil	5.85	5.78		5.77		5.91		6.12	
Lutavit C Aquastab 35 %	0.01	0.01		0.01		0.01		0.01	
Phosphate	0.83	0.70		0.65		0.75		0.65	
Choline	0.27	0.27		0.27		0.27		0.27	
Lysine HCl	0.36	0.09				0.20			
Mineral mix[d]	0.10	0.10		0.10		0.10		0.10	
Vitamin premix[d]	0.10	0.10		0.10		0.10		0.10	
Wheat starch[b]	15.95	16.77		13.60		17.66		14.65	
Soy lecithin	2.00	2.00		2.00		2.00		2.00	
Ytrium oxide[e]	0.02	0.02		0.02		0.02		0.02	
Brewer's spent grain						7.50		15.00	
Brewer's spent yeast		10.00		20.00					
Total Fish meal	15.00	15.00		15.00		15.00		15.00	
Total Vegetable meal	44.78	40.58		34.78		***50.90***		***52.92***	
FM/FO	15/7.7	15/7.5		15/7.4		15/7.7		15/7.7	
		Dried	**Hydrolysed**	**Dried**	**Hydrolysed**	**Dried**	**Hydrolysed**	**Dried**	**Hydrolysed**
Crude protein (% DW)	**45.46**	**43.57**	**44.52**	**44.86**	**43.23**	**44.07**	**44.19**	**43.48**	**43.70**
Crude fat (%DW))	**15.50**	**15.65**	**16.24**	**16.41**	**16.00**	**16.25**	**16.92**	**16.38**	**17.43**

Table 1.-Formulation of feeds used in the inclusion trial, the same formulation was used for dried and hyrolysed spent yeast (SY) and spent grain (SG). Changes in corn gluten, wheat gluten and soybean meal, to account for total plant meal inclusion, were made to ensure diets were isonitrogenous. Total vegetable meal includes the amounts of spent grain added to DSG and HSG feeds

a Super Prime LT fishmeal Corpesca, Chile

b Cargill, Brenntag, Spain

c Eurocoyal, Barcelona, Spain

d Tecnovit, Tarragona, Spain

e Sigma, Spain

Ingredient (g)	CTRL	DSY	HSY	DSG	HSG
Soy bean meal	5.00	5.00	5.00	2.00	1.51
Wheat gluten	25.00	17.84	18.13	21.44	20.91
Soycomeal	16.70	8.54	8.96	19.01	20.00
Fish meal[a]	15.00	10.00	10.00	10.00	10.00
Fish oil[c]	7.92	7.31	6.98	7.01	7.04
Soya oil	6.72	6.40	6.71	6.72	6.73
Phosphate	1.09	0.92	0.91	0.93	0.91
DL-Methionine	0.07	0.07	0.07	0.06	0.07
Choline	0.23	0.26	0.26	0.26	0.26
Lysine HCl	0.43			0.18	0.18
Vitamin premix[d]	0.10	0.10	0.10	0.10	0.10
Mineral mix[d]	0.10	0.10	0.10	0.10	0.10
Wheat Starch[b]	19.44	11.26	10.59	10.00	10.00
Lecithin	2.00	2.00	2.00	2.00	2.00
Brewer's spent yeast		30.00			
Spent grain				20.00	
Spent grain hydrolysate					20.00
Brewer's spent yeast hydrolysate			30.00		
Total Fish meal	15.00	10.00	10.00	10.00	10.00
Total Vegetable meal	46.70	31.38	32.09	***62.45***	***62.42***
FM/FO	15/7.9	10/7.3	10/7.0	10/7.0	10/7.0
Crude Protein (% DW)	42.98	40.37	42.24	42.59	41.84
Crude fat (% DW)	16.85	16.38	16.58	17.82	17.39

Table 2.- Formulation of feeds used in the validation trial, the same formulation was used for dried and hyrolysed yeast and spent grain. Changes in wheat gluten, wheat gluten and soybean meal, to account for total plant meal inclusion, were made to ensure diets were isonitrogenous. Total vegetable meal includes the amounts of spent grain added to DSG and HSG feeds

a Super Prime LT fishmeal Corpesca, Chile

b Cargill, Brenntag, Spain

c Eurocoyal, Barcelona, Spain

d Tecnovit, Tarragona, Spain

	Initial weight (g)		Final weight (g)		HSI		SGR		RGR		FCR		PER	
	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD
CTRL	94.56	9.20	183.03	17.80	2.90	0.38	0.93	0.04	93.49	5.23	1.44	0.08	1.53	0.08b
DSY10%	94.34	7.93	187.51	18.84	3.09	0.62	0.97	0.03	98.74	4.45	1.37	0.07	1.68	0.08ab
DSY20%	94.48	8.55	190.14	20.81	2.85	0.39	0.99	0.00	101.28	0.18	1.33	0.01	1.68	0.01ab
HSY10%	94.55	8.51	183.68	20.35	3.33	0.52	0.94	0.01	94.26	0.88	1.43	0.01	1.67	0.02ab
HSY20%	94.30	9.48	190.93	19.56	3.22	0.58	0.99	0.03	102.40	4.00	1.32	0.05	1.76	0.06a
DSG7.5%	94.42	9.17	184.17	18.46	2.86	0.29	0.94	0.00	95.06	0.32	1.42	0.00	1.60	0.01ab
DSG15%	94.59	8.91	187.41	18.95	2.73	0.40	0.96	0.02	98.18	2.51	1.37	0.04	1.66	0.04ab
HSG7.5%	94.90	10.21	188.15	22.05	2.72	0.51	0.96	0.02	98.26	2.30	1.37	0.02	1.66	0.03ab
HSG15%	94.55	9.50	185.35	19.27	2.50	0.45	0.95	0.01	96.03	1.47	1.40	0.02	1.63	0.02ab
DY-ABN10%	94.25	10.57	188.37	23.35	3.06	0.32	0.96		97.74		1.35		1.70	
DY-ABN20%	94.38	10.11	184.73	21.65	2.90	0.56	0.95		95.73		1.41		1.60	
HY-ABN10%	94.64	8.30	187.13	17.78	3.13	0.52	0.96		97.74		1.38		1.69	
DY-ABN20%	94.19	9.51	192.48	16.81	3.29	0.36	1.01		104.35		1.29		1.76	
ANOVA			P=0.681		P=0.082		P=0.134		P=0.133		P=0.140		P=0.049	

Table 3.- Initial and final weight of seabream in the inclusion trial and results in specific growth rate (SGR), relative growth rate (RGR), food conversion ratio (FCR) and protein efficiency ratio (PER). Different letters indicate statistically significant differences (ANOVA)

CTRL: Control diet, DSY: Dried spent yeast, HSY: Hydrolysed spent yeast, DSG: Dried spent grain, HSG: Hydrolysed spent grain, DY-ABN: Dried commercial yeast from ABN, HY-ABN: Hydrolysed commercial yeast from ABN

Fish fed commercial yeast from ABN were not included in the statistics, only 1 tank was used per treatment

Apparent Digestibility Coefficients of Feeds

	CTRL	DSY20%	HSY20%	DSG15%	HSG15%	DYABN20%	HYABN20%
Protein	95.54	94.01	93.62	94.04	93.10	88.48	90.46
Lipids	92.67	92.36	92.08	91.38	90.05	85.21	89.20

Apparent Digestibility Coefficients of Ingredients

	D-Yeast	H-Yeast	D-Spent grain	H-Spent grain	D-Yeast ABN	H-Yeast ABN
Protein	93.22	85.53	78.66	62.19	58.65	69.71

Table 4.- Apparent digestibility of feeds and ingredients used in the inclusion trial
DSY: Dried spent yeast, HSY: Hydrolysed spent yeast, DSG: Dried spent grain, HSG: Hydrolysed spent grain, DYABN: Dried commercial yeast from ABN, HYABN: Hydrolysed commercial yeast from ABN

	MUSCLE				LIVER			
	Total Lipids (% DW)		Protein (% DW)		Total Lipids (% DW)		Protein (% DW)	
	Av	SD	Av	SD	Av	SD	Av	SD
CTRL	7.99	0.29c	71.79	0.54b	41.96	0.39ab	19.57	0.02a
DSY 10%	10.45	0.13b	71.73	1.81b	43.28	2.56b	17.37	0.17b
DSY 20%	10.85	0.98b	72.17	2.54b	39.34	1.45c	**18.99**	**0.54a**
HSY 10%	**16.22**	**0.13a**	70.46	2.93b	42.10	2.19b	17.51	0.017b
HSY 20%	8.93	0.26c	74.47	6.70ab	42.77	1.22b	17.35	0.20b
DSG 7.5%	8.19	0.68c	76.39	0.28ab	46.45	0.51ab	**18.23**	**0.14a**
DSG 15%	7.59	0.02c	78.12	0.42ab	46.90	0.98ab	**19.01**	**0.25a**
HSG 7.5%	11.76	0.22b	**81.62**	**3.30a**	38.46	1.59c	**19.67**	**0.04a**
HSY 15%	9.26	0.15c	78.44	0.70ab	44.92	0.31ab	**19.50**	**0.46a**
DY-ABN 10%	9.34	0.01c	**82.53**	**3.60a**	**49.30**	**0.35a**	16.12	0.17c
DY-ABN 20%	9.35	0.25c	72.42	3.14b	47.51	0.06ab	16.78	1.64c
HY-ABN 10%	12.48	0.42b	**82.07**	**1.80a**	45.67	0.19ab	17.91	0.55b
HY-ABN 20%	**17.40**	**0.83a**	78.99	4.34ab	47.01	1.02ab	17.47	0.33b
	P<0.001		P<0.001		P<0.001		P<0.001	

Table 5.- Protein and lipid content (% dry weight, DW) of the muscle and liver of gilthead seabream fed the experimental diets. Different letters indicate significant differences (ANOVA)

	Control		HSG 7.5%		HSG 15%		DSG 7.5%		DSG 15%		DSY 10%		DSY 20%		HSY 10%		H2Y 20%		DYABN 10%		DYABN 20%		HYABN 10%		HYABN 20%	
	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD
Total FAs (mg/g Lipids)	614.7	27.9	606.1	6.1	640.4	1.5	619.3	0.7	607.0	13.2	614.2	2.3	604.4	1.2	616.5	6.8	632.7	0.6	541.2	18.2	597.6	37.0	552.2	11.9	561.7	30.7
16:0	18.8	0.1	18.3	0.7	17.9	0.2	18.1	0.1	18.0	0.0	17.5	0.6	17.5	0.7	18.7	0.2	17.7	0.1	17.6	0.5	17.7	0.1	17.1	0.2	17.7	0.1
18:0	4.7	0.4	4.1	0.0	4.1	0.0	4.0	0.2	4.0	0.1	4.1	0.3	4.1	0.0	4.1	0.1	3.9	0.1	4.0	0.1	4.6	0.2	4.2	0.3	4.5	0.1
Total SAT	**26.0**	**0.3a**	**24.3**	**0.8ab**	**24.2**	**0.3ab**	**24.3**	**0.4ab**	**24.1**	**0.1ab**	**23.6**	**0.9ab**	**23.7**	**1.0ab**	**25.3**	**0.3b**	**23.3**	**0.1a**	**23.7**	**0.3ab**	**24.3**	**0.0ab**	**23.1**	**0.3b**	**23.8**	**0.5ab**
16:1	2.7	0.2	2.5	0.2	2.3	0.0	2.5	0.2	2.5	0.2	2.6	0.1	2.7	0.0	3.4	0.4	3.1	0.4	2.8	0.3	2.8	0.2	2.5	0.0	2.9	0.0
18:1n-9	24.3	1.8	23.5	0.1	23.8	0.1	23.9	0.1	23.7	0.7	24.3	0.6	24.7	0.4	24.5	0.5	24.7	0.2	24.4	0.5	24.5	0.0	24.8	0.1	24.5	0.3
Total MUFA	**28.4**	**1.9**	**27.3**	**0.1**	**27.4**	**0.2**	**27.6**	**0.3**	**27.4**	**1.0**	**28.1**	**0.6**	**28.5**	**0.5**	**29.4**	**0.9**	**29.0**	**0.3**	**28.5**	**0.1**	**28.5**	**0.2**	**28.5**	**0.1**	**28.6**	**0.2**
18:2n-6	33.3	0.9	35.4	0.7	36.2	0.3	35.00	0.2	35.4	0.6	33.7	0.2	33.7	0.5	29.4	0.1	33.4	0.8	33.7	0.0	33.0	0.1	34.2	0.1	33.5	0.6
Total n-6 PUFA	**33.9**	**1.0b**	**36.0**	**0.6a**	**36.8**	**0.3a**	**35.6**	**0.1a**	**36.0**	**0.6a**	**34.4**	**0.3b**	**34.4**	**0.4b**	**30.3**	**0.7c**	**34.1**	**0.7b**	**34.4**	**0.1b**	**33.6**	**0.2b**	**35.2**	**0.3a**	**34.2**	**0.7b**
18:3n-3	3.0	0.2	3.4	0.1	3.3	0.0	3.4	0.1	3.2	0.1	3.3	0.1	3.3	0.0	3.1	0.2	3.3	0.0	3.1	0.2	3.3	0.1	3.3	0.1	3.3	0.1
20:5n-3	3.5	0.6b	3.7	0.1b	3.2	0.3b	3.6	0.2b	3.4	0.2b	4.2	0.1ab	3.9	0.1ab	4.7	0.1a	4.2	0.4ab	4.0	0.0ab	4.0	0.2ab	3.8	0.3ab	4.0	0.2ab
22:6n-3	5.3	0.4b	5.4	0.2b	5.1	0.1b	5.4	0.4b	5.7	0.5ab	6.3	0.1a	6.2	0.0a	7.1	0.0a	6.1	0.1a	6.2	0.2a	6.1	0.2a	6.0	0.2a	6.0	0.1a
Total n-3 PUFA	**11.7**	**1.2b**	**12.4**	**0.1b**	**11.7**	**0.4b**	**12.5**	**0.8b**	**12.4**	**0.4b**	**13.8**	**0.0ab**	**13.4**	**0.2ab**	**14.9**	**0.3a**	**13.5**	**0.5ab**	**13.4**	**0.3ab**	**13.5**	**0.0ab**	**13.1**	**0.7a**	**13.4**	**0.1ab**
Total PUFA	**45.6**	**2.2**	**48.4**	**0.7**	**48.5**	**0.1**	**48.1**	**0.6**	**48.4**	**0.9**	**48.2**	**0.3**	**47.8**	**0.6**	**45.2**	**0.7**	**47.6**	**0.2**	**47.8**	**0.2**	**47.2**	**0.2**	**48.3**	**0.4**	**47.6**	**0.8**

Table 6.- Fatty acid composition (% of total FAs, only main fatty acids and totals are included) of the feeds used in the inclusion trial. Different letters indicate significant differences (ANOVA P<0.05)

DSY: Dried spent yeast, HSY: Hydrolysed spent yeast, DSG: Dried spent grain, HSG: Hydrolysed spent grain, DYABN: Dried commercial yeast from ABN, HY:abn Hydrolysed commercial yeast from ABN

	CTRL		DSY 10%		DSY 20%		HSY 10%		HSY 20%		DSG 7.5%		DSG 15%		HSG 7.5%		HSG 15%		DYABN 10%		DYABN 20%		HYABN 10%		HYABN 20%	
	Av	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**
Total FAs (mg/g Lipids)	780.3	16.7	717.7	22.9	674.5	45.0	757.8	16.8	673.4	35.2	831.3	48.9	798.3	41.2	802.0	82.2	751.0	30.9	780.3	16.7	717.7	22.9	674.5	45.0	757.8	16.8
16:0	16.2	0.4	14.2	0.4	15.7	0.5	15.0	0.1	15.1	0.4	16.5	1.1	16.2	0.2	16.7	0.4	17.1	1.1	14.6	0.5	13.9	0.0	15.6	0.3	15.4	0.3
18:0	4.1	0.1	4.7	0.1	4.5	0.5	4.4	0.0	4.5	0.8	4.1	0.1	4.1	0.1	3.7	0.1	3.8	0.3	5.1	0.3	5.0	0.2	4.6	0.0	4.4	0.1
Total SAT	**21.1**	**0.6c**	**26.2**	**0.1ab**	**28.3**	**0.5a**	**27.7**	**0.6ab**	**26.6**	**2.2b**	**21.8**	**1.5c**	**21.1**	**0.2c**	**21.9**	**0.5bc**	**22.3**	**1.0c**	**29.3**	**0.1**	**27.3**	**1.0**	**22.4**	**0.4**	**23.7**	**0.2**
16:1	4.0	0.3	4.4	0.3	4.6	0.1	4.9	0.1	4.3	0.4	4.1	0.1	3.7	0.1	4.6	0.0	4.1	0.0	3.8	0.2	4.0	0.3	4.3	0.1	4.6	0.1
18:1n-9	31.2	0.4	29.8	0.0	28.7	1.6	30.9	0.4	28.8	1.8	32.1	1.0	30.5	0.1	33.5	0.0	31.2	1.2	26.3	0.3	28.0	0.8	33.8	0.1	34.7	0.3
Total MUFA	**36.5**	**0.2a**	**35.1**	**0.3b**	**34.3**	**1.5b**	**36.8**	**0.6ab**	**33.9**	**2.1b**	**37.3**	**1.0ab**	**35.4**	**0.2b**	**39.4**	**0.1a**	**36.3**	**1.1b**	**30.9**	**0.5**	**32.9**	**1.1**	**39.2**	**0.0**	**40.4**	**0.4**
18:2n-6	20.8	0.2	20.9	0.1	20.3	1.7	19.2	0.1	21.1	0.7	21.8	0.4	22.3	0.0	22.8	0.0	23.7	0.5	19.7	0.1	21.0	0.5	22.8	0.0	21.6	0.5
Total n-6 PUFA	**22.1**	**0.0ab**	**20.9**	**0.1bc**	**20.3**	**1.7bc**	**19.2**	**0.1c**	**21.1**	**0.7b**	**23.2**	**0.4a**	**23.7**	**0.1a**	**22.8**	**0.0a**	**23.9**	**0.4a**	**19.7**	**0.1**	**21.0**	**0.5**	**22.8**	**0.0**	**21.6**	**0.5**
18:3n-3	1.9	0.1	1.5	0.0	1.6	0.2	1.6	0.1	1.7	0.2	2.0	0.1	2.0	0.0	2.1	0.1	2.2	0.1	1.4	0.1	1.6	0.0	2.1	0.1	2.0	0.0
20:5n-3	3.8	0.3	3.6	0.1	3.2	0.7	3.8	0.1	4.0	0.2	3.7	0.2	4.0	0.2	3.2	0.3	3.3	0.2	3.9	0.1	3.5	0.0	3.4	0.1	3.1	0.4
22:6n-3	13.0	0.0a	9.3	0.2b	9.6	0.8b	7.9	0.2	9.9	0.1b	12.1	0.0a	12.4	0.2a	9.5	0.0b	10.9	0.5ab	9.7	0.1	9.3	0.1	8.9	0.1	8.1	0.4
Total n-3 PUFA	**20.3**	**0.4a**	**15.7**	**0.4bc**	**15.2**	**0.8c**	**14.3**	**0.4c**	**16.7**	**0.0b**	**17.8**	**0.8b**	**19.9**	**0.1a**	**15.9**	**0.4bc**	**17.6**	**0.6b**	**16.2**	**0.3**	**15.5**	**0.1**	**15.6**	**0.3**	**14.3**	**0.1**
Total PUFA	**42.4**	**0.4a**	**36.6**	**0.3b**	**34.6**	**2.1b**	**33.5**	**0.3b**	**37.8**	**0.6b**	**40.9**	**2.5a**	**43.6**	**0.0a**	**38.7**	**0.4b**	**41.5**	**0.1a**	**35.9**	**0.1**	**36.5**	**0.4**	**38.5**	**0.3**	**35.9**	**0.6**

Table 7.- Fatty acid composition (% of total FAs, only main fatty acids and totals are included) of the muscle of the fish collected at the end of inclusion trial. Different letters indicate significant differences (ANOVA P<0.05)

DSY: Dried spent yeast, HSY: Hydrolysed spent yeast, DSG: Dried spent grain, HSG: Hydrolysed spent grain, DYABN: Dried commercial yeast from ABN, HYABN: Hydrolysed commercial yeast from ABN

	CTRL		DSY10%		DSY20%		HSY10%		HSY20%		DSG7.5%		DSG15%		HSG7.5%		HSG15%		DYABN10%		DYABN20%		HYABN10%		HYABN20%	
	Av	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**
Total FAs (mg/g Lipids)	739.1	72.7	798.9	5.4	841.1	18.6	765.3	14.7	808.7	90.9	746.2	35.0	821.5	4.0	839.7	0.2	821.2	44.8	739.1	72.7	798.9	5.4	841.1	18.6	765.3	14.7
16:0	14.2	0.1	15.5	0.2	16.5	0.4	15.7	0.3	16.3	0.9	14.8	0.3	14.7	1.0	15.0	0.2	14.7	0.1	15.9	0.2	15.1	0.0	17.0	1.0	14.8	0.0
18:0	4.8	0.1	5.1	0.1	5.2	0.1	5.4	0.2	5.9	0.0	4.5	0.0	4.9	0.3	5.0	0.3	4.7	0.3	5.4	0.1	5.1	0.1	5.1	0.2	5.1	0.1
Total SAT	**20.1**	**0.1b**	**22.1**	**0.1ab**	**23.4**	**0.2a**	**22.3**	**0.1ab**	**23.8**	**0.9a**	**20.7**	**0.5ab**	**21.8**	**0.2ab**	**21.2**	**0.5ab**	**20.7**	**0.3a**	**22.6**	**0.1ab**	**21.4**	**0.1ab**	**23.5**	**1.3a**	**21.2**	**0.2ab**
16:1	5.1	0.1	5.4	0.2	5.8	0.1	5.9	0.0	5.6	0.1	5.3	0.2	5.1	0.3	4.7	0.1	4.8	0.2	5.4	0.2	5.2	0.1	5.8	0.8	5.1	0.3
18:1n-9	39.5	0.2	41.0	0.1	39.4	0.3	42.2	0.3	42.2	0.9	38.7	0.3	36.5	1.1	37.8	0.1	37.2	0.8	40.7	1.3	39.9	0.6	41.2	0.4	41.5	0.2
Total MUFA	**45.8**	**0.1ab**	**47.8**	**0.1ab**	**46.5**	**0.4ab**	**49.4**	**0.2a**	**48.9**	**0.7a**	**45.4**	**0.2ab**	**42.8**	**1.4b**	**43.9**	**0.0b**	**43.3**	**0.7b**	**47.5**	**0.8a**	**46.2**	**0.6ab**	**48.3**	**1.0a**	**48.0**	**0.3a**
18:2n-6	20.9	0.2	18.3	0.0	17.9	0.8	16.1	0.0	18.1	1.0	20.4	0.0	22.5	0.3	21.8	0.0	22.5	0.1	17.6	0.2	19.7	0.2	17.7	0.3	18.7	0.1
18:3n-6	1.6	0.4	1.2	0.2	0.0	0.0	1.2	0.0	0.0	0.0	1.8	0.1	1.4	0.1	1.9	0.1	1.7	0.1	1.3	0.2	1.5	0.1	0.2	0.0	1.3	0.0
Total n-6 PUFA	**22.5**	**0.6ab**	**19.5**	**0.2b**	**17.9**	**0.8b**	**17.3**	**0.1b**	**18.1**	**1.0b**	**22.2**	**0.1ab**	**23.8**	**0.4a**	**23.7**	**0.1a**	**24.2**	**0.2a**	**18.8**	**0.0b**	**21.1**	**0.2ab**	**18.0**	**0.3b**	**20.0**	**0.0b**
18:3n-3	1.7	0.1	1.6	0.0	1.8	0.0	1.3	0.0	1.4	0.0	1.8	0.0	1.7	0.0	1.7	0.1	2.1	0.1	1.6	0.0	1.6	0.1	1.7	0.1	1.6	0.0
20:5n-3	2.2	0.1	2.1	0.2	1.7	0.7	2.3	0.1	1.0	0.2	2.1	0.3	2.1	0.9	2.1	0.3	2.0	0.2	2.0	0.1	2.0	0.2	1.9	0.6	2.0	0.1
22:6n-3	6.5	0.0	5.7	0.2	6.3	0.5	6.0	0.0	5.7	0.5	6.5	0.0	6.5	0.4	6.2	0.5	6.6	0.3	6.3	0.6	6.2	0.3	5.6	1.6	6.0	0.2
Total n-3 PUFA	**11.6**	**0.4**	**10.6**	**0.0**	**11.2**	**0.2**	**10.9**	**0.1**	**9.2**	**0.6**	**11.6**	**0.4**	**11.6**	**1.5**	**11.2**	**0.3**	**11.9**	**0.6**	**11.0**	**0.8**	**11.2**	**0.5**	**10.2**	**2.6**	**10.8**	**0.5**
Total PUFA	**34.1**	**0.2**	**30.1**	**0.2**	**30.1**	**0.6**	**28.3**	**0.2**	**27.3**	**1.6**	**33.8**	**0.4**	**35.4**	**1.2**	**34.9**	**0.4**	**36.0**	**0.4**	**29.8**	**0.7**	**32.3**	**0.8**	**28.2**	**2.3**	**30.8**	**0.5**

Table 8.- Fatty acid composition (% of total FAs, only main fatty acids and totals are included) of the liver of the fish collected at the end of inclusion trial. Different letters indicate significant differences (ANOVA P<0.05)

DSY: Dried spent yeast, HSY: Hydrolysed spent yeast, DSG: Dried spent grain, HSG: Hydrolysed spent grain, DYABN: Dried commercial yeast from ABN, HYABN: Hydrolysed commercial yeast from ABN

	Initial weight (g)		Final weight (g)		HSI			VSI			SGR			RGR			FCR			PER		
	Av	SD	Av	SD	Av	SD		Av	SD		Av	SD		Av	SD		Av	SD		Av	SD	
CTRL	114.80	16.72	176.10	25.65	2.81	0.96	a	7.69	1.43	ab	0.86	0.074	ab	53.40	5.05	b	2.38	0.29	ab	0.99	0.11	bc
DSY	112.71	14.81	178.99	23.52	3.19	0.66	a	8.25	1.26	a	0.92	0.056	a	58.80	3.81	a	2.04	0.05	c	1.21	0.03	a
HSY	114.18	14.94	177.20	23.19	3.14	0.66	a	7.76	1.06	ab	0.87	0.080	ab	55.20	3.62	b	2.32	0.29	b	1.03	0.12	b
DSG	110.95	23.39	169.53	35.73	2.28	0.93	b	7.26	0.77	b	0.85	0.054	b	52.80	3.51	b	2.49	0.37	ab	0.96	0.13	c
HSG	114.51	17.83	169.48	26.38	2.27	0.77	b	7.36	0.94	b	0.78	0.025	c	48.00	4.94	c	2.45	0.04	ab	0.98	0.02	c
ANOVA			p=0.311		p<0.001			p<0.001			p<0.001			p<0.001			p<0.001			p<0.001		

Table 9.- Initial and final weight of seabream in the validation trial and results in specific growth rate (SGR), relative growth rate (RGR), food conversion ratio (FCR) and protein efficiency ratio (PER). Different letters indicate statistically significant differences (ANOVA)

CTRL: Control diet, DSY: Dried spent yeast, HSY: Hydrolysed spent yeast, DSG: Dried spent grain, HSG: Hydrolysed spent grain

	MUSCLE				LIVER			
	Total Lipids (% DW)		Total Protein (%DW)		Total Lipids (% DW)		Total Protein (%DW)	
	Av	SD	Av	SD	Av	SD	Av	SD
CTRL	**8.34**	**0.01a**	81.57	3.92	35.09	0.23b	20.32	2.50
DSY 30%	**8.21**	**0.03a**	82.06	4.15	32.84	0.03c	23.19	2.21
HSY 30%	6.88	0.18c	88.15	2.17	29.60	0.34e	25.15	3.17
DSG 20%	7.28	0.14b	87.23	2.55	**37.47**	**0.49a**	19.78	2.86
HSG 20%	**8.17**	**0.34a**	83.01	4.30	31.19	0.70d	24.54	3.05
ANOVA	P<0.001				P<0.001			

Table 10.- Protein and lipid content (% dry weight, DW) of the muscle and liver of gilthead seabream fed the validation diets. Different letters indicate significant differences (ANOVA)

Fatty acids (% Total)	CTRL		DSY30		HSY30		DSG20		HSG20	
	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD
Total FAS (mg/g Lipids)	684.15	10.23	697.28	14.16	687.18	8.42	683.14	11.93	716.6	12.5
16:0	18.29	0.13	17.45	0.13	17.02	0.13	17.69	0.07	17.53	0.13
18:0	4.75	0.04	4.08	0.04	4.17	0.02	4.04	0.18	4.20	0.02
Total saturated	26.36	0.16a	24.53	0.16b	24.07	0.12b	24.44	0.03b	24.47	0.12b
16:1	3.99	0.01	4.23	0.01	4.05	0.14	3.23	0.08	3.08	0.14
18:1n-9	23.13	0.04	23.52	0.04	23.49	0.14	23.12	0.28	23.16	0.14
Total monounsaturated	28.82	0.07ab	29.15	0.07a	28.98	0.34ab	27.70	0.14b	27.62	0.34b
18:2n-6	28.67	0.10	29.17	0.10	29.07	0.35	31.27	0.13	32.10	0.35
18:3n-6	0.40	0.02	0.15	0.02	0.15	0.04	0.14	0.03	0.08	0.04
Total n-6 PUFA	29.13	0.10b	29.72	0.10b	29.60	0.37b	31.76	0.10a	32.49	0.37a
18:3n-3	3.38	0.07	3.77	0.07	3.70	0.08	3.82	0.00	3.98	0.08
20:5n-3	4.41	0.09	4.73	0.09	4.87	0.02	4.42	0.12	4.24	0.02
22:6n-3	5.71	0.13	5.98	0.13	6.60	0.20	5.66	0.18	5.08	0.20
Total n-3 PUFA	15.68	0.22c	16.59	0.22b	17.35	0.10a	16.11	0.07b	15.42	0.10c
Total PUFA	44.81	0.11	46.30	0.11	46.95	0.47	47.87	0.17	47.91	0.47

Table 11.- Fatty acid composition (% Total FAs, only main fatty acids and totals are included) of the feeds used in the validation trial. Different letters indicate significant differences

DST: Dried spent yeast; HSY: Hydrolysed spent yeast, DSG: Dried spent grain, HSG: Hydrolysed spent grain

Fatty acids (% Total)	CTRL		DSY 30		HSY 30		DSG 20		HSG 20	
	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD
Total FAs (mg/g Lipids)	670.25	12.52	631.68	3.54	641.85	0.98	634.12	8.64	654.81	12.52
16:0	16.42	0.55b	17.71	0.10ab	18.05	0.14ab	17.68	0.25ab	18.41	0.15a
18:0	4.20	0.08	4.66	0.03	4.78	0.05	4.53	0.06	4.07	0.01
Total saturated	23.20	0.51b	25.09	0.10ab	25.22	0.29a	24.60	0.37b	25.06	0.17ab
16:1	3.42	0.21	3.90	0.03	3.71	0.21	3.03	0.07	3.63	0.03
18:1n-9	28.36	0.17	28.17	0.21	26.64	0.19	26.90	0.08	28.47	0.18
Total monounsaturated	33.12	0.00a	33.50	0.25a	31.64	0.05b	31.28	0.04b	33.47	0.13a
18:2n-6	22.05	0.10	18.72	0.12	18.81	0.20	20.59	0.10	20.06	0.10
Total n-6 PUFA	23.28	0.05a	19.96	0.11c	20.10	0.17c	21.93	0.01ab	21.22	0.06b
20:5n-3	4.16	0.05	4.48	0.08	4.95	0.02	4.43	0.19	4.43	0.02
22:6n-3	10.78	0.22b	11.67	0.11b	12.92	0.06a	12.44	0.06a	10.62	0.26b
Total n-3 PUFA	20.40	0.45	21.44	0.05	23.04	0.07	22.19	0.31	20.25	0.36
Total PUFA	43.68	0.50a	41.40	0.15b	43.14	0.24ab	44.12	0.33a	41.47	0.30b

Table 12.- Fatty acid composition (% Total FAs, only main fatty acids and totals are included) of the muscle of the fish used in the validation trial. Different letters indicate significant differences

DSY: Dried spent yeast; HSY: Hydrolysed spent yeast, DSG: Dried spent grain, HSG: Hydrolysed spent grain.

Fatty acids (% Total)	CTRL		DSY 30		HSY 30		DSG 20		HSG 20	
	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD
Total FAs (mg/g Lipids)	753.08	18.97	694.4	16.75	698.66	1.90	683.24	19.20	686.57	5.81
16:0	16.88	0.23	16.10	0.07	16.13	0.13	16.83	0.20	16.59	0.11
18:0	5.26	0.01	6.15	0.29	5.54	0.05	5.20	0.03	5.01	0.08
Total saturated	24.69	0.34	24.75	0.34	24.29	0.03	24.69	0.25	24.30	0.10
16:1	4.23	0.08	4.49	0.04	4.62	0.06	4.07	0.08	3.96	0.14
18:1n-9	35.51	0.04	36.57	0.12	35.12	0.14	32.95	0.20	32.71	0.21
Total monounsaturated	41.04	0.07a	42.27	0.24a	40.97	0.13a	38.36	0.26b	37.98	0.12b
18:2n-6	20.98	0.11	18.31	0.16	19.00	0.07	21.26	0.07	20.58	0.01
Total n-6 PUFA	22.21	0.10a	19.56	0.12b	20.11	0.08b	22.50	0.19a	21.91	0.03ab
20:5n-3	2.13	0.09	2.71	0.09	2.89	0.03	2.57	0.06	2.92	0.08
22:6n-3	5.35	0.27b	6.16	0.02b	6.90	0.04a	6.86	0.22a	7.83	0.00a
Total n-3 PUFA	12.05	0.38b	13.42	0.02b	14.63	0.08a	14.45	0.31a	15.81	0.01a
Total PUFA	34.26	0.28b	32.98	0.10b	34.74	0.16ab	36.95	0.51a	37.72	0.02a

Table 13.- Fatty acid composition (% Total FAs, only main fatty acids and totals are included) of the liver of the fish used in the validation trial. Different letters indicate significant differences

DST: Dried spent yeast; HSY: Hydrolysed spent yeast, DSG: Dried spent grain, HSG: Hydrolysed spent grain.