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# Brewery by-products (yeast and spent grain) as protein sources in rainbow trout (*Oncorhynchus mykiss*) feeds

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A trial was conducted to analyze the effect of the inclusion of yeast and spent grain obtained from breweries in feeds for rainbow trout (*Oncorhynchus mykiss*), taking into account the availability of these by-products, produced in large quantities in Europe. The ingredients were assayed in both dried and hydrolyzed format and compared with a commercial dried or hydrolyzed yeast. According to the results, the inclusion of 20% yeast and 15% spent grain in the feed, formulated with only 15% inclusion of fish meal, produced similar results in growth among all the groups, a food conversion significantly lower for the control and spent grain formulated feeds, and rainbow trout muscle composition similar to the fish fed with a control commercial feed and showed a protein digestibility of 87%–89% without differences with the commercial yeast. Hydrolysis of the ingredients had no effects on the protein digestibility of the feeds. Protein digestibility of the ingredients was lower for spent grain. An inclusion rate not higher than 15% for spent grain is recommended. These industrial by-products can be a good source to reduce the use of plant-based ingredients and increase the sustainability of both sectors, brewery industry, and aquaculture.

## KEYWORDS

Brewery by-products, valorisation, circular economy, protein digestibility, protein substitution, rainbow trout, fillet quality

## Introduction

According to Food and Agriculture Organization (FAO) statistics for 2020 total aquaculture production in 2018 accounted for 82.1 million tonnes (51.3 million inland and 30.8 million of marine aquaculture). The contribution of aquaculture to fish production reached 46% in 2018 with inland aquaculture, producing the highest

quantities of farmed fish (57.2% of world total) mainly produced in freshwater (FAO, 2020). Whole fish and fish by-products are used in high quantities to produce fish meal and fish oil for animal feeding, and according to FAO, 2020, the sustainability of aquaculture depends on the use of other new ingredients to replace these fish-derived products. New ingredients need to be highly digestible to boost fish performance and reduce waste production, and they must be available in regular quantities and at competitive prices (Gatlin et al., 2007). Several protein sources have been used in recent years in experimental aquafeeds for freshwater and marine finfish, mostly derived from microalgae (Sarker et al., 2020) or insects (Cardinaletti et al., 2019; Randazzo et al., 2021a; Randazzo et al., 2021b). Terrestrial animal by-products have also been used although they are not considered acceptable by consumers (Naylor et al., 2009). As feed covers the largest cost of production in aquaculture that can reach 50%–70% of fish farmers production costs like in salmon (Ashe and Oglend, 2016), reducing the feed cost without compromising the quality or health of cultured fish may lead to a significant reduction in the total cost. That is the reason why, in the last decade, the interest in industrial by-products recovery, especially in those locally available and low-cost, to be used as alternative ingredients in feed, has increased significantly (Barrows et al., 2008).

More than 1.95 billion hectoliters of beer are produced worldwide (Conway, 2019), generating large amounts of organic waste, mostly spent yeast (BSY), and grain (BSG) that are considered high-quality ingredients for feeds due to their high content of protein, approximately 20% dry weight (DW) in the case of BSG (Robertson et al., 2010; Aliyu and Bala, 2011; McCarthy et al., 2013) and 40%–50% DW for BSY (Mussatto et al., 2006; Thomas and Rahman, 2006; Mussatto, 2009; Levic et al., 2010; Zhou et al., 2018).

In Europe (Eurostat, 2020) over 34 billion liters of beer containing alcohol were produced in 2020, equivalent to approximately 77 liters per inhabitant. BSG accounts for approximately 85% of all residues produced by the brewing industry (Aliyu and Bala, 2011), whereas BSY accounts for maximum 15% of total by-products generated (Kerby and Vriesekoop, 2017). These wastes from breweries are reused as terrestrial animal feed or for the production of bioethanol (Djuragic et al., 2010; Buffington, 2014). Several publications in recent years have identified these by-products as alternative ingredients in feeds for farmed fish in Europe (Oliva-Teles and Gonçalves, 2001; Cheng et al., 2004; Kaur and Saxena, 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).

Brewers' spent grain is the major (approximately 85% in weight) by-product of beer industry (Mussatto et al., 2006). It has been used not only as human and livestock food (Murdoch et al., 1981; Faccenda et al., 2017; Mussatto et al., 2014) but also as feed ingredient for the ongrowing of crustaceans (Muzinic et al., 2004) and fish (Yamamoto et al., 1994; Cheng et al., 2004; Kaur

and Saxena, 2004; Campos et al., 2018; Jayant et al., 2018) due to its high content in protein and fiber as well as lipids, minerals, and vitamins (Mussatto et al., 2006; Mussatto et al., 2014).

Brewers' spent yeast is the second largest by-product from brewing, and its disposal is considered an environmental problem. Several authors (Oliva-Teles and Gonçalves, 2001; Ebrahim and Abou-Seif, 2008; Ozório et al., 2012; Sealey et al., 2014) cited this by-product as a potential alternative to fish meal in the feed for cultured fish, porcine, and ruminants (Huige, 2006). Brewer's yeast is a source of protein, vitamins, and minerals (Ovie and Eze, 2014), and other bioactive compounds such as  $\beta$ -glucans, mannan oligosaccharides, vitamins, minerals, and nucleic acids (Ferreira et al., 2010) are also present in high quantities in this by-product.

The digestibility of these two ingredients has already been published by Nazzaro et al. (2021). The main objectives of the present study were as follows: 1) to establish the inclusion rate of hydrolyzed and non-hydrolyzed BSG and BSY and 2) to validate their use as aquafeed ingredients for rainbow trout (*Oncorhynchus mykiss*), as a model for carnivorous freshwater fish.

## Materials and methods

### Ingredients and experimental diet preparation

Mahou San Miguel, a Spanish brewery located in Lérida (Spain), provided the by-products that were stabilized before its inclusion in aquafeeds as in Nazzaro et al. (2021) and hydrolyzed as in San Martín et al. (2020). Four ingredients were obtained: (1) dried spent yeast (DSY; *Saccharomyces cerevisiae*), (2) hydrolyzed spent yeast (HSY), (3) dried spent grain (DSG), and (4) hydrolyzed spent grain (HSG) with a moisture lower than 10%. A commercial dried and hydrolyzed yeast was obtained from Aplicaciones Biológicas a la Nutrición S.L. (ABN, Madrid, Spain), being included in the feeds at the same rate as BSY, to compare the digestibility of this commercial yeast with that obtained from BSY and to evaluate its effects on fish growth.

The diets were formulated and extruded (4-mm die diameter, 5- to 6-mm pellet diameter) at Institute of Agrifood Research and Technology (IRTA, Mas Bové, Tarragona, Spain). The extrusion (Rosal extruder, model RS50, Spain) was carried out at 700 rpm with a final temperature of 110°C at 140 kg/h using 20% water vapor to reach 85°C–90°C at precondition. The diets were formulated using a commercial fish meal (Super Prime 70 LT, Corpesca, Spain) to meet the nutritional requirements of rainbow trout (FAO, 2020) (Table 1). Yttrium oxide ( $Y_2O_3$ , Sigma Aldrich, Spain) was used as an inert marker ( $0.2 \text{ g kg}^{-1}$ ) for the evaluation of digestibility. All the feeds were iso-proteic and iso-lipidic and were formulated including 10% and 20% of DSY, HSY, dried yeast from ABN company

TABLE 1 Formulation of feeds used in the trial.

Ingredient (g)	CTRL	DSY 10	HSY 10	DSY 20	HSY 20	DSG 7.5	HSG 7.5	DSG 15	HSG 15
Soy bean meal	6.00	4.06	4.06	1.00	1.00	6.00	6.00	2.52	2.52
Wheat gluten	21.78	19.52	19.52	16.78	16.78	20.40	20.40	18.40	18.40
Soycomeal <sup>f</sup>	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00
Fish meal <sup>a</sup>	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Fish oil <sup>c</sup>	7.75	7.50	7.50	7.38	7.38	7.74	7.74	7.72	7.72
Soya oil	5.85	5.78	5.78	5.77	5.77	5.91	5.91	6.12	6.12
Lutavit C Aquastab 35 %	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Dicalcium Phosphate <sup>e</sup>	0.83	0.70	0.70	0.65	0.65	0.75	0.75	0.65	0.65
Choline chloride <sup>f</sup>	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Lysine HCl	0.36	0.09	0.09			0.20	0.20		
Mineral mix <sup>d</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix <sup>d</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Wheat starch <sup>b</sup>	15.95	16.77	16.77	13.60	13.60	17.66	17.66	14.65	14.65
Soy lecithin	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Yttrium oxide <sup>c</sup>	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Brewer's spent grain						7.50	7.50	15.00	15.00
Brewer's spent yeast		10.00	10.00	20.00	20.00				
Total Fish meal	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Total Vegetable meal	44.78	40.58	40.58	34.78	34.78	50.90	50.90	52.92	52.92
FM/FO	15/7.7	15/7.5	15/7.5	15/7.4	15/7.4	15/7.7	15/7.7	15/7.7	15/7.7
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
Water (%)	91.50	91.55	91.60	91.70	91.90	91.60	92.10	92.00	92.20
Ash (%DW)	5.30	5.35	5.70	5.80	5.60	5.65	5.70	5.30	5.50

The same formulation was used for dried and hydrolyzed yeast and spent grain. Changes in corn gluten, wheat gluten, and soybean meal, to account for total plant meal inclusion, were made to ensure diets were isonitrogenous. CTRL, control diet; DSY, dried spent yeast; HSY: hydrolyzed spent yeast; DSG, dried spent grain; HSG, hydrolyzed spent grain; DYABN, dried commercial yeast from ABN; HYABN, hydrolyzed commercial yeast from ABN. 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.

F. Andres Pinaluba S.A., Spain.

(DYABN), and hydrolyzed yeast from ABN (HYABN) and 7.5% and 15% of DSG and HSG to the basal mixture (Table 1). Feeds including the commercial yeast from ABN have the same formulation and inclusion rate (10% and 20%) than the feeds with brewery SY included. This new ingredients were included in the feeds replacing gluten and plant-derived meals.

## Fish rearing and fecal collection

Rainbow trout specimens were obtained from Aiguanatura dels Ports (Tarragona, Spain) transported to IRTA by road and kept in quarantine for 14 days and fed using the *Premium* commercial feed for rainbow trout from Skretting. Trouts were distributed in twenty-two 200-L fiber glass tronco-conical tanks

with a purge system to collect uneaten feed pellets, in groups of 15 fish (body weight  $77.90 \pm 8.97$  g) per tank. The tanks were supplied with filtered freshwater in a recirculation system (IRTAMar<sup>TM</sup>) and maintained at 20°C with natural light and photoperiod and salinity of 1.5 ppt.

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

$$RGR = (Wf - Wi) / Wi \times 100$$

$$SGR = (\ln Wf - \ln Wi) / t \times 100$$

where  $W_i$  and  $W_f$  are the fish weight at the beginning ( $W_i$ ) and at the end ( $W_f$ ) of the feeding period, and  $t$  is the time (days) between  $W_f$  and  $W_i$ .

Five fish per tank were dissected, and liver was weighted to calculate hepatosomatic index (HSI) as in the following formula:

$$HSI = \text{Weight of liver of fish} / \text{Body weight of fish} \times 100$$

The experimental diets were randomly assigned to the tanks and fed in triplicates, once per day, during 60 days using Arvotec (Finland) automatic feeders. The amount of feed was calculated according to the theoretical weight of the fish and the feeding tables provided by Skretting. Every day, the feed provided was registered, as well as the uneaten feed, collected every day from the bottom center drain of the tanks, dried in an oven for 24 h, and weighted to calculate feed conversion ratio (FCR) and protein efficiency ratio (PER).

$$FCR = \text{Feed consumed} / (\text{Final} - \text{Initial weight})$$

$$PER = (\text{Final} - \text{Initial weight}) / \text{Protein consumed}$$

In the case of the fish fed with 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).

Feces were collected by abdominal stripping (as described by Nazzaro et al., 2021) in anesthetized fish (MS 222, 47 ppm, 5-min exposure, Pharmaq, Spain), in alternate days during 2 weeks, to collect enough quantities for chemical and Yttrium analyses, before the final sampling and carried out in all the tanks fed with the control and brewery by-products ingredients. Fecal samples were freeze-dried and stored at  $-20^{\circ}\text{C}$  until chemical analyses.

In the final sampling (day 60), five fish per tank were eviscerated, and the weight of viscera and liver was recorded. Samples of whole liver and muscle taken using a standardized cut (2 cm wide cut behind the dorsal fin on the left lateral side of the fish) were collected and kept at  $-20^{\circ}\text{C}$  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 DY and 15% for SG)

$$ADC(\%) = 100 \times (1 - (\text{dietary } Y_2O_3\text{ level} / \text{feces } Y_2O_3\text{ level}) \times (\text{feces nutrient or energy level} / \text{dietary nutrient or energy level}))$$

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

$$ADC_{SG}(\%) = ADC_{test} + [(ADC_{test} - ADC_{ref}) \times ((0.85 \times D_{ref}) / (0.15 \times D_{ing}))]$$

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

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

The feces samples were dehydrated by freeze-drying (LyoAlfa 6, Telstar, USA) before chemical analyses to avoid nutritional losses or alterations. The biochemical analyses of the diets, ingredients, feces, and muscle and liver of the fish were performed in duplicates according to the standard methods of the Association of Official Analytical Chemists (AOAC, 2006). Dry matter ( $105^{\circ}\text{C}$  for 14 h, AOAC 925.09), ash incineration in a muffle furnace (Nabertherm, Germany  $500^{\circ}\text{C}$  for 5 h, AOAC, 942.05), crude protein (Dumas's procedure using Nitrogen analyser FP-528 Leco, USA, AOAC 968.06, with N correction factor of 6.25), and crude fat (Büchi Extraction System B-811, Switzerland, AOAC 920.39) were analyzed in all the samples obtained. Acid catalyzed transmethylation (Christie, 1982) and purification of methyl esters (TLC plates) were carried out before the fatty acid analysis carried out by gas-liquid chromatography (Thermo TraceGC, Villalta et al., 2005). Yttrium oxide content in diets and feces was determined according to Garantun-Tjeldsto et al. (2006) by inductively coupled plasma-mass spectrometry (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.

Initial and final weight ( $n = 60$ ), HSI ( $n = 3$ ), growth rates (SGR and RGR,  $n = 3$ ), feed conversion (FCR,  $n = 3$ ), protein efficiency (PER,  $n = 3$ ), biochemical composition of muscle and liver ( $n = 3$ ), and apparent digestibility coefficient (ADC) data ( $n = 2$ ) 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

Table 1 shows the formulation and proximate composition of the feeds used in the study including brewery by-products, and the formulation used for the inclusion of spent yeast (SY) was also used for the formulation using yeast from a commercial company (ABN, Spain). Table 2 shows the composition of the brewery by-products used in the trial.

**TABLE 2** Proximate composition and amino acid profile of the ingredients derived from the brewery industry [spent yeast (SY) and spent grain (SG)] dried (D) and hydrolyzed (H), used in the formulation of the feed.

	DSY	HSY	DSG	HSG
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 (ppm)	2.76	5.61	0.43	1.43
$\beta$ -glucan (g/kg DM)	80.9	90.4	0.88	0.00
<i>Essential amino acids (g/kg DM)</i>				
Arginine	25.16	23.81	12.83	10.70
Histidine	12.10	11.45	6.52	5.61
Lysine	31.74	28.86	9.57	7.34
Threonine	24.31	23.47	9.13	8.05
Isoleucine	23.46	22.68	10.22	8.05
Leucine	35.14	33.69	24.78	18.45
Valine	26.54	25.27	12.72	10.50
Methionine	8.17	7.75	5.11	3.87
Phenylalanine	23.04	22.46	14.46	11.31
<i>Non-essential amino acids (g/kg DM)</i>				
Tyrosine	17.20	15.72	9.57	8.05
Aspartic acid	47.88	45.82	17.17	14.47
Glutamic acid	60.30	58.28	52.39	36.90
Alanine	29.41	28.30	15.11	11.72
Glycine	18.90	18.30	8.80	7.95
Proline	22.30	21.22	25.11	18.24
Hydroxyproline	<0.3	<0.3	<0.3	<0.3
Serine	25.37	24.26	11.20	8.77

Final weight was significantly higher for DSY and HSY groups (Table 3), although SGR and RGR were not significantly different among the groups of fish fed with brewery by-products (DSY, HSY, DSG, and HSG) included at two different levels and those fed with the control diet (CTRL). FCR and PER results were significantly lower for the CTRL and HSG-fed groups, whereas those fed with spent yeast (DSY and HSY) show the highest values.

ADCs of the feeds and ingredients are shown in Table 4. Digestibility of protein was 86.6%–89.9% in the experimental diets, being higher in the case of CTRL. The ADC of lipids varied from 80.9% to 88.7%, and the lowest digestibility was found for CTRL and the highest for the spent yeast (both dried and hydrolyzed), although no significant differences were detected (ANOVA,  $P = 0.053$ ,  $n = 2$ ).

Protein digestibility coefficients were higher than 75% for SY and the commercial yeast (YABN), whereas SG had statistically significant lower values between 50% and 65%. No improvement

in digestibility of the ingredients was obtained after hydrolysis of yeast and spent grain.

Muscle and liver protein and lipid content results are shown in Table 5. A significantly higher protein content was recorded in the muscle of fish fed with HSY included at 20% and HYABN commercial yeast included at 10%, showing values approximately 71%–72%, and the lowest values were obtained in the fish from DSG, HSG, and CTRL groups. Lipids were higher in the muscle (10% lipids) of fish from DSY 20 group, whereas the lowest values were found in CTRL, HSG 7.5, and HYABN 10 (5.6% lipids). In the liver, the highest protein content (52%–54% DW) was recorded in DSG 7.5 and HSG 15 groups, whereas it was lower in those fed with DSY and HSY included at 10% and 20%, as well as in the fish fed with HYABN at 10%. Lipid content was higher in the liver of fish fed with DYABN included at 10% (15.5% lipids) and lower in those fed with HSY at 10% (9% lipids) with the rest of the groups showing levels between 11% and 14%.

**TABLE 3** Initial and final weight of rainbow trout used in the inclusion trial (n = 60 per treatment) and results in hepatosomatic index (HSI; five fish per tank, n = 3), specific growth rate (SGR; n = 3), relative growth rate (RGR; n = 3), feed conversion ratio (FCR; n = 3), and protein efficiency ratio (PER; n = 3).

	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	79.33	8.65	174.28	34.56 b	2.03	0.61 b	1.29	0.05	119.44	6.67	1.29	0.05 b	1.19	0.07 b
DSY10%	77.10	9.33	217.88	48.84 a	4.71	2.05 a	1.70	0.19	200.95	8.53	1.81	0.05 a	2.01	0.09 a
DSY20%	77.42	10.07	219.68	28.75 a	3.76	0.88 ab	1.71	0.09	184.13	15.25	1.71	0.09 a	1.84	0.15 a
HSY10%	78.82	9.60	217.37	45.87 a	4.26	1.28 ab	1.66	0.05	175.33	8.80	1.66	0.05 a	1.75	0.09 a
HSY20%	77.56	9.67	222.80	31.48 a	4.46	1.44 a	1.73	0.04	187.30	7.42	1.73	0.04 a	1.87	0.07 a
DSG7.5%	77.95	8.80	193.38	37.55 ab	2.94	1.51 ab	1.49	0.11	148.11	16.11	1.49	0.11 ab	1.48	0.16 ab
DSG15%	77.63	7.52	192.90	29.14 ab	2.88	0.88 ab	1.49	0.03	148.48	5.30	1.49	0.03 ab	1.48	0.05 ab
HSG7.5%	77.39	8.79	178.40	38.85 b	2.14	0.53 b	1.37	0.04	130.66	5.53	1.37	0.03 b	1.31	0.06 b
HSG15%	77.92	8.37	175.73	39.02 b	2.32	0.53 b	1.33	0.15	125.08	20.14	1.33	0.15 b	1.25	0.20 b
DYABN10%	77.41	9.55	206.19	62.02 ab	3.30	0.73 ab	1.61		166.37		1.61		1.66	
DYABN20%	78.13	10.06	228.63	43.21a	2.50	0.07 ab	1.76		192.62		1.76		1.93	
HYABN10%	77.19	8.10	216.36	35.36 ab	4.07	0.85 ab	1.69		180.31		1.69		1.80	
HYABN20%	78.15	10.45	202.94	38.58 ab	3.47	0.79 ab	1.56		159.67		1.56		1.60	
ANOVA			P < 0.001		P < 0.001		P=0.007		P=0.07		P < 0.001		P < 0.001	

Different letters indicate statistically significant differences (ANOVA,  $P < 0.05$ ,  $n = 3$ ). CTRL, control diet; DSY, dried spent yeast; HSY, hydrolyzed spent yeast; DSG, dried spent grain; HSG, hydrolyzed spent grain; DYABN, dried commercial yeast from ABN; HYABN, hydrolyzed commercial yeast from ABN. Fish fed with commercial yeast from ABN were not included in the statistics; only one tank was used per treatment.

Tables 6, 7, 8 show the fatty acid profile of feeds, muscle, and liver, respectively. Muscle and liver fatty acid content reflected the fatty acid composition of the feeds used. Figure 1 summarizes the fatty acid profile of the muscle with all the groups showing similar profiles although the muscle of fish fed with spent yeast (dried or hydrolyzed) included at 20% shows a lower N-3 PUFA content and higher MUFA, the fish fed with spent grain show higher levels of N-6, and those fed with DSY included at 10% show a profile closer to the CTRL.

## Discussion

In the last years, considerable efforts have been made to look for alternative ingredients to fish meal and oil, and most of the

research focused in looking for sustainable ingredients to reduce wastes. Several products such as fish trimmings (Stevens et al., 2018), insects (IPIFF, 2018), algae (Loveday, 2019), and by-products derived from the processing industry and microbial biomass (Hua et al., 2019) have centered most of this research. Brewery by-products have also been considered as good ingredients for marine and freshwater fish culture, because of their content of protein (40%–50% for spent yeast), lipids, vitamin B2,  $\beta$ -glucans, and mannan-oligosaccharides. Recently, a review by Agboola et al. (2021) about the use of yeast in feed production and fish aquaculture suggested the need for a large-scale production of yeast at affordable cost. In the case of breweries, spent yeast and spent grain are produced in very high quantities, but they need to be dewatered and dried before being used in aquafeeds. San Martín et al. (2020) designed these

**TABLE 4** Apparent digestibility coefficients of feeds and ingredients (Av $\pm$ SD) used in the inclusion trial.

### Apparent Digestibility of the Feed

	CTRL	DSY 20%	HSY 20%	DSG 15%	HSG 15%	DYABN 20%	HYABN 20%
Protein	89.86 $\pm$ 1.70	86.63 $\pm$ 2.35	86.80 $\pm$ 2.40	87.62 $\pm$ 2.66	86.63 $\pm$ 2.44	87.71 $\pm$ 1.23	87.15 $\pm$ 1.17
Lipids	80.87 $\pm$ 1.67	87.44 $\pm$ 1.76	88.65 $\pm$ 1.65	84.20 $\pm$ 1.81	82.07 $\pm$ 1.53	85.86 $\pm$ 2.05	86.14 $\pm$ 2.06
Apparent Digestibility Coefficients of Ingredients							
	DSY 20%	HSY 20%	DSG 15%	HSG 15%	DYABN 20%	HYABN 20%	
Protein	74.52 $\pm$ 1.62a	75.37 $\pm$ 1.47a	65.10 $\pm$ 2.90b	49.93 $\pm$ 3.23c	78.62 $\pm$ 1.27a	76.09 $\pm$ 1.35a	

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. Different letters indicate significant differences (ANOVA,  $P < 0.001$ ,  $n = 2$ ).

TABLE 5 Protein and lipid content (% dry weight, DW) of the muscle and liver of rainbow trout fed the experimental diets.

	MUSCLE				LIVER			
	Total Lipids (% DW)		Protein (% DW)		Total Lipids (% DW)		Protein (% DW)	
	Av	SD	Av	SD	Av	SD	Av	SD
CTRL	5.62	0.22 d	62.13	0.57 c	14.84	0.21 ab	45.09	1.56 b
DSY 10%	6.67	0.22 c	69.83	3.27 ab	11.02	0.21 c	38.97	1.00 c
DSY 20%	10.74	0.02 a	64.97	0.98 abc	11.34	0.57 c	38.67	0.46 c
HSY 10%	7.74	0.03 c	64.66	1.82 abc	9.59	0.14 d	39.11	0.83 c
HSY 20%	9.33	0.03 b	72.3	3.00 a	11.63	0.11 c	39.75	0.18 c
DSG 7.5%	8.88	0.39 b	67.3	3.81 abc	13.32	0.34 b	52.14	0.33 a
DSG 15%	7.24	0.05 c	61.43	2.46 c	13.87	0.14 b	44.36	0.81 b
HSG 7.5%	5.61	0.19 d	60.47	3.64 c	13.4	0.17 b	48.66	1.67 b
HSY 15%	8.13	0.35 bc	68.43	0.80 abc	14.42	0.47 b	54.24	0.33 a
DYABN 10%	9.35	0.54 b	65.41	4.06 abc	15.47	0.32 a	47.17	0.36 b
DYABN 20%	6.71	0.21 c	62.08	0.64 b	11.6	0.03 c	41.58	1.25bc
HYABN 10%	5.63	0.23 d	71.05	1.08 a	10.97	0.01 c	38.65	1.08 c
HYABN 20%	9.16	0.32 b	62.75	2.89 b	11.97	0.28 c	45.93	0.01 b
ANOVA	P<0.001		P<0.001		P<0.001		P<0.001	

Different letters indicate significant differences (ANOVA,  $P < 0.05$ ,  $n = 3$ ). 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.

processes of dewatering and drying, reducing energy consumption, being more economical and environmentally sustainable, and the quantities of DSY and grain can now provide a higher contribution in satisfying the protein demand of the global aquafeed industry. This was one of the objectives of the project LIFE-Brewery together with the trials carried out with gilthead sea bream (Estévez et al., 2021) and the present trial with rainbow trout, to show that, once these brewery by-products are processed, they can be used as ingredients in aquafeeds for high-value fish such as salmon, rainbow trout, or European sea bass and gilthead seabream. The main objective of the present study was to evaluate the inclusion of these products in rainbow trout feeds and re-evaluate the digestibility of both ingredients and feeds. Thus, the ADC values obtained in the present study were higher than those published by Nazzaro et al. (2021) probably as a consequence of the improvements introduced in the mechanical dewatering and drying processes, reducing the manipulation of spent yeast.

Brewer's yeast can be considered a good replacer of fish meal and plant-based proteins (Rumsey et al., 1990; Rumsey et al., 1991; Oliva-Teles and Gonçalves, 2001) in aquafeeds, and it has been included in commercial diets for several fish species, including salmonids (Ferreira et al., 2010). Similar to the results of the present study in rainbow trout, Rumsey et al. (1991) found that, when brewer's yeast was included at up to 25% of the diet, fish growth and feed conversion were better than with the control feed. Higher levels of brewer's yeast were less palatable, but fish did not appear to be adversely affected. More recent studies using grain distiller's dried yeast from the ethanol

industry in feeds for rainbow trout showed that 37.5% replacement of fish meal protein and 18% replacement of total dietary protein did not reduce fish performance (Hauptman et al., 2014; Sealey et al., 2015).

Spent grain is the main solid waste produced by the beer industry (Farcas et al., 2017) reaching up to 85% of breweries by-products. It is daily produced in large breweries, and, due to its high moisture content and transport cost, it is generally used in the neighborhood of the breweries as animal feed or as a compost. There are not many publications related to the use of this raw material in aquafeeds, but, in a publication by Hertrampf and Piedad-Pascual (2012), they recommended not to include it in high amounts in the feeds due to its high fiber content and the low pelletizing ability and poor durability of the pellet. It has been mostly used in freshwater fish feeds (carp and Nile tilapia; Kaur and Saxena, 2004) with good results in growth and conversion. Cheng et al. (2004) found similar results as in the present trial using spent grain in the diet for rainbow trout, whereas other authors (Overland et al., 2013; Welker et al., 2014) found also that using dried grains (mostly maize) from the fuel ethanol industry replacing plant ingredients in diets for rainbow trout has good results in terms of growth and conversion, improved the protein digestibility of the diets, and did not have any effect on intestine structure and function.

The results obtained in the present study show that SY inclusion levels up to 20% produced very good results in rainbow trout growth, with final weights significantly higher than those of CTRL group, although no differences in SGR and RGR were observed. FCR and PER had significantly higher values for the

TABLE 6 Fatty acid composition (% of total FAs, only main fatty acids and totals are included) of the feeds used in the trial.

	<u>CTRL</u>		<u>DSY 10%</u>		<u>DSY 20%</u>		<u>HSY 10 %</u>		<u>HSY 20%</u>		<u>DSG 7.5%</u>		<u>DSG 15%</u>		<u>HSG 7.5%</u>		<u>HSG 15%</u>		<u>DYABN 10%</u>		<u>DYABN 20%</u>		<u>HYABN 10%</u>		<u>HYABN 20%</u>	
	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)	614.69	27.91	614.24	2.31	604.38	1.19	616.51	6.75	632.68	0.62	619.25	0.72	607.02	13.15	606.12	6.07	640.44	1.47	541.24	18.19	597.55	36.97	552.15	11.87	561.70	30.71
14:0	1.67	0.15	1.07	0.14	1.16	0.34	1.50	0.00	1.36	0.00	1.45	0.34	1.31	0.01	1.33	0.29	1.25	0.06	1.30	0.04	1.18	0.06	1.05	0.26	1.06	0.23
16:0	18.80	0.10	17.54	0.63	17.53	0.68	18.68	0.16	17.70	0.10	18.08	0.10	18.03	0.01	18.26	0.69	17.87	0.23	17.58	0.46	17.69	0.14	17.10	0.17	17.65	0.08
18:0	4.68	0.37	4.14	0.25	4.10	0.04	4.14	0.13	3.86	0.06	4.04	0.16	3.95	0.07	4.14	0.04	4.11	0.04	3.99	0.07	4.59	0.16	4.17	0.26	4.51	0.14
22:0	0.26	0.02	0.24	0.02	0.22	0.04	0.24	0.01	0.29	0.09	0.26	0.01	0.28	0.00	0.24	0.07	0.28	0.02	0.22	0.00	0.21	0.04	0.25	0.03	0.24	0.01
Total SAT	25.96	0.32 a	23.59	0.87 ab	23.67	1.02 ab	25.33	0.27 ab	23.33	0.12 ab	24.25	0.37ab	24.11	0.07 ab	24.32	0.80 ab	24.15	0.26 ab	23.73	0.33 ab	24.32	0.02 ab	23.12	0.29 b	23.80	0.53 ab
16:1	2.67	0.16	2.58	0.11	2.73	0.04	3.40	0.37	3.09	0.38	2.50	0.18	2.45	0.23	2.47	0.17	2.30	0.00	2.84	0.27	2.83	0.16	2.51	0.03	2.87	0.01
18:1n-9	24.31	1.81	24.27	0.63	24.67	0.38	24.46	0.45	24.70	0.17	23.91	0.07	23.74	0.66	23.54	0.12	23.78	0.09	24.43	0.45	24.49	0.01	24.79	0.07	24.53	0.33
Total MUFA	28.39	1.85	28.14	0.58	28.54	0.46	29.41	0.93	29.01	0.25	27.63	0.27	27.43	1.02	27.26	0.06	27.36	0.21	28.46	0.14	28.46	0.20	28.52	0.08	28.61	0.24
18:2n-6	33.27	0.92	33.66	0.20	33.67	0.51	29.37	0.05	33.40	0.78	34.99	0.19	35.36	0.57	35.38	0.74	36.19	0.29	33.66	0.04	32.97	0.09	34.15	0.06	33.45	0.62
18:3n-6	0.61	0.07	0.43	0.02	0.36	0.07	0.61	0.28	0.43	0.09	0.29	0.04	0.40	0.01	0.33	0.09	0.35	0.02	0.40	0.17	0.40	0.08	0.71	0.26	0.37	0.04
20:4n-6			0.32	0.05	0.33	0.01	0.34	0.04	0.29	0.02	0.27	0.03	0.28	0.03	0.28	0.01	0.24	0.03	0.34	0.00	0.27	0.00	0.34	0.03	0.33	0.01
Total n-6 PUFA	33.88	0.98 b	34.41	0.27 b	34.35	0.43 b	30.32	0.37 c	34.12	0.67 b	35.56	0.12 a	36.04	0.59 a	35.99	0.64 a	36.78	0.30 a	34.40	0.14 b	33.64	0.17 b	35.20	0.30 a	34.16	0.67 b
18:3n-3	3.01	0.16	3.25	0.05	3.33	0.04	3.12	0.21	3.25	0.02	3.38	0.12	3.15	0.06	3.36	0.12	3.31	0.02	3.10	0.19	3.34	0.05	3.25	0.11	3.28	0.05
20:5n-3	3.45	0.64 b	4.20	0.05 ab	3.91	0.12 ab	4.73	0.10 a	4.16	0.44 ab	3.64	0.16 b	3.39	0.16 b	3.65	0.06 b	3.22	0.28 b	3.95	0.02 ab	3.95	0.17 ab	3.76	0.29 ab	3.99	0.23 ab
22:6n-3	5.27	0.38 b	6.31	0.11 a	6.16	0.02 a	7.05	0.03 a	6.09	0.10 a	5.44	0.39 b	5.73	0.54 ab	5.37	0.15 b	5.14	0.05 b	6.21	0.18 a	6.08	0.22 a	6.04	0.18 a	6.00	0.08 a
Total n-3 PUFA	11.73	1.19 b	13.83	0.02 ab	13.40	0.15 ab	14.90	0.29 a	13.49	0.53 ab	12.52	0.75 b	12.37	0.35 b	12.38	0.09 b	11.68	0.35 b	13.37	0.33 ab	13.54	0.04 ab	13.11	0.66 ab	13.39	0.10 ab
Total PUFA	45.61	2.17	48.23	0.29	47.76	0.57	45.22	0.66	47.61	0.15	48.08	0.63	48.41	0.94	48.37	0.73	48.45	0.05	47.77	0.19	47.18	0.21	48.31	0.37	47.55	0.77

Different letters indicate significant differences (ANOVA,  $P < 0.05$ ,  $n = 3$ ). 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.



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 the trial.

	<u>CTRL</u>		<u>DSY 10%</u>		<u>DSY 20%</u>		<u>HSY 10%</u>		<u>HSY 20%</u>		<u>DSG 7.5%</u>		<u>DSG 15%</u>		<u>HSG 7.5%</u>		<u>HSG 15%</u>		<u>DYABN 10%</u>		<u>DYABN 20%</u>		<u>HYABN 10%</u>		<u>HYABN 20%</u>	
	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)	634.92	5.71	612.17	7.80	650.01	21.16	654.08	17.07	643.00	7.31	648.14	8.07	630.52	27.46	657.32	12.91	659.71	12.91	622.60	6.81	661.74	3.10	648.85	12.06	608.02	7.72
16:0	20.09	0.57	20.95	0.06	20.03	0.08	21.71	0.25	20.27	0.47	21.15	0.27	19.65	0.07	20.70	0.15	20.23	0.25	20.16	0.00	20.28	0.67	21.42	0.34	20.35	0.16
18:0	4.92	0.12	4.75	0.05	5.07	0.07	4.93	0.12	5.26	0.13	4.80	0.09	4.90	0.02	4.72	0.03	4.89	0.06	5.00	0.24	4.90	0.13	5.02	0.12	5.21	0.07
Total SAT	27.03	0.40c	28.18	0.13bc	27.45	0.06c	29.30	0.19a	28.03	0.66b	28.58	0.14bc	26.76	0.04c	27.64	0.30c	27.31	0.22c	27.61	0.10c	27.36	1.07bc	28.84	0.19b	27.86	0.28bc
16:1	2.70	0.15	3.94	0.02	4.12	0.05	4.60	0.12	4.05	0.16	3.81	0.04	2.91	0.04	3.03	0.17	2.89	0.02	4.43	0.08	3.42	0.12	3.50	0.07	4.06	0.05
18:1n-9	21.94	0.83	21.66	0.14	26.02	0.22	23.08	0.30	24.81	0.23	23.42	0.05	22.10	0.38	21.43	0.12	22.48	0.16	24.51	0.19	23.21	0.11	20.27	0.07	25.93	0.10
18:1n-7	2.73	0.12	3.16	0.13	3.51	0.07	3.68	0.16	3.52	0.28	3.16	0.36	3.15	0.27	2.68	0.66	3.13	0.30	3.38	0.06	2.99	0.35	2.93	0.04	3.26	0.21
Total MUFA	28.64	0.89c	30.07	0.06c	35.22	0.23a	32.81	0.25b	33.93	0.62ab	31.73	0.38bc	29.52	0.05c	28.42	0.39c	29.78	0.17c	33.75	0.16ab	31.07	0.31b	28.07	0.18c	34.81	0.31a
18:2n-6	19.21	0.35	18.67	0.01	19.62	0.29	16.06	0.04	19.68	0.43	20.49	0.22	21.06	0.10	21.11	0.00	21.53	0.19	18.93	0.30	17.91	1.10	16.78	0.37	18.78	0.14
20:4n-6	1.02	0.02	0.76	0.00	0.70	0.04	0.79	0.05	0.67	0.03	0.76	0.06	0.82	0.00	0.94	0.04	0.72	0.09	0.68	0.02	0.87	0.21	0.96	0.01	0.72	0.02
Total n-6 PUFA	20.98	0.27b	19.80	0.03c	20.74	0.22b	17.26	0.08c	20.74	0.46ab	21.81	0.29a	22.38	0.10a	22.61	0.01a	22.80	0.25a	19.95	0.30c	19.14	1.30c	18.12	0.41c	19.94	0.05c
18:3n-3	1.87	0.07	2.03	0.02	2.09	0.12	1.78	0.04	1.99	0.18	2.06	0.06	2.14	0.05	1.89	0.02	2.12	0.11	2.07	0.03	2.23	0.11	1.80	0.10	2.03	0.01
20:4n-3	0.39	0.03	0.31	0.02	0.33	0.00	0.28	0.01	0.29	0.01	0.26	0.01	0.33	0.04	0.28	0.05	0.28	0.01	0.29	0.01	0.29	0.01	0.32	0.00	0.26	0.01
20:5n-3	2.87	0.23 a	2.23	0.09c	1.79	0.06d	2.20	0.10c	1.93	0.02cd	2.30	0.00c	2.37	0.00c	2.56	0.05b	2.18	0.10c	1.85	0.03d	2.19	0.08c	2.45	0.03b	1.76	0.03d
22:5n-3	0.92	0.01	0.75	0.06	0.63	0.00	0.71	0.07	0.67	0.02	0.76	0.04	0.78	0.02	0.84	0.01	0.73	0.00	0.61	0.03	0.76	0.03	0.72	0.02	0.62	0.01
22:6n-3	16.81	1.25b	16.11	0.42b	11.27	0.23c	15.15	0.44b	11.88	0.66c	11.91	0.07c	15.18	0.11b	15.17	0.15b	14.25	0.05b	13.46	0.26bc	16.39	0.45b	19.25	0.89a	12.25	0.02c
Total n-3 PUFA	23.35	1.56a	21.95	0.22b	16.59	0.04c	20.63	0.36b	17.31	0.82c	17.89	0.22a	21.35	0.09b	21.33	0.08b	20.11	0.30b	18.72	0.32	22.39	0.55	24.97	0.76	17.39	0.01
Total PUFA	44.33	1.29	41.75	0.19	37.33	0.18	37.88	0.44	38.04	1.28	39.70	0.51	43.73	0.01	43.94	0.09	42.91	0.05	38.67	0.02	41.53	0.75	43.09	0.35	37.33	0.04
DHA/EPA	5.85	0.03a	7.24	0.49b	6.31	0.34ab	6.90	0.52a	6.16	0.42ab	5.17	0.03a	6.42	0.04ab	5.93	0.18ab	6.54	0.27ab	7.27	0.001b	7.48	0.46b	7.86	0.45b	6.94	0.14b
EPA/ARA	2.81	0.16	2.95	0.12	2.56	0.22	2.78	0.31	2.89	0.17	3.05	0.24	2.90	0.01	2.72	0.05	3.04	0.50	2.74	0.03	2.58	0.53	2.54	0.04	2.44	0.11
n-3/n-6	1.11	0.09b	1.11	0.01b	0.80	0.01a	1.20	0.01b	0.83	0.02a	0.82	0.00a	0.95	0.01a	0.94	0.00a	0.88	0.02a	0.94	0.03a	1.17	0.11b	1.38	0.07c	0.87	0.00a

Different letters indicate significant differences (ANOVA,  $P < 0.05$ ,  $n = 3$ ). 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

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 the trial.

	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)	538.51	4.70	512.01	2.37	542.67	4.44	525.88	6.76	524.95	5.35	522.46	6.73	523.34	10.17	538.31	1.86	524.15	3.97	531.38	2.20	539.89	21.46	569.52	7.86	578.82	13.70
16:0	16.80	0.21	15.28	0.23	15.38	0.10	16.76	0.19	15.73	1.06	18.33	0.19	19.51	0.26	18.69	0.54	20.23	0.59	20.90	0.91	18.12	0.46	15.84	0.09	15.61	0.41
18:0	7.48	0.36	7.14	0.19	7.48	0.07	7.24	0.00	7.08	0.08	8.94	0.14	8.85	0.14	8.23	0.05	9.53	0.05	9.94	0.14	8.06	0.04	6.91	0.14	8.01	0.01
Total SAT	25.30	0.53b	23.87	0.07b	23.88	0.13b	25.29	0.28b	24.11	1.50b	28.29	0.37ab	29.37	0.25ab	27.83	0.68ab	30.74	0.57a	32.04	1.17a	27.01	0.64ab	24.03	0.21b	24.45	0.43b
16:1	1.35	0.08	3.26	0.10	2.71	0.04	2.46	0.03	2.65	0.26	1.78	0.07	1.99	0.14	1.66	0.09	1.46	0.06	3.85	0.69	1.91	0.46	2.97	0.12	2.31	0.28
18:1n-9	13.44	0.62	17.44	0.10	16.67	0.21	15.81	0.14	17.67	0.23	14.11	0.19	18.61	0.36	14.40	0.15	15.22	0.42	24.75	0.45	15.53	0.27	15.83	0.10	14.38	0.07
18:1n-7	2.05	0.00	3.08	0.04	2.97	0.19	3.30	0.00	2.82	0.16	2.46	0.12	2.69	0.05	2.40	0.23	2.44	0.21	3.85	0.03	2.79	0.22	3.13	0.04	2.45	0.11
Total MUFA	19.49	0.99c	26.49	0.01b	25.43	0.02b	25.59	0.21b	26.36	0.21b	21.09	0.11bc	25.96	0.45b	20.59	0.37c	21.48	0.69bc	36.24	1.17a	23.26	0.65b	24.82	0.04b	21.98	0.05bc
18:2n-6	9.26	0.24	7.78	0.00	7.62	0.10	6.12	0.03	7.94	0.62	8.71	0.06	13.25	0.49	10.91	0.09	10.89	0.66	9.04	0.20	8.23	0.06	6.31	0.00	7.88	0.12
20:4n-6	4.20	0.07	4.18	0.09	4.84	0.11	4.19	0.06	4.26	0.35	4.78	0.07	3.27	0.14	4.14	0.08	4.21	0.10	2.61	0.22	4.63	0.17	4.63	0.09	5.50	0.05
Total n-6 PUFA	15.58	0.01bc	15.01	0.55bc	14.88	0.13bc	12.45	0.04c	14.50	0.85bc	15.75	0.01bc	18.97	0.33a	16.93	0.01ab	17.56	0.93a	13.88	0.55bc	15.24	0.53bc	13.07	0.10bc	16.13	0.17b
18:3n-3	0.55	0.00	0.36	0.01	0.36	0.02	0.32	0.00	0.46	0.02	0.45	0.01	0.83	0.03	0.63	0.01	0.61	0.08	0.46	0.00	0.35	0.02	0.29	0.03	0.33	0.03
20:5n-3	2.75	0.01a	1.95	0.06b	1.98	0.03b	1.92	0.01b	1.97	0.13b	2.68	0.08a	1.95	0.02b	2.64	0.10a	2.36	0.04a	1.10	0.38d	1.76	0.09c	1.71	0.10c	1.93	0.01b
22:5n-3	0.78	0.01	0.59	0.01	0.56	0.02	0.56	0.02	0.64	0.01	0.70	0.04	0.52	0.01	0.67	0.06	0.55	0.05	0.31	0.09	0.49	0.01	0.54	0.05	0.56	0.01
22:6n-3	33.46	1.39a	30.27	0.41a	30.65	0.11a	32.52	0.01a	30.35	0.68a	29.02	0.41a	20.64	1.03b	28.85	0.91ab	24.92	0.11b	14.73	1.38c	30.27	0.63ab	33.99	0.18a	32.75	0.62a
Total n-3 PUFA	37.74	1.40a	33.26	0.47a	33.69	0.10a	35.43	0.02a	33.55	0.83a	33.02	0.43a	24.11	1.03b	32.98	1.06a	28.59	0.20ab	16.69	1.86c	32.98	0.71ab	36.63	0.08a	35.70	0.64a
Total PUFA	53.32	1.41	48.27	0.07	48.56	0.23	47.89	0.02	48.05	1.67	48.77	0.43	43.08	0.70	49.91	1.04	46.15	1.12	30.57	2.41	48.22	1.24	49.69	0.18	51.83	0.46
DHA/EPA	12.15	0.48a	15.55	0.30b	15.48	0.20b	16.90	0.11bc	15.42	0.65b	10.83	0.18a	10.56	0.40a	10.93	0.05a	10.57	0.15a	14.01	3.55b	17.21	0.51bc	19.86	1.21bc	16.95	0.26bc
EPA/ARA	0.66	0.01ab	0.47	0.01ab	0.41	0.00ab	0.46	0.01ab	0.24	0.30a	0.56	0.01b	0.60	0.02b	0.64	0.01ab	0.56	0.00b	0.42	0.11ab	0.38	0.01ab	0.37	0.03ab	0.35	0.00ab
n-3/n-6	2.42	0.09b	2.22	0.11b	2.26	0.01b	2.85	0.01b	2.32	0.01b	2.10	0.03b	1.27	0.08a	1.95	0.06ab	1.63	0.07a	1.20	0.09a	2.16	0.03b	2.80	0.01b	2.21	0.06b

Different letters indicate significant differences (ANOVA,  $P < 0.05$ ,  $n = 3$ ). 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

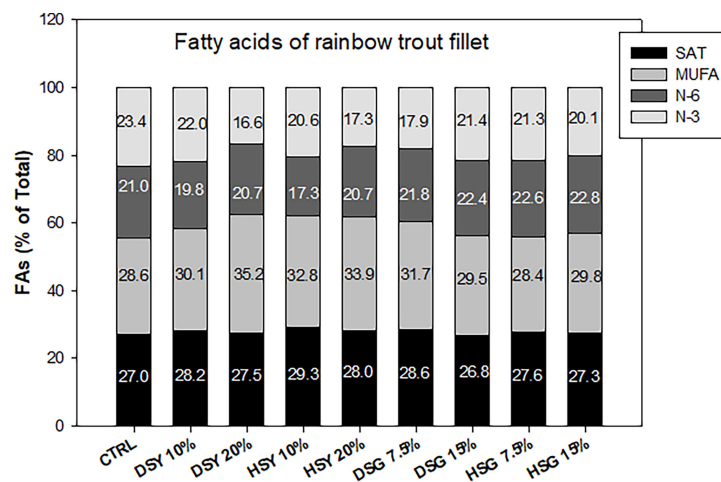


FIGURE 1

Fatty acid composition of the muscle of rainbow trout fed with the experimental diets. SAT, saturated; MUFA, monounsaturated; N-3, omega-3; N-6, omega-6 fatty acids.

fish fed with SY probably due to its lower digestibility compared with CTRL. In the case of SG, the results in final weight were similar to CTRL and SY-fed fish especially in the case of DSG, without differences in SGR and RGR, and, in this case, FCR was similar to that found for the CTRL group. In all the cases, the inclusion of brewery derived by-products did not affect fish final composition. Muscle composition at the end of the study shows a higher protein content in the fish fed with HSY included at 20% and a higher lipid levels in the fish fed with DSY included at 20%, as well as omega-3 fatty acid profile similar to those found in the fish fed with the control/commercial feed. In a previous study carried out with gilthead seabream (Estévez et al., 2021) using feeds with SY included up to 30%, very good results in growth and conversion were obtained even when the fish meal content was reduced to 10% in the formulation. However, in the same study using SG included up to 20%–30%, the growth of seabream and feed digestibility were reduced due to the high fiber content of SG. In that case, the final recommendation for seabream was the inclusion of 30% brewers' SY with a reduction in the use of fish meal, whereas for SG, the inclusion should not be higher than 15%.

As a conclusion, the inclusion of 20% of brewers' SY or 15% SG in the feed for carnivorous freshwater fish (rainbow trout) resulted in a higher final weight without differences in growth rates (SGR and RGR) than that obtained using a commercial feed, showing also good protein digestibility and food conversion. Final muscle nutritional composition was very similar to the results obtained for the control group, including the fatty acid composition. These by-products are produced in large quantities in Europe, and considering the results obtained, they can be considered a potential new source of protein and increase the sustainability of both brewery and aquaculture

sectors. The valorization of Brewers' by-products as ingredients for the formulation of aquafeeds has an important favorable effect both on brewers and aquaculture environmental impact. In this regard, the comparison between the aquafeeds obtained using these by-products as ingredients and commercial aquafeed showed significant benefits, including a 6% reduction in climate change (Iñarra et al., 2022).

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

## Ethics statement

The animal study was reviewed and approved by Generalitat de Catalunya Ethics Committee.

## Author contributions

AE carried out the trials with the fish, took samples and analysed the fillet and liver and wrote the manuscript; DS and BI work with the dewatering and drying of the spent yeast and spent grain and helped in writing the manuscript; LP formulated and prepared the feeds used in the trial and collaborate in writing the paper. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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