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Effects of Eco-Organic Feed on Growth Performance, Biometric Indices, and Nutrient Retention of Gilthead Seabream (*Sparus aurata*)

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Abstract: This study examined how eco-organic feed affects the growth performance, nutrient efficiency, feed utilisation, and body composition of gilthead seabream. Six different diets were tested, including a control diet (CONT) without organic ingredients and four diets with 100% organic ingredients: trout (TRO), seabass (SBS), poultry (POU), and mix (MIX), along with a control organic diet (ORG) containing organic ingredients and 30% fishmeal. The experiment lasted 70 days, and the fish were fed twice a day, starting with an initial weight of 60.5 g. The results showed that the highest growth rates were observed in fish fed the ORG and CONT diets containing fishmeal. Conversely, the POU diet resulted in the lowest growth rate, survival rate, and highest value for feed conversion ratio (FCR). Almost all essential amino acid efficiency values were high in fish fed the ORG and CONT diets. Still, significant differences were noted in the retention efficiency of fatty acids across all diets. The retention efficiency was higher in the CONT diet, followed by the ORG diet. However, the economic conversion rate was lower for CONT, SBS, TRO, and MIX. Overall, using organic diets of animal origin impacted the growth performance of gilthead seabream, but it is still a promising approach.

Keywords: sustainable aquaculture; organic diets; amino acids; organic fish; organic production; fishmeal substitution

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1. Introduction

The improvement of aquaculture sustainability is an issue of increasing importance. The lack of fish meal has forced the aquaculture sector to seek a more reliable solution for the environment and fishery resources. Advances in aquaculture sustainability are gradually reducing the amount of wild fishmeal used in aquafeeds. These advances have manifested in various ways, such as creating standards and regulations for protecting the environment or creating or caring for the environment in production processes and reducing and managing waste [1]. Organic aquaculture is a term usually understood as synonymous with ecological aquaculture and is a comprehensive method of farming fish and other marine species that adheres to organic principles [2]. The exact definition of organic may vary depending on the certification system with specific rules regarding production methods, and only products that follow the guidelines are allowed to use certified organic labels.

In most organic systems, such as EU regulatory processes, the preference for organic consideration of the raw ingredients is for the use of by-products coming from certified organic farms [3]. Organic aquaculture is a relatively new food-producing sector [4]. The first

common carp (*Cyprinus carpio*) standard was established in Austria in 1994 [5]. According to the European Market Observatory for Fisheries and Aquaculture (EUMOFA) [6], overall organic aquaculture output in the EU 27 was estimated at 74,032 tonnes in 2020, accounting for 6.4% of the total EU aquaculture production. Production has grown by 60% from 2015 (46,341 tonnes at the EU 27 level in 2015), while European nations represent approximately 20% of the world's organic aquaculture. However, some European nations have decreased production lately [7]. The fundamental organically produced species, arranged by significance, are salmon, mussels, carp, trout, seabass, and seabream [8]. Specifically, for organic aquaculture, the Regulation mentions that it is a relatively new production sector, and the number of aquaculture production units converting to organic production is expected to rise. This will generate new experience, technical knowledge, and advances in ecological aquaculture that must be reflected in production standards [9]. The scope of organic farming is still minimal regarding the primary Mediterranean-farmed species: seabass and seabream [10]. Organic feeds result from a farming system that does not use synthetic fertilizers, pesticides, growth regulators, or livestock feed additives. Organic legislation generally prohibits irradiation and using genetically modified organisms (GMOs) or products derived from or containing GMOs [11].

Organic seabream production has been hampered mainly by economic concerns, such as the higher cost of production feed prices, which have deterred consumers and producers [10]. According to recent consumer preference studies, the Mediterranean has much potential for organic seabream production [12,13]. Furthermore, expanding organic aquaculture production is limited by the need for more organic feed, particularly for carnivorous species. Indeed, the EU organic legislation imposes minimums on the source of organic ingredients for the formulation of nutritionally balanced diets [14,15]. Only some ingredients must be organically certified (60% in the EU); only the plant ingredients must be organic. The limitations of the protein ingredients that can be used for organic feeds are one of the main challenges. Currently, most organic labels are allowed to use non-organic fishmeal, although the use of fishmeal from sustainable fisheries is required. However, according to the regulation (EU) 2018/848 [16], transformed animal proteins (TAPs) can be used, and TAPs of organic origin would be considered organic. To reduce reliance on conventional fishmeal and fish oil, fisheries and aquaculture by-products are an excellent sustainable aquafeed option [17,18]. Using by-products from organic production would open the door to an increase in the percentage of the minimum organic raw ingredients used in the organic formulation. There are few studies in which organic feed has been used in carnivorous fish species. The regulatory limitations are summarised as all vegetable ingredients must be organic, a maximum of 60% of vegetable ingredients are allowed, and the absence of synthetic amino acids. Fish meal can be used as long as it comes from sustainability-certified fisheries or organic production. The use of non-synthetic amino acids is allowed. Therefore, the fundamental protein source with the scope of sustainability should be fishmeal from organic aquaculture or sustainable fisheries, and, due to the lack of availability, it is different. With these limitations, the availability of good ecological protein sources suitable for carnivorous fish is complicated. The main challenges for the supply of feed ingredients for the organic production of carnivorous fish are to increase the diversity of ingredients available to balance the amino acid profile without synthetic amino acids and to identify new sources suitable for the supply of eicosapentaenoic acid (EPA, 20:5n3) and docosahexaenoic acid (DHA, 22:6n3) [14].

Even fewer studies compare conventional feeding with organic feeding. Sardinha et al. [19] in sea bream, Pascoli et al. [20] in seabass, or Di Marco et al. [21] in seabass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) compare organic with conventional feeding. However, in these studies, organic feeds have conventional fishmeal as a protein source. The stress and immunological markers in the fish were similar [20], while the fillet fatty acid composition varied depending on the diet [22]. The by-product meals used in this study need to be better studied. More protein sources from organic sources must be prioritised in research. The main alternatives are using TAPs from organic cattle and generating a circular economy,

using the remains of other species of organic fish, and avoiding cannibalism to generate organic meals with nutritionally optimal fatty acids and amino acid profiles. The purpose of this research was to specifically determine the effect that organic feeds have on the growth of gilthead seabream and to see how new organic raw materials, such as poultry, remains of trout, and remains of seabass, affect growth and nutritional and biometric parameters. The goal was to better understand organic feeds as alternative ecological sources for gilthead seabream. These products may have a role in developing organic aquaculture in the Mediterranean to make it more sustainable.

2. Materials and Methods

2.1. Production System

The growth trial was carried out in a recirculating saltwater system (65 m^3 capacity), with a rotary mechanical filter and a gravity biofilter (about 6 m^3), and eighteen cylindrical fiberglass tanks (1750 L, three per treatment). Aeration was installed in all tanks at the Laboratory of Aquaculture (Animal Science Department at Universitat Politècnica de València, Valencia, Spain). The temperature was held constant at 21 ± 1 °C, the dissolved oxygen level was 8.7 ± 1.6 mgL^{-1}, the salinity was 33.3 ± 2.4 g L^{-1}, the pH was 7.49, nitrates were 104.2 mg L^{-1}, nitrites was 0.38 mg L^{-1}, and the ammonium level was 0.22 mg L^{-1}. The photoperiod was regular throughout the experiment, and all tanks had similar lighting conditions.

2.2. Fish and Trial Design

Organic seabream juveniles from the Sonrionansa S.L. fish farm located in Pesues (Cantabria) were used for the study. The fish were transported to Universitat Politècnica de València and distributed in experimental tanks. All fish were acclimated to laboratory conditions two weeks before the feeding experiment. At the start of the trial, all fish were weighed individually to calculate the initial body weight and the initial biomass in each tank. A group of 720 fish (average weight 60.5 g) was distributed in 18 experimental tanks with a stocking rate of 22.9 fish/m^3 (40 fish per tank). The test lasted 70 days; six diets were tested in triplicate, shown in Table 1: the control diet (CONT, without organic ingredients and 30% commercial fishmeal content), the organic control diet (ORG, with organic ingredients and 30% commercial fish meal content), and four diets with 100% organic ingredients: the TRO diet (with organic trout meal as a protein source), the SBS diet (with remains of organic seabass as a protein source), the POU diet (with organic poultry meal as a protein source), and the MIX diet (containing three equal parts of the feed from the organic remains of seabass, trout, and poultry). From Monday to Saturday, the fish were hand-fed twice daily (9:00 and 17:00 h) until apparent satiation. The fish were starved on Sunday. The pellets were progressively dispersed, enabling all fish to consume and the overall amount of feed distributed was recorded. Every 30 days, all fish were weighed. The fish were anesthetised with 10 mg L^{-1} clove oil (Guinama®, Valencia, Spain) containing 87% eugenol before being weighed, and were not fed the previous day.

Table 1. Ingredients and formulation of the experimental diets.

	CONT	ORG	TRO	SBS	MIX	POU	PRICE
Ingredients (g/kg DM)							EUR $tonn^{-1}$
Fish meal [1]	300	300					1933
Organic trout meal [2]			400		140		750
Organic seabass meal [3]				405	140		750
Organic poultry meal [4]					140	435	750
Wheat [5]	180						473
Organic wheat [6]		22	216	84	120	119	515
Organic pea [7]			111	54	118	102	550
Organic corn [8]		8					515
Organic spelled bran [9]		10					1950

Table 1. *Cont.*

	CONT	ORG	TRO	SBS	MIX	POU	PRICE
Wheat gluten [10]	120						1750
Soybean [11]	214						746
Organic soybean [12]		504	150	433	270	272	1355
Fish oil [13]	50	67	50		50	50	3671
Soybean oil [14]	100						1558
Organic soybean oil [15]		59	53				1815
Calcium phosphate [16]	23	20					3500
Vegetable methionine [17]	3		10	14	12	12	2220
Vitamin and mineral mix [18]	10	10	10	10	10	10	5000
Price [19] (EUR kg $^{-1}$)	1.51	1.76	1.03	1.04	1.07	1.07	

[1] Fish meal (91.70% DM, 74.30% CP, 12.40% CL, 14.30% ash). [2] Organic trout meal (95.71% DM, 76.71% CP, 17.36% CL, 9.43% ash) (Naturix, Valderrebollo, Guadalajara). [3] Organic seabass meal (98.83% DM, 44.91% CP, 40.93% CL, 17.83% ash) (Andrómeda, España). [4] Organic poultry meal (97.48% DM, 58.45% CP, 27.50% CL, 16.06% ash). [5] Wheat (87.82% DM, 11.41% CP. 1.76% CL, 1.63% ash). [6] Organic wheat (92.40% DM 12.70% CP, 2.30% CL, 1.70% ash) (Piensos Montoya, Valencia). [7] Organic pea (91.80% DM 23.00% CP, 3.30% CL, 2.10% ash) (Piensos Montoya, Valencia). [8] Organic corn (86.40% DM 8.3% CP, 3.30% CL, 1.10% ash) (Piensos Montoya, Valencia). [9] Organic spelt bran (80.48% DM 17.98% CP, 0.61% CL, 4.26% ash) (Piensos Montoya, Valencia). [10] Gluten (93.33% DM 81.00% CP, 0.86% CL, 0.86% ash). [11] Soybean (88.13% DM 49.90% CP, 2.20% CL, 7.08% ash). [12] Organic soybean (92.30% DM 53.40% CP, 4.30% CL, 5.90% ash) (Piensos Montoya, Valencia). [13] Fish oil (Industrias Afines, S.L. (Arpo), Polígono industrial A Veigadaña, Rúa as Baloutas, de Abaixo, 24, 36416, Pontevedra). [14] Soybean oil (refined soybean oil, Casimiro Perez Sl, Gabriel Miró, 16, 18, 03804 Alcoi, Alicante). [15] Organic soybean oil (Clearspring Ltd., Acton Park Estate, London W3 7QE, United Kingdom). [16] Calcium phosphate. [17] Vegetable methionine (Adibio S.L., Edificio Galileo, C/Enebros 74, 2ª planta, 44002 Teruel, España). [18] Vitamin and mineral mix (g kg^{-1}): premix: 25; choline 10; DL-a-tocopherol, 5; ascorbic acid, 5; $(PO4)_2Ca_3$, 5. Premix composition: retinol acetate, 1,000,000 IU kg^{-1}; calcipherol, 500 IU kg^{-1}; DL-a-tocopherol, 10; menadione sodium bisulphite, 0.8; thiamine hydrochloride, 2.3; riboflavin, 2.3; pyridoxine hydrochloride, 15; cyanocobalamine, 25; nicotinamide, 15; pantothenic acid, 6; folic acid, 0.65; biotin, 0.07; ascorbic acid, 75; inositol, 15; betaine, 100; polypeptides, 12. [19] Prices in EUR kg^{-1}.

2.3. Diets and Feeding

Six diets, isoproteic (45%) and isolipidic (18.8%), were formulated (Table 1). The CONT and ORG diets included 30% non-organic fishmeal because no organically certified commercial fishmeal exists. The ORG diet differed from the CONT diet in using organic ingredients: in ORG, unlike in CONT, the use of non-organic ingredients was avoided to the maximum. Four alternative diets (TRO, SBS, POU, and MIX) were also designed to avoid using non-organic ingredients. Vegetable methionine of sustainable origin was added to the organic diets to meet the needs of gilthead seabream because it is a limiting amino acid for growth [23]. The control diet also had added methionine; however, this was of synthetic origin. The diets were prepared using a semi-industrial twin-screw extruder (CLEXTRAL BC 45, St. Etienne, France). A screw speed of 100 rpm, temperature of 110 °C, and pressure of 40–50 atm were the processing conditions. Conventional fish oil from sustainable fisheries was used because organic fish oil has not yet been certified.

2.4. Proximate Composition and Amino Acid Analysis

The raw materials were chemically analysed. At the start of the experiment, five fish were sampled, triturated, and homogenised before being analysed for chemical composition. At the end of the experiment, five fish per tank were randomly sampled to determine the biometric parameters and were triturated and homogenised before being analysed for the proximate body composition. The dietary ingredients and the whole body of fish fed the six experimental diets were analysed according to AOAC (1990) [24] procedures: dry matter (105 °C to constant weight), ash (550 °C to constant weight), crude protein (N × 6.25) using the Kjeldahl method after acid digestion (2300 Kjeltec Analyzer Unit), and crude lipid extracted with diethyl ether (ANKOM XT10) using the Dumas principle.

The diet (Table 2) and body fish amino acid content were analysed using a Waters HPLC system that included two pumps (Model 515; Waters), an autosampler (Model 717; Waters), a fluorescence detector (Model 474; Waters), and a temperature control module,

as described by Bosch et al. [25]. After hydrolysis, an internal standard of aminobutyric acid was introduced. AQC (6aminoquinolylNhydroxysuccinimidyl carbamate) was used to derive the amino acids.

Table 2. Amino acid composition of experimental diets (g kg^{-1} DM).

	CONT	ORG	TRO	SBS	MIX	POU
EAAs						
Arginine	37.4	45.3	31.8	33.5	37.3	36.6
Histidine	13.9	14.7	9.7	9.2	11.2	11.0
Isoleucine	25.5	27.2	19.9	17.8	21.2	21.7
Leucine	43.0	44.5	32.9	30.0	34.9	36.9
Lysine	34.8	42.3	32.6	27.8	33.9	32.2
Methionine	12.9	9.8	9.9	8.0	9.9	9.8
Phenylalanine	27.7	27.8	20.0	18.9	21.3	21.9
Threonine	22.8	24.7	19.9	16.9	20.4	20.2
Valine	30.2	31.9	24.4	21.5	25.3	25.8
NEAAs						
Alanine	28.1	31.1	26.2	23.5	26.9	27.4
Aspartic acid	47.6	62.3	46.0	41.1	49.9	46.0
Cysteine	5.9	4.8	4.2	3.4	4.2	4.7
Glutamic acid	123.1	97.0	76.8	71.4	81.6	90.0
Glycine	29.3	31.1	35.2	30.0	33.8	32.7
Proline	37.3	28.1	25.1	23.5	26.4	28.2
Serine	24.9	26.8	21.8	20.4	22.9	22.0
Tyrosine	18.1	19.7	13.9	12.6	14.6	14.6
EAA/EAA	7.9	8.9	8.1	8.1	8.3	8.1

EAA: essential amino acids; NEAA: non-essential amino acids.

After oxidation with performic acid, methionine and cysteine were identified as methionine, sulphone, and cysteine acid, respectively. The Waters AcQ isolated the amino acids using a C18 reverse-phase column with a 150 mm × 3.9 mm tag. All analyses were carried out in duplicate.

2.5. Growth and Nutrient Efficiency Indices

After the experiment, the growth and nutrient efficiency indices were calculated using the tank as the experimental unit. The specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), economic conversion ratio (ECR), survival (S), productive protein value (PPV), and productive fat value (PFV) were all determined, with consideration given to the monthly biomass reports of any deceased fish [26].

2.6. Biometric Indices

After the feeding trial, every fish was weighed on its own. Biometric indices were determined by randomly slaughtering five fish from each tank, fifteen per treatment, using a lethal bath of clove oil (150 mg L^{-1}). The samples collected from each tank were combined and kept at −30 °C for further analysis. The fish's overall weight and length, as well as the weights of its internal organs, visceral fat, and liver, were measured to determine the condition factor (CF), viscerosomatic (VSI), visceral fat (VFI), and hepatosomatic (HSI) indices [26].

2.7. Fatty Acid Analysis

Total lipid fatty acid methyl esters (FAMEs) were produced directly, as stated by O'Fallon [27]. A focus gas chromatograph (Thermo, Milan, Italy) with a split/splitless injector and a flame ionisation detector was used to analyse the FAMEs. A fused silica capillary column SPTM 2560 (Supelco, PA, USA) was used to separate the methyl esters (100 m × 0.25 mm × 0.2 µm film thickness). Helium was used as the carrier gas, with a linear velocity of 20 cm/seg. A split ratio of 1/100 was used to inject the samples. The

starting oven temperature was set at 140 °C for five minutes, and then increased to 240 °C at a rate of 4 °C/min for another 30 min. The temperature of the detector and injector were both set at 260 °C. Individual fatty acids were identified by comparing retention periods to Supelco-supplied fatty acid methyl ester standards. Only fatty acids with a minimum concentration of 0.1 percent were considered. To quantify the fatty acids, we calculated the g of fatty acids per 100 g of a sample using the sample weight data from the analysis and measured using C13:0 as an internal standard. Table 3 shows the fatty acid content of the trial diets.

Table 3. Fatty acid composition of the experimental diets (g kg^{-1} in DM).

	CONT	ORG	TRO	SBS	MIX	POU
SFA						
(C13:0)	3.7	3.8	3.5	3	3.5	4.3
(C14:0)	3.7	13.5	3.3	5.3	5.3	8.9
(C15:0)	0.4	0.7	0.4	0.6	0.5	0.6
(C16:0)	26.6	28.9	31.3	27.8	32	33.8
(C17:0)	0.5	0.5	0.5	0.5	0.6	0.6
(C18:0)	0.6	0.4	0.6	0.5	0.5	0.5
MUFA						
(C16:1)	4.2	5.3	4.2	6	6.2	6.2
(C17:1)	0.2	0.3	0.3	0.4	0.4	0.3
(C18:1n9c)	40.4	28.6	49.5	41	49.1	42.8
(C18:1(n-7)	3.8	2.3	4.3	4.3	4.7	3.8
(C20:1)	2.3	1.6	0.1	7	7.3	3.9
(C24:1)	0.6	0.4	0.6	0.7	0.7	0.7
PUFA						
(C18:2n6c) LA	63.1	26.2	57.6	40.5	42.9	42.6
(C18:3n3) LNA	9.3	5.2	7.9	6.9	6.9	6
(C20:2)	0.4	0.2	0.6	0.9	1	0.5
(C20:3n6)	0.1	0.1	0.2	0.2	0.2	0.2
(C20:4n6) ARA	0.8	0.4	1.9	0.7	1.3	1.2
(22:4n-6)	0.1	0	0	0	0	0
(22:5n-3)	0.1	0	0.2	0.1	0.1	0.1
(20:5n-3) EPA	6	3.5	4.5	5.7	5.1	4.6
(22:6n-3) DHA	9.9	6.1	6.8	11.8	10	7.7

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; LA: linoleic acid; LNA: linolenic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; ARA: arachidonic acid.

2.8. Statistical Analysis

Growth data, feed utilisation, and all other data obtained were evaluated using analysis of variance (ANOVA), with the initial live weight as a covariate [28]. The Newman–Keuls test assessed specific diet differences at $p < 0.05$ (Statgraphics, Statistical Graphics System, Version Plus 5.1, Herndon, VA, USA). The tank means were the statistical unit.

2.9. Ethical Statement

This study followed European Union Council Directive 2010/63/EU, which establishes the basic requirements for animal protection during experimentation, and Spanish state law (Spanish Royal Decree 53/2013), which governs animal use in experimentation and other scientific objectives. The Ethics Committee of the Polytechnic University of Valencia (UPV) approved the experimental methodology. The fish were examined every day. In addition, after sedation with clove oil dissolved in water (0.01 mg/L of water) to minimise animal suffering, the health state of the fish was determined through observation. An excess of clove oil (150 mg L^{-1}) was used to euthanise the animals, which were dissected.

3. Results

3.1. Fish Growth

Figure 1 shows the average growth of the gilthead seabream fed the different experimental diets. The gilthead seabream fed the CONT and ORG diets obtained the highest average weight after the 70-day trial period ($p < 0.05$).

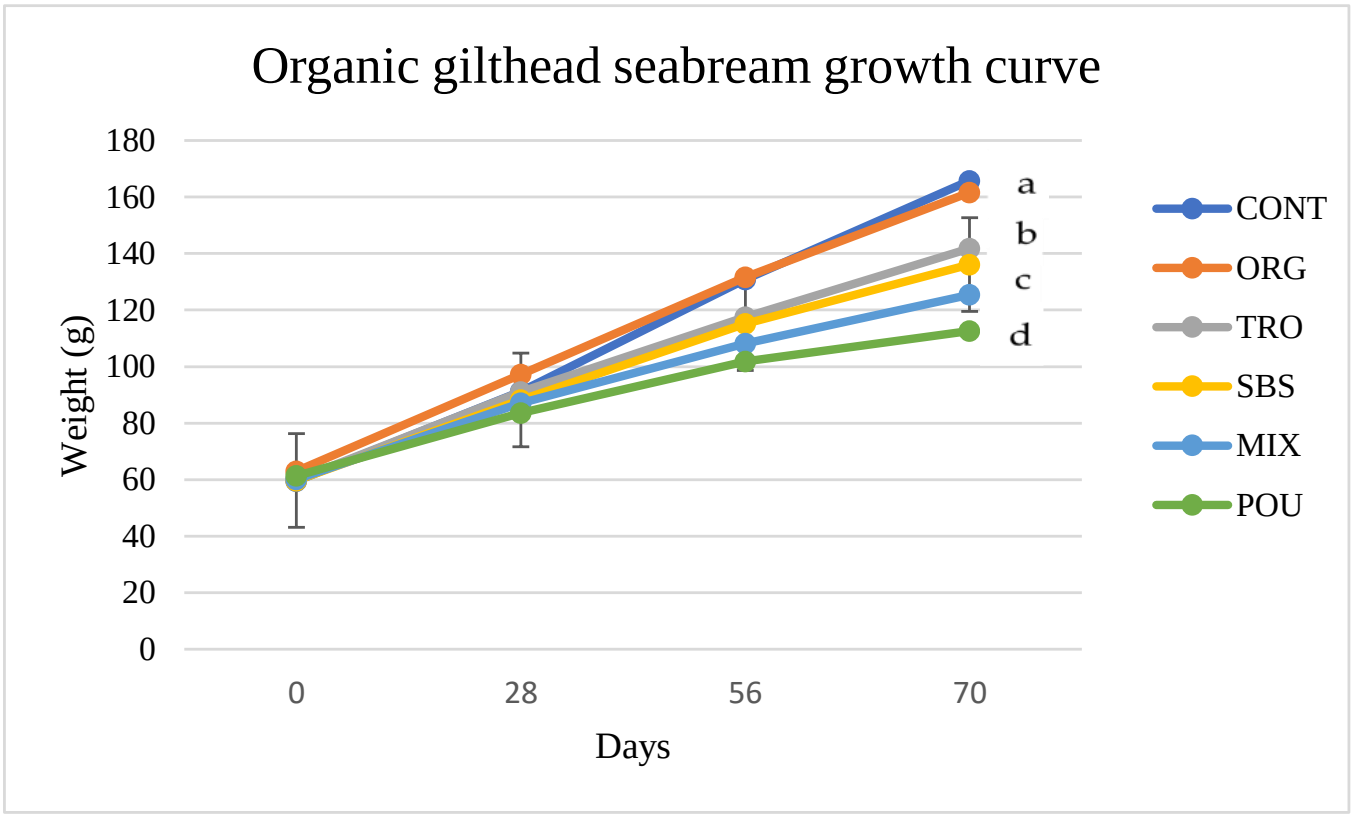

Figure 1. Evolution of the average body weight of gilthead seabream fed on the experimental diets. The values that do not share the same letter differ significantly ($p < 0.05$). Values represented as mean ± standard error (n=3). Different superscript in each sampling means significant differences ($p < 0.05$). Test Newman-Keuls.

Regarding organic diets without fishmeal, the fish fed the TRO and SBS diets showed the highest growth ($p < 0.05$), followed by the MIX diet. Finally, POU exhibited the lowest growth. As reported in Table 4 and Figure 1, the best growth occurred in the two control diets containing 30% fishmeal, CONT and ORG (165.8 and 161.6 g, respectively), regardless of whether the rest of the ingredients included were organic. The fish that grew the best were those fed organic fish, TRO and SBS (141.8 and 136.1, respectively). The worst growth occurred with diets containing poultry meals, either alone (POU) or in a mixture (MIX) (125.4 and 112.6, respectively). Significant differences ($p < 0.05$) were observed in the SGR, showing the highest value in the fish fed the CONT diet (1.46%). Fish fed the POU diet presented the lowest value (0.87%).

Table 4. Overall performance of gilthead seabream fed the organic experimental diets.

	CONT	ORG	TRO	SBS	MIX	POU	SEM
Initial weight (g) [1]	59.5	63.0	59.9	59.7	60.1	61.3	1.30
Final weight (g)	165.8 [a]	161.6 [a]	141.8 [b]	136 [b]	125.4 [c]	112.6 [d]	3.80
Mortality (%)	3.3 [b]	4.2 [b]	5 [b]	7.5 [b]	8.2 [b]	13.3 [a]	1.44
SGR (%/day) [2]	1.46 [a]	1.35 [b]	1.23 [c]	1.18 [c]	1.05 [d]	0.87 [e]	0.02
FI(g/100gfish/day) [3]	2.25 [b]	2.32 [b]	2.65 [a]	2.50 [ab]	2.46 [ab]	2.57 [ab]	0.07
FCR [4]	1.7 [d]	1.9 [c]	2.3 [b]	2.3 [b]	2.4 [b]	2.9 [a]	0.08
ECR [5]	2.60 [b]	3.36 [a]	2.40 [b]	2.35 [b]	2.57 [b]	3.13 [a]	0.08

For each treatment, the values are represented as mean ± standard error (n = 3). The values that do not share the same letter differ significantly ($p < 0.05$). Newman–Keuls test. [1] Initial weight was considered covariable for final weight and specific growth rate. [2] Specific growth rate (SGR) = 100 × ln (final weight/initial weight)/days. [3] Feed intake (FI) (g 100 g fish^{-1} day^{-1}) = 100 × feed consumption (g)/average biomass (g) × days. [4] Feed conversion ratio (FCR) = feed consumption (g)/weight gain (g). [5] Economic conversion ratio (ECR) = feed consumption (g) × price (EUR/kg)/weight gain (g).

Fish fed the POU diet exhibited the highest mortality (13.33%) ($p < 0.05$), while those fed the CONT, ORG, TRO, SBS, and MIX diets presented mortality rates (3.33 to 8.25%) without significant differences ($p > 0.05$). Regarding the feed conversion rate, fish fed the POU diet presented the highest value of FCR (2.92%), and fish fed the CONT and ORG diets showed the lowest (1.72% and 1.91, respectively) ($p < 0.05$). The fish fed diets containing organic fish remains (TRO, SBS, MIX) showed FCR without significant differences between them ($p > 0.05$). The TRO diet was higher (FI 2.65%) than the CONT and ORG diets (2.25 and 2.32, respectively), and the highest was in the TRO diet (2.65%) ($p < 0.05$). If the economic conversion rate is observed (ECR), the ORG and POU diets showed significant differences (3.36 and 3.13 EUR/kg, respectively) ($p < 0.05$) compared to the rest of the diets, which ranged from 2.35 to 2.60 EUR/kg.

3.2. Biometric Indices of Gilthead Seabream Fed Experimental Diets

The biometric indices after the trial period are presented in Table 5. No significant differences ($p > 0.05$) were found in any biometric parameters calculated according to the diet, except for the condition factor. The CF presented significant differences; fish fed the MIX diet had the lowest value (1.77%), and fish fed the ORG diet had the highest value (2.02%) ($p < 0.05$).

Table 5. Biometric indices of gilthead seabream fed the experimental diets.

	CONT	ORG	TRO	SBS	MIX	POU	SEM
VSI (%) [1]	6.1	9.0	5.5	6.6	5.9	6.4	0.87
HSI (%) [2]	1.2	0.9	0.8	0.8	0.8	0.9	0.16
VFI (%) [3]	1.6	1.9	1.2	0.9	1.0	0.9	0.47
CF (g/cm^3) [4]	2.0 [ab]	2.0 [a]	1.9 [abc]	1.9 [bc]	1.7 [c]	1.8 [bc]	0.13

For each treatment, values are represented as mean ± standard error (n = 9). The values that do not share the same letter differ significantly ($p < 0.05$). [1] Viscerosomatic index (VSI) (%) = (visceral weight (g)/total fish weight (g)) × 100. [2] Hepatosomatic index (HIS) (%) = (liver weight (g)/total fish weight (g)) × 100. [3] Visceral fat index (VFI) (%) = (visceral fat (g)/total fish weight (g)) × 100. [4] Condition factor (CF) (g/cm^3) = (total fish weight (g)/length^3 (cm)) × 100.

3.3. Proximate Body Composition and Nutrient Efficiency Retention

The results of the final body composition and nutrient retention efficiency are shown in Table 6. No significant differences were found for dry matter, protein, and ash between the treatments ($p > 0.05$). The fat was significantly lower in the POU diet (9.39%) compared to the CONT diet (14%) ($p < 0.05$).

Table 6. Body composition (% wet weight) and protein efficiency retention of gilthead seabream fed the experimental diets.

	Initial	CONT	ORG	TRO	SBS	MIX	POU	SEM
Dry matter (%)	30.0	31.8	30.4	29.9	29.6	29	28.1	1.41
Protein (%)	17.0	16.7	16.7	16.7	16.5	16.7	17.5	0.33
Fat (%)	9.0	14.0^{a}	12.3 ^{ab}	11.8 ^{ab}	11.8 ^{ab}	10.5 ^{b}	9.4 ^{b}	0.42
Ash (%)	3.6	1.5	1.6	1.7	1.6	2.0	2.2	0.09
PPV (%) [1]		23.1 ^{a}	21.0 ^{a}	19.1 ^{ab}	19.9 ^{ab}	19.3 ^{ab}	16.1 ^{b}	0.43
PFV (%) [2]		54.2 ^{a}	41.6 ^{b}	37.1 ^{bc}	31.4 ^{bc}	26.7 ^{cd}	18.2 ^{d}	2.64

For each treatment, values are represented as mean ± standard error (n = 3). The values that do not share the same letter differ significantly ($p < 0.05$). Newman–Keuls test. [1] Productive protein value (PPV %) = protein retained (final fish protein × final biomass (g)) × 100 − initial fish protein x initial biomass (g)/protein ingested (kg ingested food ×% crude protein). [2] Productive fat value (PFV %) = fat retained (final fish fat × final biomass (g)) × 100 − initial fish fat × initial biomass (g)/fat ingested (kg ingested food × % crude fat).

Concerning protein efficiency retention (PPV), there were no differences between the CONT, ORG, TRO, SBS, and MIX diets ($p > 0.05$). On the other hand, the fish fed the POU diet obtained the lowest value of PPV (16.11%) ($p < 0.05$). Regarding the EAA (Figure 2), it can be seen that almost all of the lowest values for the essential amino acids were obtained in the fish fed the POU diet ($p < 0.05$). The highest retention efficiency for phenylalanine, isoleucine, and leucine was observed in fish fed the SBS diet (16.56, 22.51, and 21.20%, respectively) ($p < 0.05$). The CONT diet showed the highest retention efficiency of Lys, Arg, Thr, and Val (29.74, 23.24, 19.21, and 20.05, respectively) ($p < 0.05$).

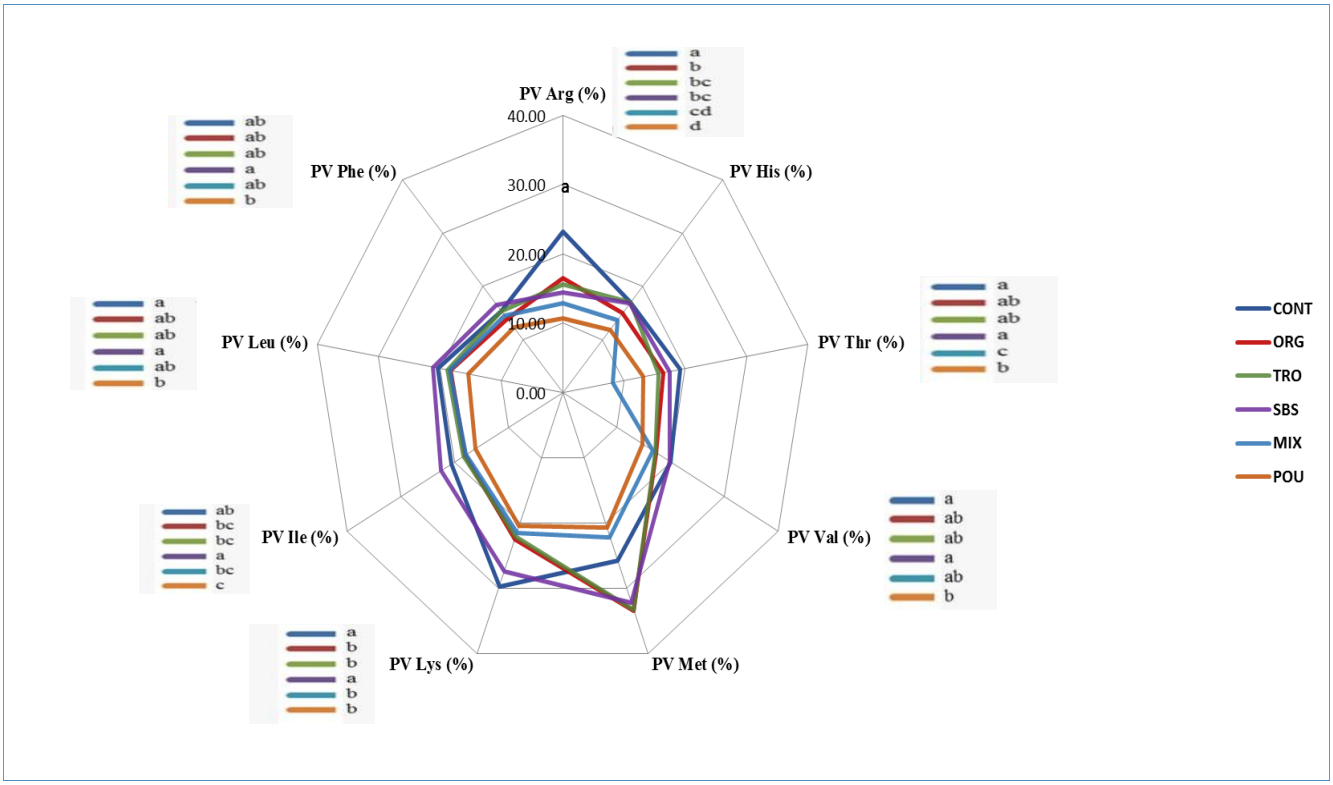

Figure 2. Productive value of essential amino acids (n = 3) for gilthead seabream fed experimental diets. For each treatment, the values are represented as mean ± standard error (n = 3). The values that do not share the same letter differ significantly ($p < 0.05$). Newman–Keuls test. Productive values of amino acids (%) = (fish amino acid gain (g) × 100)/amino acid intake (g).

The results of the productive values of the primary fatty acids (g/100 g of wet weight) in the whole body of gilthead seabream fed different diets are shown in Table 7. The

efficiency of retention of the fish fed the CONT diet was observed to have the highest values in most fatty acids ($p < 0.05$). Different organic ingredients in the diets showed significant changes in retention efficiency between treatments of several saturated fatty acids (SFAs) ($p < 0.05$). The retention efficiency of fish fed the POU diet was the lowest ($p > 0.05$). The retention efficiency of the monounsaturated fatty acids (MUFAs) showed significant differences ($p < 0.05$) between diets where the CONT diet obtained the highest value significantly (C16:1, C18:1n9t, and C18:1n9c), except C20:1 (Table 7). The retention efficiency of linoleic acid (C18:2n6c) and linolenic acid (18:3n3) was the highest ($p < 0.05$) in the fish fed the TRO diet (54.7 and 52.1, respectively). The lowest values ($p < 0.05$) were found for the POU diet. No significant differences ($p > 0.05$) were found for eicosapentaenoic acid (EPA, 20:5n3) or docosahexaenoic acid (DHA, 22:6n3). Regarding the omega 3/omega 6 ratio, no significant differences ($p > 0.05$) were observed.

Table 7. Productive values of fatty acids in gilthead seabream fed the experimental diets (g/100 g of wet weight).

	CONT	ORG	TRO	SBS	MIX	POU	SEM
SFA [1]							
(C13:0)	12.3 ^{b}	18.5 ^{b}	67.6 ^{a}	12.8 ^{b}	14.0 ^{b}	9.7 ^{b}	3.49
(C14:0)	60.9	57.4	39.1	50.2	42.5	46.4	4.68
(C15:0)	53.4	54.4	38.6	39.7	46.5	35.2	5.56
(C16:0)	60.4 ^{a}	53.2 ^{ab}	50.0 ^{ab}	39.6 ^{bc}	40.5 ^{bc}	28.6 ^{c}	4.55
(C17:0)	68.6 ^{a}	64.7 ^{a}	44.6 ^{ab}	47.4 ^{ab}	46.7 ^{ab}	32.2 ^{b}	6.72
(C18:0)	60.6 ^{a}	46.0 ^{b}	40.0 ^{bc}	39.9 ^{bc}	36.8 ^{bc}	23.5 ^{c}	3.98
MUFA [2]							
(C16:1)	98.3 ^{a}	89.7 ^{a}	48.1 ^{b}	58.8 ^{b}	55.9 ^{b}	54.8 ^{b}	5.54
(C17:1)	87.3	92.3	56.7	67.9	66.2	56.9	18.15
(C18:1n9c)	95.6 ^{a}	70.1 ^{c}	86.9 ^{b}	95.7 ^{a}	73.9 ^{c}	73.6 ^{c}	9.46
(18:1n-7)	66.2 ^{a}	55.1 ^{ab}	45.4 ^{bc}	43.6 ^{bc}	43.9 ^{bc}	33.5 ^{c}	4.29
(C20:1)	57.3 ^{ab}	68.7 ^{a}	34.8 ^{b}	32.7 ^{b}	34.3 ^{b}	49.7 ^{ab}	6.38
PUFA [3]							
(C18:2n6c) LA [4]	43.8 ^{ab}	40.1 ^{ab}	54.7 ^{a}	39.1 ^{ab}	43.3 ^{ab}	29.3 ^{b}	3.95
(C18:3n3) LNA [5]	45.1 ^{ab}	34.8 ^{ab}	52.1 ^{a}	36.0 ^{ab}	37.1 ^{ab}	27.0 ^{b}	4.14
(C20:2)	84.5 ^{a}	68.8^{b}	38.5 ^{c}	39.8 ^{c}	30.7 ^{c}	22.6 ^{d}	4.48
(C20:3n6)	42.0 ^{a}	35.5 ^{ab}	30.2 ^{ab}	22.5 ^{b}	26.8 ^{ab}	28.5 ^{ab}	3.82
(C20:4n6) ARA [6]	47.8	48.7	37.4	43	46.2	37	6.84
(22:4n-6)	22	42.2	27.4	43.4	26.9	16.9	7.55
(22:5n-3)	98.3	95.1	70.1	71.5	61.8	67.8	10.58
(20:5n-3) EPA [7]	31.5	32	40	28.7	33.2	28.5	4.32
(22:6n-3) DHA [8]	53.5	53	49.2	45.9	50.4	48	9.07
ω-3/ω-6 ratio	1.6	1.4	1.3	1.4	1.1	1.6	0.16

For each treatment, values are represented as means (n = 3). The values that do not share the same letter differ significantly ($p < 0.05$). Productive values of fatty acids (%) = (fish fatty acid gain (g) × 100)/fatty acid intake (g). [1] SFA: saturated fatty acids. [2] MUFA: monounsaturated fatty acids. [3] PUFA: polyunsaturated fatty acids. [4] LA: linoleic acid. [5] LNA: linolenic acid. [6] ARA: arachidonic acid. [7] EPA: eicosapentaenoic acid. [8] DHA: docosahexaenoic acid.

4. Discussion

According to Craig and McLean, the need for certified protein sources significantly hinders the growth of organic aquaculture [29]. There is still much discussion about the certifiability of by-catch from commercial fisheries, by-products, and processing wastes from aquaculture, fish, and meat processing industries as ingredients for organic aquafeed. The acceptability and availability of amino acids in these products are also questionable [30]. Vegetable protein sources pose challenges, especially for feeding higher-level carnivores such as seabream. They contain antinutritional factors and have low biological value due to essential amino acid deficiencies and poor digestibility [31]. Furthermore, including non-organic certified plant ingredients instead of fish ingredients in fish feeds also brings about

the presence of undesirable substances [32]. Some commonly used pesticides in land-based agriculture have been identified in aquatic feeds. For instance, a recent extensive analysis of aquafeeds has revealed the potential presence of chlorpyrifos-methyl (CPM) [33]. A survey of commercially available aquatic feeds conducted in 2017 reported CPM levels ranging from 11 to 26 µg/kg [34]. On average, approximately 5–10% of the examined feed samples had CPM levels exceeding the detection limit.

This study observed the highest final weight in fish fed the CONT and ORG diets without significant differences ($p > 0.05$). In these diets, 30% of commercial fishmeal was used as a protein source. These high-quality fish meals are known to be the best protein source for fish thanks to their high digestibility and because their amino acid composition is very close to the need profile of most carnivorous aquaculture species [35,36]. Regarding the amino acid profile, in both the CONT and ORG diets, there is a greater quantity of essential amino acids compared to other diets, which also must impact the final growth results.

For the organic diets without a commercial fish meal, the TRO and SBS diets exhibited better growth compared to MIX and POU diets. However, previous studies of diets produced with the remains of the rest of the seabass and trout cannot be found. The growth results observed in the fish fed the TRO and SBS diets can be explained by the nature of the diet. Fish protein has an amino acid profile that closely matches the nutritional needs of the fish, which likely contributes to their improved growth compared to the control diet. The differences between the control diet and the TRO and SBS meal diets can be attributed to the fact that the raw materials used are the remains of these species. The remains may affect factors such as protein availability or the processing of these raw materials, leading to variations in growth outcomes. Using trout meal and seabass meal by-products promotes resource efficiency, waste reduction, and the establishment of a circular economy. One of the key advantages of using these by-products is their positive environmental impact. Instead of discarding them, incorporating them into other products or processes minimises the need for additional resources and waste disposal. This approach fosters a more sustainable production cycle and contributes to environmental conservation [37]. There is currently no commercial organic supply chain for trout and seabass. These certified organic meal products should be manufactured in dedicated organic meal factories. The current availability of organic seabass and trout is insufficient to justify these factories' existence. However, if such products were established, it could greatly enhance the profitability of organic production.

It is worth noting that the fish from the MIX treatment, which included poultry meal in its composition, obtained a lower final weight than those containing organic ingredients and aquaculture proteins (TRO and SBS). The presence of poultry meal in the MIX treatment affected the growth of the gilthead seabream and may have impacted protein availability. Moreover, the lowest final weight was obtained with the POU treatment.

According to Regulation (EU) 2018/848 [16], Part III, paragraph (e) of Section 3.1 regarding feeding aquaculture animals, it dictates that "growth factors and synthetic amino acids will not be used." Consequently, using amino acids is not allowed commercially in organic aquaculture. Its application in diet formulation at the production level would not be feasible unless sustainable plant amino acids are used, such as vegetable methionine, in the present work, even though its efficiency is lower (at the time of the design of the experiment, when the commercial company that provided the diet-only had vegetable methionine).

Concerning the fatty acids in the diet, they do not seem to be the determining factor in the present study, given the amount of fish oil in this diet. Likewise, it depends on the percentage of inclusion in the diet and its quality, as mentioned above. In the present study, the feed intake was numerically higher in the organic diet groups than in the control group. However, the differences were not statistically significant. The fact that the FI and FCR were higher in the organic diet groups may indicate that the organic diets' nutrients were unbalanced. Hence, the animals needed to increase their feed intake to compensate for

deficient nutrients, such as essential amino acids. This may be the result of the origin of the raw materials. Regarding the FCR, the highest values and those statistically different from the rest of the treatments were registered in the fish fed the POU diet. Because of the above and in agreement with the study by Karapanagiotidis et al. [38], a 100% replacement of fishmeal for poultry meal significantly increased the FCR and reduced the efficiency of feed utilisation. However, if the ECR is observed, the ORG and POU diets resulted in a higher investment of money to produce fish. The higher price of the ORG diet and the high FCR of the POU diet causes this worsening of the ECR. On the other hand, the growth of the CONT, TRO, SBS, and MIX diets entails a similar ECR: the better FCR of the CONT diet is compensated by the lower prices of the organic diets made with by-products. In addition, mortality was higher in the POU treatment than in other studies [38], where no difference in mortality was evident between the treatments. This was possibly a consequence of the lower appetite of these fish, providing justification for their worse growth.

Some studies on seabream and seabass have been published that compare conventional diets with organic diets, obtaining better growth in organic diets [21,39] since these diets were formulated with a higher percentage of fish meal (63 and 56%) than the conventional ones (50 and 20%).

Regarding body composition and nutrient retention efficiencies, the fat was significantly lower in the poultry treatment diets (POU and MIX) compared to the ORG diet, which differs from the study by Sabbagh et al. [40,41], where no differences were found. However, it agrees with the findings of other studies in which a higher inclusion of poultry meal led to a decrease in body fat [38], possibly due to the lower growth obtained with this diet.

The CONT and ORG treatments show higher percentages for protein retention efficiency (PPV) and fat retention efficiency (PFV). This means that they use a higher proportion of proteins and lipids in their diet for their growth, and consequently, more significant growth is manifested. On the other hand, the PPV was significantly lower in the POU treatment and was similar to the MIX treatment, which is related to the low growth of the fish fed these types of diets. The essential amino acid profile in the diet can also explain differences in amino acid retention efficiency. Some authors [42,43] noticed that protein retention efficiency decreases with the intake. Consequently, it seems logical that the efficiency retention of a single amino acid could be influenced by the feed composition, increasing the efficiency when the composition is lower. Some of the increased efficiencies observed in Figure 2 could be explained by observing the TRO diet, which is a low amount of histidine (9.70 g/kg), but has the highest retention efficiency for this EAA (16.98%). The same trend is evident in the diet SBS with phenylalanine, where there is a low amount of this amino acid (18.90 g/kg), and the retention efficiency is the highest (16.56%). In general, many of the high retention efficiencies of gilthead seabream could be due to a lower amino acid content. The fact that EAAs with higher concentrations in the organic diets have lower retentions in fish suggests that the EAA profile needs to be well balanced. Instead of being used for protein synthesis, these excessive dietary EAAs were catabolised. This results in the lower retention of EAAs with high concentrations in the organic diets.

The retention efficiency of fatty acids in seabream fed experimental diets is directly related to the fatty acid profile in different diets, as has been seen in other species such as *Salmo salar* [44,45] or *Dicentrarchus labrax* [46]. Even though the dietary profile of fatty acids differs by the type of feeding of the fish, the results agree with the studies carried out by other authors, where saturated fatty acids are represented mainly by C16: 0 and C18: 0, and those monounsaturated by C: 18: 1n9 [47]. The literature reports that those species that include significant amounts of linoleic acid (C18: 2n-6) or linolenic acid (C18: 3n-3) in their diet present lower concentrations of the C18: 1n-9t and C18: 1n-9 acids in their tissues [48]. The variations of these acids in the analysed species are probably multiple factors, among them the feeding of the fish, a determining element for their composition [49,50].

It is essential not to ignore the effect of lipid composition on the fatty acid composition of fish fed organic feed. From the data in Tables 3 and 7, the retention efficiency of n-6

and n-3 of fish lipids is greatly affected by the n-6 and n-3 of dietary lipids. When the dietary ratio is very high in n-6 fatty acids, fish tend to alter the proportion of PUFAs incorporated in favour of n-3 fatty acids [48]. It is common to see changes in fatty acid profiles by substituting fishmeal for other lipid sources. However, there needs to be more information on the effects of changes in the retention efficiency of fatty acids. A study carried out in *Seriola dumerili* [26] fed fish with high levels of substitution of fish oils for a mixture of vegetable oils; however, in this case, such high differences were not obtained in terms of efficiency and retention, since the differences in growth concerning the control feed were not so relevant. On the other hand, it can be stated that they are closely related to the productive values obtained for fat, which was significantly lower in fish fed POU, as well as the low retention of EPA and DHA, which, together with the lower levels of these fatty acids in the diet, could have been the trigger for mortality observed in this group.

The highest productive values of FA were observed in the fish fed the CONT diet. In these diets, commercial fish oil was used as a lipid source. These high-quality fish oils are known to be the best lipid source for fish thanks to their high digestibility and their fatty acid composition availability [51]. These results agree with other studies that show that the dietary fatty acid compositions reflect FA compositions in marine fish [52]. It is perceived that variations in the fatty acid profile of meals are primarily reflected in the fish composition [53]. The productive values of the FA of the gilthead seabream show that when an FA is at a lower dietary level, its retention efficiency will increase; the opposite occurs when there is a higher level of FA.

This study found that the best growth occurred in the two control diets containing 30% fishmeal, regardless of whether the rest of the ingredients were organic. Regarding the experimental diets, the fish fed the TRO diet showed the highest growth, followed by the SBS and MIX diets; finally, the POU diet showed the lowest growth. The fish fed the POU diet exhibited the highest mortality, while those fed the CONT, ORG, TRO, SBS, and MIX diets presented similar mortality rates. Regarding nutrient retention efficiency, different organic ingredients in the diets showed significant changes in the retention efficiency of several fatty acids between the treatments. However, no significant differences were found in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Using trout meal and seabass meal by-products in production offers various benefits, including resource efficiency, waste reduction, and promoting a circular economy. Incorporating these by-products into other products or processes minimises environmental impacts and conserves resources. The availability of these raw materials depends on factors related to fish farming, fisheries management, and market demand. Implementing sustainable practices and establishing collaborations within the industry is crucial for maintaining a reliable supply chain. However, the specific growth outcomes can be influenced by factors such as the composition of the diets and the presence of certain raw materials such as poultry meal.

One of the main factors impeding organic production growth is the higher cost of organic feed. However, this does not have to be the case. The present study demonstrates that organic feed can be obtained at competitive prices by utilising by-products from other organic farms. Based on economic indices, completely replacing fishmeal with more organic alternatives containing organic fish by-products is a promising alternative to feeding farmed fish organically. Total replacement and some efficiency parameters appear to affect growth, but slightly enough to still be economically convenient. The findings provide insights into the potential benefits of using organic ingredients in aquaculture diets. Therefore, it is recommended to continue increasing the knowledge in this sector to mitigate the impact of extractive fishing and more aquaculture sustainability, as well as the experimental conclusions that can be drawn.

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Institutional Review Board Statement: The animal study protocol was approved by the Ethics and Animal Welfare Committee of the Polytechnic University of Valencia (UPV) (protocol code 2021/VSC/PEA/0268 type 2) following Royal Decree 53/2013 and the European Directive 2010/63/EU on the protection of animals used for scientific research, to minimise the suffering of animals.

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