Apparent digestibility coefficients of brewer's by-products used in feeds for rainbow trout (*Oncorhynchus mykiss*) and gilthead seabream (*Sparus aurata*)

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Abstract

A trial was conducted to test the effect of partial replacement of fishmeal by two brewery industry byproducts, yeast and spent grain, included in isoproteic (41% CP) and isolipidic (22 % CL) diets for gilthead sea bream (*Sparus aurata*) and rainbow trout (*Oncorhyncus mykiss*), having in mind the availability of these byproducts. A first step before an ingredient is included in a commercial feed is to evaluate the nutritional quality of these raw materials by measuring their digestibility. Thus, the apparent digestibility coefficients of the diets and ingredients were determined after a 30 days feeding trial and faecal collection. Apparent digestibility coefficients of these by products in the case of rainbow trout varied between 75 and 88% whereas for gilthead seabream was between 71 and 88%. According to the results obtained, the inclusion of 20-30% of brewers' spent yeast and spent grain in the feed for carnivorous fish either from fresh (rainbow trout) or marine (gilthead seabream) gave similar results to a feed with fish meal as the main protein source and show a good protein, lipid and amino acid digestibility. 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 byproducts (trimmings) and increase the sustainability of both sectors, brewery industry and aquaculture.

Introduction

Aquaculture is one of the animal production industries with the highest growth worldwide, providing most fish for human consumption than capture fisheries since 2014 (FAO, 2018). Fish and fish products from world fisheries are processed into fishmeal and fish oil for the aquaculture sector in a significant, but declining, proportion (Green & Pearsall, 2018).They are considered excellent sources of high-quality protein, containing an

optimal amino acid profile, long-chain omega-3 fatty acids, essential minerals and vitamins A, D and B (Gatlin et al., 2007), as well as high palatability and, initially, low cost and high availability (Oliva-Teles & Gonçalves, 2001). Currently, the most expensive component in aqua feeds is the protein fraction, due to the expansion of the aquaculture industry and the over-dependence and increasing demand of marine sources for feed production (Cheng et al., 2004). These prices show an increasing trend in the future, highly influencing the cost of intensive crops, which nowadays range between 40-50% of the total operating budget. However, the sustainability of aquaculture not only requires economic feasibility, but also reduction of the environmental impact. From the overfishing of the forage fish species used for feed production to the eutrophication of water column, due to overfeeding and lixiviation of aquaculture feedstuffs (Naylor et al., 2009), aquaculture suitability depends on the reduction of fishmeal use (Couto et al., 2016). This has special importance in carnivorous species of farmed fish, such as seabream (*Sparus aurata*) or rainbow trout (*Oncorhynchus mykiss*), which use protein as the main source of energy, preferably to lipids or carbohydrates (Green & Hardy, 2008).

Thus, the economic and environmental sustainability of aquaculture depends to a large extent 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 and ease of handling and storage (Gatlin et al., 2007). Additionally, criteria for the selection and application of new foods are based on the health and performance of fish, as well as benefits for human health, and acceptance by the consumer (Naylor et al., 2009). In this way, alternative protein sources to fishmeal have been used in diets, but all of them have some feature that limits their use and inclusion level in aqua-feeds for piscivores (Naylor et al., 2009). Among those, 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, accentuating the environmental impact of aquaculture (Muzquiz & Wood, 2009; Naylor et al., 2009). Terrestrial animal by-products have a better nutritional composition for fish feed and are widely available at low cost in markets, but lack consumer acceptance (Naylor et al., 2009). In this sense, the economic and environmental interest in industrial by-products recovery, for the development of alternative sources of protein, 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, with a world production exceeding 1.95 billion hectolitres in 2017 (Statista, 2019), generates large amounts of waste and by-products with great potential for its recovery as high nutritional value products (Aliyu & Bala, 2011). Large breweries, producing 1,000HL beer per day, generate 40 tons of by-products per day to remove (Thomas & Rahman, 2006) and even small breweries must have careful waste management to ensure their economic and environmental feasibility. Since the bulk of organic waste, arising as brewer spent grain (BSG) and spent yeast (BSY), has been categorized as high quality food (Mussatto, et al., 2006; Thomas & Rahman, 2006; Mussatto, 2009; Levic et al., 2010; Robertson et al., 2010; Zhou et al., 2018), leading breweries are seeking suitable ways to minimize losses and optimize production by increasing yields (Jurado & Sorensen, 2012). In the case of Europe 40 billion litres were produced in 2018 (Eurostat, 2019) generating 7 million tons of BSG and 0.9 million tons of BSY conventionally reused as animal feed and, in some cases, bioethanol production (Djuragic et al, 2010; Buffington, 2014). In this way, research efforts in Europe 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, which is obtained after the mashing process of barley grains, corresponding to around 85% of waste generated by this industry (Mussatto et al 2006). 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). BSG has a rich essential amino acid composition (Aliyu & Bala, 2011), is a good source of unsaturated fatty acids (Thavasiappan et al., 2016), and has health benefits due to its content in biological active compounds such as polyphenols, flavonoids and β-Glucans (Farças et al, 2015; Tang et al., 2009). Even so, the chemical composition of BSG can 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 that, marketed as dry and inactive yeast (dead yeast cells), has been identified 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 since the early 1990's 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 nutrients and 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).

As a first step before an ingredient is included in a commercial feed, it is essential to evaluate the nutritional quality of these raw materials by measuring their digestibility. Thus, the objective of this study was to determine the apparent digestibility coefficients (ADC) in vivo of the protein fraction of BSG and BSY, for possible commercial use in diets for rainbow and gilthead seabream.

Materials and Methods

The experiment was designed to evaluate *in vivo* apparent digestibility coefficients (ADCs) of crude protein, in dried and hydrolysed, brewer spent grain (BSG) and brewer spent yeast (BSY). BSG and BSY, as experimental ingredients, were obtained from European breweries and tested in gilthead seabream (*Sparus aurata*), as a model of marine fish, and rainbow trout (*Oncorhynchus mykiss*) as a model of freshwater fish in South European countries.

Ingredients and experimental diet preparation

The two by-products evaluated were obtained from Mahou-San Miguel (Lérida, Spain) European brewery. The brewery by-products were treated and stabilized before its inclusion in aquafeeds. The enzymatic hydrolysis pre-treatment was developed by AZTI (Bilbao, Spain) and all the process of drying were carried out in Riera Nadeu (Barcelona, Spain) by combining mechanical dewatering to reduce the humidity below 60%, using a filter centrifuge for BSG and a decanter centrifuge for BSY, followed by a thermal flash drying (RINA-JETS-1008) to reduce the moisture content to 10% (San Martin et al., 2020, in press). Hence, four test ingredients were obtained: (1) dried spent yeast (D-BSY), (2) hydrolysed spent yeast (H-BSY), (3) dried spent grain (D-BSG) and (4) hydrolysed spent grain (H-BSG) with a moisture lower than 10%.

Five diets were formulated 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 rainbow trout and gilthead seabream, in order to produce a reference diet (REF), whereas in the other 4 diets the amount of fish meal was reduced to allow the incorporation of these new ingredients (see 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. Four iso-proteic and iso-lipidic test diets were formulated and produced by including 20% of H-BSG and D-BSG and 30% of H-BSY and D-BSY to the basal mixture. Formulation of the reference and experimental diets is shown in Table 1.

Fish rearing and faecal collection

The digestibility trial was conducted at IRTA Sant Carles de la Ràpita (Tarragona, Spain). Rainbow trout (*Oncorhynchus mykiss*) specimens were obtained from Aigua Natura (Tarragona, Spain) and kept in quarantine for 14 days, prior to the experiment, in 1500-L fibre glass tanks fed a commercial diet. After acclimation, groups of 20 fish (body weight 206.05 ± 41.18 g) were randomly distributed in fifteen 500-L fibre glass tanks and maintained at 15ºC in open circulation water system with natural light and photoperiod. Gilthead seabream specimens were obtained from Andromeda Group (Castellón, Spain), transported to IRTA and kept in quarantine for 14 days as in the case of rainbow trout. Seabreams were randomly distributed in fifteen 500-L fibre glass tanks, with individual faeces sedimentation columns in the outlet of each tank, in groups of 25 fish (body weight 253.01 ± 27.68). Tanks were supplied with filtered seawater in a recirculation system (IRTAMar™) and maintained at 20ºC with natural light and photoperiod.

At the end of the trial, 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.

Experimental diets were randomly assigned to the tanks and fed in triplicate during the whole assay (30 days) using automatic feeders to satiation and feed conversion ratio (FCR) calculated. Fish were fed 110 g once daily for two weeks before faecal collection. Faecal samples were obtained from rainbow trout in one collection by manual stripping,

5-6 h post-feeding, after anaesthetizing the fish. In gilthead seabream, faeces were collected from the sedimentation columns, before feeding, for three alternate days. The procedures for faecal collection were recommended by Skretting ARC. Both rainbow trout and gilthead seabream were fed once a day during the collection period 5-6 h before faecal collection. In the case of gilthead seabream the tanks were cleaned the day before the collection, to ensure the non-presence of uneaten feed in the faecal collectors. Faecal samples were stored at -20ºC until chemical analyses.

The ADCs of the experimental diets were calculated according to Maynard et al. (1979):

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)

2.3 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 and faeces were performed according to standard methods of Association of Official Analytical Chemists (AOAC, 2006). All the samples were analysed for dry matter (105ºC for 14 h, AOAC 925.09), ash by incineration in a muffle furnace (Nabertherm, Germany 500ºC for 5 h, AOAC, 942.05); crude protein by Dumas procedure (Nitrogen analyser FP-528 Leco, USA, AOAC 968.06), crude fat using a Büchi Extraction System B-811 (Büchi, Switzerland, AOAC 920.39); gross energy was determined using an adiabatic bomb calorimeter (using the DIN 51900 rule); and phosphorus content by molibdovanadate spectrophotometric method (AOAC 965.17). Crude fibre content of the ingredients was analysed using an Ankom fibre analyser (Ankom, USA) based on filter bags technology (AOAC 962.09); starch by the enzymatic method (AOAC 996.11) and aminoacid profile

by HPLC-DAD detection (Butikofer et al., 1991). Beta glucan analysis of yeast and spent grain was carried out as in McCleary & Codd (1991) using Megazyme kits (Megazyme, Ireland). Vitamin B2 was analysed by HPLC and UV detection (445 nm) after extraction with a mixture of acetic acid, water and diluted sulphuric acid (Esteve et al, 2001). 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

Growth 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.

In the case of protein digestibility in the diets t-test was also performed, using Sigma Plot 12.0 program, in order to compare the results obtained with yeast (dried and hydrolysed), spent grain (dried and hydrolysed) and between the two species (rainbow trout and seabream).

Results

The biochemical composition of the test ingredients is shown in Table 2. Crude protein levels in brewers by products was 50% higher in BSY than in BSG and ranged from 217.92 to 478.50 g kg^{-1}. Hydrolysis of spent grain and spent yeast reduced the crude protein concentration by about 12% (29.15 g Kg^{-1}) in H-BSG and around 3% (15.39 g Kg^{-1}) in H-BSY. Hydrolysed H-BSG and H-BSY also had lower amino acid concentration as a result of low crude protein concentration. Amino acid concentration varied among test ingredients and, either essential or non-essential, were almost 50% lower in BSG than in BSY, with the exception of Glutamic acid and Proline, with similar amounts in both byproducts. Hydrolysis of crude fibre in BSG reduced its content (187.83 g Kg^{-1} in D-BSG) by about 12% (21.89 g Kg^{-1}), being higher than in BSY, which ranged from 6.79 g Kg^{-1} to 6.96 g Kg^{-1} (in D-BSY and H-BSY respectively). Starch was found to exist in very different proportions among test ingredients, which was 16% higher in both D-BSY and H-BSY than in BSG. Levels of crude fat were 25% lower in D-BSY and H-BSY, compared with those in spent grains (BSG). Vitamin B2 content was two times higher in H-BSY than in D-BSY showing brewer's yeast as one of the richest raw materials in this vitamin. β-glucan content was very high in the ingredients derived from yeast (D-BSY

and H-BSY) and low or non-existent in those derived from spent grain (D-BSG, H-BSG) probably due to the action of β-Glucanase during brewing process (Ellis et al, 1997).

The chemical composition of the reference (REF) and test diets is given in Table 1 together with the formulation of the feeds. The 20 and 30% inclusion of BSG and BSY, respectively, resulted in isoproteic (419.80 to 412.80 g Kg^{-1}) and isolipidic (224.04 to 218.42 g Kg^{-1}) experimental diets. REF diet and those containing H-BSY and D-BSY, showed significantly higher levels of carbohydrates, if compared with diets including H-BSG and D-BSG. All diets had similar gross energy values, which ranged from 18.89 to 18.49 MJ kg^{-1}.

Tables 3 and 4 show the results in growth of rainbow trout and gilthead seabream at the end of the feeding period. Although the growth trial can be considered too short, at the end of the 30 days trial both rainbow trout and seabream showed differences depending on the feed. Thus, final weight, SGR and RGR in rainbow trout were not significantly different between the fish fed brewery byproducts (D-BSY, H-BSY and D-BSG) and those fed the reference diet (REF), with the exception of diet H-BSG that gave a significantly lower fish growth. Similar results were also obtained with gilthead seabream, no significant differences were found in final weight, SGR or RGR among the groups with the only exception of D-BSG group that showed a significantly lower final weight ($P=0.002$) than the rest of the groups. Feed conversion ratios (FCR) results in both species were not significantly different among the groups.

Tables 5, 6 and 7 show the ADC results of the diets and ingredients used for trout and seabream. There were significant differences in the ADC of protein and lipids among experimental diets and ingredients. Digestibility of protein was high in all the experimental diets, from 75.99% to 84.12% in rainbow trout, with H-BSY showing significantly lower values compared to D-BSG and REF diets.

In the case of gilthead seabream, experimental diets including BSG show the highest digestibility percentages of crude protein (84-85%), apart from the REF diet (90.34%). Digestibility of protein was always significantly lower in BSY based diets than in BSG based diets in both species, although the inclusion level was lower (20%) in the latter.

The inclusion of hydrolysed ingredients (H-BSG and H-BSY) slightly enhanced the ADCs of the diets in the case of seabream but no effect was detected in rainbow trout.

Apparent digestibility of lipids was always higher in the REF diet for both fish species. In rainbow trout ADCs were significantly lower in D-BSY and H-BSG. In the case or

seabream, no significant differences could be found among the lipids ADCs of the experimental diets.

Protein digestibility coefficients of experimental ingredients are presented in Table 7. The crude protein ADC of experimental ingredients varied from 67.70% to 45.55% in rainbow trout and from 47.00% to 34.09% in gilthead seabream. Hydrolysis helped to improve the digestibility of both by-products (spent yeast and spent grain) only in the case of gilthead seabream but not in the case of rainbow trout that showed better digestibility coefficients for dried BSY and BSG. T test was also performed to study the effect of hydrolysis on the digestibility of the ingredients. In the case of rainbow trout hydrolysis helped to increase the digestibility and significant differences were observed between D-BSY vs H-BSY and D-BSG vs H-BSG (Student's t test $P<0.05$) whereas in the case of seabream no significant differences could be detected in the digestibility after hydrolysis. On the other hand digestibility coefficients of the ingredients were significantly higher for rainbow trout compared to sea bream, except for the H-BSG (see Table 7)

The apparent digestibility coefficients of individual amino acids of experimental ingredients are presented in Table 8. Amino acid ADCs varied between test ingredients with values higher for rainbow trout than for sea bream, and higher for the amino acids of the spent grain than those of yeast. Methionine ADC was very low in both species fed spent yeast and not detected when hydrolysed spent yeast was used as ingredient.

Discussion

Fishmeal is becoming a limited feed source to be used as the main protein ingredient in carnivorous fish feeds and several new protein sources are being tested as alternatives, most of them derived from plants that generally are not well accepted because they contain carbohydrates that have 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), byproducts derived from the processing industry and microbial biomass (Hua et al., 2019). Brewery-derived byproducts, due to their huge production amounts per year, can be considered part of the last group of products (industrial byproducts 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.

However, before including a new protein ingredient in a commercial aquaculture feed, it is essential to evaluate the nutritional quality and the first step is the evaluation of

apparent digestibility and check the effects on the growth and welfare of different fish species. Previous work carried out with lactic, bakers and brewers yeasts in isoproteic fishmeal based diets (Metailler & Huelvan, 1993; Rumsey et al, 1991, 1992; Oliva-Teles & Gonçalves, 2001) for trout and/or sea bass did not find any effect of high inclusion levels (up to 30%) on fish growth or feed intake, and, when negative effects were found in fish performance (Rumsey et al, 1990), it was probably caused by the use of intact yeast cells because not all the intracellular ingredients were available to the fish compared to disrupted cells. The same results were observed in the present study, that although it was not designed to evaluate growth (only 30 days trial) it shows that the inclusion of 20% spent grain and 30% spent yeast did not cause any problem in fish growth, being no significantly different to that obtained in the control (REF) group. In the case of rainbow trout only the fish fed the diet H-BSG showed a lower final weight and growth, but in the case of seabream no differences with the control group were observed. Although both ingredients have a low content of protein and high content of fibre and carbohydrates, the results indicate that the inclusion levels used were adequate for both species.

As Campos et al (2018) discussed, the ADC of an ingredient reflects the capability of a certain species to utilize its nutrients, predicting its potential as feedstuff. In the present study the ADC of protein in the diets were 70-80% for the two species assayed and the two products (spent yeast and spent grain) used in the formulations and the ADC results were similar to those obtained by Oliva-Teles and Gonçalves (2001) or Campos et al (2018) in sea bass. However the ADC of protein of brewery spent yeast, were much lower (see table 7) than those reported by these authors (90% in the case of Oliva-Teles & Gonçalves, 2001 and 87.9% in the case of Campos et al., 2018), and similar to the digestibility reported by Metailler & Huelvan (1993, 53%). This low protein digestibility of yeast observed in both species, but specially in the case of gilthead seabream might be due to the high manipulation of the yeast during the dewatering and drying processes used (San Martin et al., 2020 in press) before being including in the feed, a process that have been improved recently with better results in feed inclusion, fish acceptability and digestibility results (San Martin pers. com.). Differences in ADC values in both species and specially lower ADC values for seabream can also be derived from the different ways used to collect faeces from gilthead seabream (collection column in the tank, in some cases underestimate ADC) and rainbow trout (stripping, in some cases overestimate ADC). However, we need to consider the sources of yeast that other authors used in their studies, in some cases yeast was alive (Tovar-Ramirez et al., 2004), laboratory-cultured (Santacroce et al., 2012), obtained from breweries but without indicating the

form and/or type of yeast (Oliva-Teles & Gonçalves, 2001; Castro et al., 2013) or processed and hydrolysed (Campos et al., 2018) and used not only as a protein source but as a probiotic with immunostimulant properties. Campos et al (2018) observed a better digestibility of yeast when it was hydrolysed and a peptide fraction of >3000 Da was used for sea bass. In the present study this positive effect of hydrolysis on the yeast protein ADC was only observed in the case of seabream but not in rainbow trout and no differences were detected when spent grain was included hydrolysed in the feed.

The amino acid content of yeast is similar to the values reported by Campos et al (2018) and very low when they are compared to the amino acid profile of fish meal (Cho & Kim, 2011), especially in terms of methionine. Individual amino acid ADCs were high for both ingredients and both species, except for methionine due to its low content. In the case of spent grain the ADC values were always between 85-90% for both species, indicating a good uptake by the two fish species used in this study.

In the case of lipid digestibility of the diets it was high for both ingredients and in both species in a rank between 70 to 82%, and slightly higher for rainbow trout. Hydrolysis of spent grain and spent yeast had only effects on the lipid ADC of seabream. Similar results were published by Campos et al (2018) that observed a higher lipid digestibility when the yeast was used after hydrolysis.

In the case of spent grain, not much information about protein, amino acids or lipid ADC can be found in fish. This ingredient has only been used for the ongrowing of freshwater fish (carp and Nile tilapia, Kaur & Saxena, 2004) with good results in growth and conversion but without any previous digestibility study. It has been used as an excellent ingredient for ruminants because it has a high nutritional value, high content of protein, fibre and energy and promote increased milk production and milk fat yield (Mussatto et al., 2006). In the present study, one of the few studies using spent grain as an ingredient in aquaculture feeds, BSG dried or hydrolysed has shown to have good effects in the growth of rainbow trout and sea bream, high protein digestibility included in the diet at 20% and have an amino acid digestibility coefficients in the rank of 70-96% for most amino acids in both species.

As a conclusion, the inclusion of 20-30% of brewers' spent yeast and spent grain in the feed for carnivorous fish either from fresh (rainbow trout) or marine (gilthead seabream) water resulted in a growth similar to that obtained using fish meal as the main protein ingredient, and show a good protein, lipid and amino acid digestibility. 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 byproducts

(trimmings) and increase the sustainability of both sectors, brewery industry and aquaculture. This results warrant a further evaluation in order to determine their maximal inclusion level to assure good growth performance of freshwater and marine fish.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Table 1 Formulation, proximate composition and gross energy content (average and standard deviation) of reference and experimental diets. Different letters indicate significant differences (ANOVA P<0.05)

[1] Super Prime LT fishmeal Corpesca, Chile
[2] Cargill, Brenntag, Spain
[3] Eurocoyal, Barcelona, Spain
[4] TecnoVit, Tarragona, Spain
[5] Sigma, Spain

Ingredients (%)	**REF**	**D-BSY**	**H-BSY**	**D- BSG**	**H-BSG**
Fish meal 70 LT[1]	60.00	40.00	42.00	50.00	50.00
Wheat starch[2]	20.95	9.45	7.45	12.45	12.45
Dried Yeast	-	30.00	-	-	-
Hydrolysed yeast	-	-	30.00	-	-
Dried spent grain	-	-	-	20.00	-
Hydrolysed spent grain	-	-	-	-	20.00
Fish oil[3]	18.00	19.50	19.50	16.50	16.50
Vit & Min Premix PV01[4]	1.05	1.05	1.05	1.05	1.05
Yttrium oxide[5]	0.02	0.02	0.02	0.02	0.02
Dry matter (g/Kg DM)	978.30±3.09	979.20±2.36	976.60±5.46	980.50±5.50	978.10±8.50
Ash (g/Kg DM)	98.80±0.98	83.20±0.77	78.70±0.76	93.60±4.24	100.60±1.07
Crude protein (g/Kg DM)	419.80±3.39	413.30±0.16	418.20±2.49	417.70±3.51	412.80±0.70
Crude fat (g/Kg DM)	218.42±3.29	223.94±1.45	224.04±5.71	219.83±2.04	221.40±1.59
Starch (g/Kg DM)	215.10±8.42b	218.50±16.36b	197.00±4.22b	130.20±9.07a	166.20±11.46a
Gross energy (MJ/Kg)	18.65±0.08	18.89±0.19	18.72±0.32	18.54±0.22	18.49±0.23

Table 2 Chemical composition and amino acid profile of the test ingredients

	D-BSY	H-BSY	D-BSG	H-BSG		D-BSY	H-BSY	D-BSG	H-BSG
Dry matter (DM, g/Kg)	941.90	890.50	920.00	981.10					
Ash (g/Kg DM)	42.36	43.46	39.13	61.05					
Crude Protein (g/Kg DM)	478.50	463.11	247.07	217.92					
Crude fat (g/Kg DM)	3.74	5.01	84.23	116.81					
Gross Energy (MJ/Kg)	19.90	19.92	21.69	20.65					
Phosphorus (g/Kg DM)	9.66	9.77	5.33	3.47					
Crude fiber (g/Kg DM)	6.79	6.96	187.83	165.94					
Starch (g/Kg DM)	218.60	225.15	39.02	34.96					
Vitamin B2 (mg/Kg DM)	2.76	5.61	0.43	1.43					
Beta-glucan (g/Kg DM)	80.9	90.4	8.8	0					
Essential amino acids (g/Kg DM)						(% protein)			
Arginine	25.16	23.81	12.83	10.70		5.26	5.14	5.19	4.91
Histidine	12.10	11.45	6.52	5.61		2.53	2.47	2.64	2.57
Lysine	31.74	28.86	9.57	7.34		6.63	6.23	3.87	3.37
Threonine	24.31	23.47	9.13	8.05		5.08	5.07	3.70	3.70
Isoleucine	23.46	22.68	10.22	8.05		4.90	4.90	4.14	3.70
Leucine	35.14	33.69	24.78	18.45		7.34	7.27	10.03	8.47
Valine	26.54	25.27	12.72	10.50		5.55	5.46	5.15	4.82
Methionine	8.17	7.75	5.11	3.87		1.71	1.67	2.07	1.78
Phenylalanine	23.04	22.46	14.46	11.31		4.81	4.85	5.85	5.19
Non-essential amino acids (g/Kg DM)						(% protein)			
Tyrosine	17.20	15.72	9.57	8.05		3.59	3.39	3.87	3.70
Aspartic Acid	47.88	45.82	17.17	14.47		10.01	9.89	6.95	6.64
Glutamic acid	60.30	58.28	52.39	36.90		12.60	12.58	21.21	16.93
Alanine	29.41	28.30	15.11	11.72		6.15	6.11	6.12	5.38
Glycine	18.90	18.30	8.80	7.95		3.95	3.95	3.56	3.65
Proline	22.30	21.22	25.11	18.24		4.66	4.58	10.16	8.37
Hydroxyproline	<0.3	<0.3	<0.3	<0.3		-	-	-	-
Serine	25.37	24.26	11.20	8.77		5.30	5.24	4.53	4.02

Table 3 Initial and final weight (average and standard deviation) and growth rates (relative –RGR- and specific growth rate –SGR-) of rainbow trout (*Oncorhynchus mykiss*) at the end of the study. Different letters indicate significant differences (ANOVA P<0.05)

	REF	D-BSY	H-BSY	D-BSG	H-BSG	ANOVA
Initial weight (g)	206.05 ± 41.18	206.05 ± 41.18	206.05 ± 41.18	206.05 ± 41.18	266.05 ± 41.18	
Final weight (g)	353.37 ± 60.85a	328.39 ± 63.25ab	337.75 ± 67.61ab	324.97 ± 51.98ab	317.43 ± 50.62n	P=0.012
SGR (%)	1.79 ± 0.18a	1.55 ± 0.08ab	1.64 ± 0.12ab	1.53 ± 0.11ab	1.44 ± 0,03b	P=0.032
RGR (%)	71.50 ± 8.95a	59.18 ± 4.04ab	63.87 ± 6.03ab	58.14 ± 5.13ab	54.15 ± 0.03b	P=0.032
FCR (%)	1.13 ± 0.15	1.37 ± 0.30	1.33 ± 0.16	1.37 ± 0.05	1.48 ± 0.19	NS

Table 4 Initial and final weight (average and standard deviation) and growth rates (relative –RGR- and specific growth rate –SGR-) of gilthead seabream (*Sparus aurata*) at the end of the study. Different letters indicate significant differences (ANOVA P<0.05)

	REF	D-BSY	H-BSY	D-BSG	H-BSG	ANOVA
Initial weight (g)	253.01 ± 27.68	253.01 ± 27,68	253.01 ± 27.68	253.01 ± 27.68	253.01 ± 27.68	
Final weight (g)	323.63 ± 39.78a	329.57 ± 40.98ab	345.19 ± 41.34ab	319.05 ± 38.76ab	328.11 ± 41.46b	P=0.002
SGR (%)	0.82 ± 0.08	0.88 ± 0.27	1.03 ± 0.13	0.77 ± 0.11	0.87 ± 0.08	NS
RGR (%)	27.91 ± 3.09	30.34 ± 10.17	36.43 ± 5.12	26.10 ± 4.24	29.68 ± 3.09	NS
FCR (%)	1.88 ± 0.20	1.92 ± 0.77	1.45 ± 0.22	2.03 ± 0.32	1.77 ± 0.19	NS

Table 5 Apparent Digestibility Coefficients (ADC, average and SD) of protein and lipid of experimental diets in rainbow trout. Different letters indicate significant differences (ANOVA, P<0.05)

Diet	Protein faeces (g/Kg)	Protein diet (g/Kg)	Protein ADC	Lipids faeces (g/Kg)	Lipids diet (g/Kg)	Lipids ADC
REF	318.20 ± 0.56	419.80 ± 3.39	84.12 ± 0.15a	122.10 ± 6.05	218.42 ± 3.29	88.29 ± 0.11a
D-BSY	295.90 ± 1.28	413.30 ± 1.16	78.73 ± 2.11ab	157.60 ± 0.11	223.94 ± 1.45	79.09 ± 2.07c
H-BSY	314.30 ± 1.46	418.20 ± 2.49	75.99 ± 1.26b	132.20 ± 3.31	234.04 ± 5.71	81.96 ± 0.95b
D-BSG	247.50 ± 0.06	417.70 ± 3.51	81.96 ± 1.04a	140.20 ± 6.11	219.83 ± 2.04	80.58 ± 1.12b
H-BSG	224.10 ± 0.21	392.80 ± 0.70	79.69 ± 0.34ab	154.40 ± 3.99	221.40 ± 1.59	75.18 ± 0.41c
ANOVA			P=0.007			P<0.001

Table 6 Apparent Digestibility Coefficients (ADC, average and SD) of protein and lipid of experimental diets in gilthead seabream. Different letters indicate significant differences (ANOVA, P<0.05)

Diet	Protein faeces (g/Kg)	Protein diet (g/Kg)	Protein ADC	Lipids faeces (g/Kg)	Lipids diet (g/Kg)	Lipids ADC
REF	198.10 ± 0.40	419.80 ± 3.39	90.26 ± 0.11a	124.70 ± 0.94	218.42 ± 3.29	88.21 ± 0.13a
D-BSY	262.40 ± 1.59	413.30 ± 1.16	71.76 ± 2.73c	142.70 ± 5.94	223.94 ± 1.45	71.66 ± 2.74b
H-BSY	223.10 ± 2.79	418.20 ± 2.49	75.01 ± 1.27c	122.80 ± 0.07	234.04 ± 5.71	75.42 ± 1.25b
D-BSG	118.20 ± 3.41	417.70 ± 3.51	84.01 ± 0.54b	114.60 ± 0.60	219.83 ± 2.04	70.55 ± 1.00b
H-BSG	87.80 ± 0.90	392.80 ± 0.70	85.22 ± 0.31b	85.80 ± 1.80	221.40 ± 1.59	74.38 ± 0.53b
ANOVA			P<0.001			P<0.001

Table 7 Apparent protein digestibility coefficients (ADC, average and SD) of brewery by-products ingredients used in the experimental diets for rainbow trout and gilthead seabream

Ingredient	ADC Rainbow trout (%)	ADC Seabream (%)	Student's t test
D-BSY	67.68 ± 6.43b	33.89 ± 8.33a	P=0.005
H-BSY	58.81 ± 3.94b	42.77 ± 3.95a	P=0.008
D-BSG	67.29 ± 8.12b	41.57 ± 4.22a	P=0.008
H-BSG	45.59 ± 2.94	46.39 ± 2.66	P=0.744

Table 8 Apparent digestibility coefficients (ADC) of individual amino acids in the test ingredients used for rainbow trout and gilthead seabream

	RAINBOW TROUT				GILTHEAD SEABREAM			
ADC (%)	**D-BSY**	**H-BSY**	**D-BSG**	**H-BSG**	**D-BSY**	**H-BSY**	**D-BSG**	**H-BSG**
Essential amino acids								
Arg	76.89	70.43	90.53	86.93	52.95	49.64	91.51	91.07
His	77.21	74.21	92.01	89.38	66.78	63.55	93.99	93.36
Lys	72.26	64.82	94.56	92.38	53.60	49.26	96.33	95.77
Thr	62.80	51.58	91.91	89.58	45.70	46.20	92.46	92.91
Iso	80.11	68.65	94.04	91.91	60.65	54.87	93.47	94.01
Leu	86.20	77.72	92.59	89.99	70.51	65.52	91.73	92.32
Val	80.05	70.09	93.06	90.78	63.69	60.52	92.58	93.03
Met	10.55	ND	56.91	45.99	38.30	ND	85.50	71.53
Phe	86.04	78.01	93.35	91.00	68.06	61.49	92.33	92.44
Non-essential aminoacids								
Tyr	81.21	73.08	93.18	90.18	60.64	54.10	93.04	92.58
Asp	52.58	36.81	87.65	84.73	39.66	35.29	91.77	92.07
Glu	87.69	78.98	92.04	89.10	73.81	68.12	91.49	91.97
Ala	75.87	62.66	90.63	87.84	55.27	48.59	89.71	89.78
Gly	54.49	40.97	82.81	77.68	25.51	27.63	85.34	84.88
Pro	87.80	80.04	87.52	84.89	77.99	71.98	86.95	87.39
Ser	67.51	54.43	91.95	89.30	53.68	45.96	92.29	92.62