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

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Abstract: This study examined how organic feed affects the growth performance, nutrient efficiency, feed utilization, 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 (TRO, SBS, POU, and 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 food 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 economical 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: gilthead seabream; 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 look for 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. Four basic principles are usually watched: health, ecology, fairness, and care. Under these principles, ecologic inputs in aquaculture must be maximized. In most organic systems, such as UE regulatory, the preference for organic consideration of the raw ingredients is for the use of by-products coming from certified organic farms. These by-products are mostly considered as the more ecological ingredients that may be used because they represent a further exploitation of an already considered organic ingredient.

These are defined as products produced based on regulations that seek the care and protection of the environment [3]. With the same thought, organic production has expanded to the cattle, poultry, and pork industries, and aquaculture has not been an exception, joining this type of responsible exploitation. 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 EUMOFA, (2022), overall organic aquaculture output in the EU 27 is estimated at 74032 tonnes in 2020, accounting for 6.4% of total EU aquaculture production. Production has grown by 60% from 2015 (46341 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 and retail prices, which have deterred consumers and producers. The higher cost is mainly based on the higher cost of fingerlings and feed [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 dietary and 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, it is allowed on most organic labels to use non-organic fishmeal. Instead, the use of fishmeal from sustainable fisheries is required. The need for more proteins of animal origin aggravates the situation over protein ingredients use. However, 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, using fisheries and aquaculture by-products is an excellent sustainable aquafeed option [16,17]. 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 summarized 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, 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 EPA and DHA [14].

Even fewer studies compare conventional feeding with organic feeding, Sardinha et al. [18] in sea bream, Pascoli et al. [19] in sea bass, or Di Marco et al. [20] in sea bream (*Dicentrarchus labrax*), and sea bass (*Sparus aurata*) compare organic feeding with conventional. However, in these studies, organic feeds have conventional fishmeal as a protein source. Stress and immunological markers in fish were similar [19], while fillet fatty acid composition varied depending on the diet [21]. The by-product meals used in this study need to be better studied.

The need for more protein sources from organic sources must be ahead. The main alternatives are using TAPs from organic cattle but also generating a circular economy, using the remains of other species of organic fish, avoiding cannibalism, to generate organic meals with more accurate fatty acids and amino acid profiles. The purpose of this research was to determine more about the effect that organic feeds have on the growth of gilthead seabream and see how new organic raw materials, such as poultry, remain 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.

2. Materials and Methods

2.1 Production System

The growth trial was carried out in a recirculating saltwater system (65 m3 capacity), with a rotary mechanical filter and a gravity biofilter (about 6 m3), 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 104.2mg L^{-1}, nitrites was 0.38 mg L$^{-1,}$ and the ammonium level was 0.22 mg L^{-1}. Throughout the experiment, the photoperiod was regular, and all tanks had similar lighting conditions.

2.2 Fish and trial design

Juveniles of organic seabream from the Sonrionansa S.L. fish farm located in Pesues (Cantabria) were used for the study. Fish were transported to Universitat Politècnica de València and distributed in experimental tanks. All fish were acclimated to laboratory conditions before the two-week 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 (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. TRO diet (with organic trout meal as a protein source) and SBS diet (with remains of organic seabass as a protein source). POU diet (with organic poultry meal as a protein source), and MIX diet (containing three equal parts of the meal from the organic remains of seabass. trout, and poultry). From Monday to Saturday, fish were hand-fed twice daily (9:00 and 17:00 h.) until apparent satiation. Pellets were progressively dispersed, enabling all fish to consume, and recording the overall amount of feed distributed. Every 30 days, all fish were weighed. Fish were previously anesthetized with 10 mg L^{-1} clove oil (Guinama®) containing 87 % eugenol. Before being weighed, the fish were not fed the previous day.

2.3 Diets and feeding

Six diets, isoproteic (44%) and isolipidic (20%) were formulated (Table 1). CONT and ORG diets include 30% non-organic fishmeal because no organically certified commercial fishmeal exists. The ORG diet differs from the CONT diet in using organic ingredients: in ORG, unlike in CONT, the use of non-organic ingredients was avoided at 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 its growth [22]. The control diet also had added methionine; however, this was of synthetic origin. The diets were prepared by a semi-industrial twin-screw extruder (CLEXTRAL BC 45, St. Etienne, France). The screw speed of 100 rpm, the temperature of 110 °C, and the pressure of 40–50 atm were the processing conditions. Conventional fish oil was used because organic fish oil is not yet certified.

2.4 Proximate composition and amino acid analysis

Raw materials were chemically analyzed. At the start of the experiment, five fish were sampled, triturate, and homogenized before being analyzed for chemical composition. At the end of the experiment, five fish per tank were randomly sampled to determine the biometric parameters and triturate and homogenized before being analyzed for the proximate body composition. Dietary ingredients and the whole body of fish fed the six experimental diets were analyzed according to AOAC (1990) [23] procedures: dry matter (105°C to constant weight), ash (550°C to constant weight), crude protein (N × 6.25) by the Kjeldahl method after acid digestion (2300 Kjeltec Analyzer Unit)), and crude lipid extracted with diethyl ether (ANKOM XT10) by the Dumas principle.

The diet, Table 2, and body fish amino acids content were analyzed 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. [24]. After hydrolysis, an internal standard of aminobutyric acid was introduced. AQC (6aminoquinolylNhydroxysuccinimidyl carbamate) was used to derivatize amino acids.

Table 1. Ingredients and formulation of the experimental diets

	CONT	ORG	TRO	SBS	MIX	POU	PRICE
Ingredients (g/kg DM)							€ $tonn^{-1}$
Fish meal[1]	300	300					1933
Organic Trout meal[2]			400		140		750
Organic Sea bass 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
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	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] (€ kg $^{-1}$)	1.51	1.76	1.03	1.04	1.07	1.07	

[1] Fish meal (91.90% DM, 73.95% CP, 11.20 % CL, 14.30% Ash).
[2] Organic Trout meal (98.60% DM, 64.60% CP, 21.7% CL, 12.40% Ash). (Naturix, Valderrebollo, Guadalajara)
[3] Organic Sea bass meal (97.50% DM, 34.50% CP, 41.70% CL, 20.83% Ash). (Andrómeda, España)
[4] Organic Poultry meal (96.48% 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 spelled 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; $(PO_4)_2Ca_3$. 5. Premix composition: retinol acetate. 1,000,000 IU kg $^{-1}$; calcipherol. 500 IU kg $^{-1}$; DL-a-tocopherol. 10; menadione sodium bisulfite. 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 (€ kg $^{-1}$).

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

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 / NEAA	7.9	8.9	8.1	8.1	8.3	8.1

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

2.6 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 (PPV)were all determined, with consideration given to the monthly biomass reports of any deceased fish.

2.7 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 their internal organs, visceral fat, and liver, were measured to determine their condition factor (CF), viscerosomatic (VSI), visceral fat (VFI), and hepatosomatic (HSI) indices.

2.8 Fatty acid analysis

Total lipid fatty acid methyl esters (FAME) were produced directly, as stated by O'Fallon [25]. A Focus Gas Chromatograph (Thermo, Milan, Italy) with a split/splitless injector and a flame ionization detector was used to analyze FAME. A fused silica capillary column SPTM 2560 (Supelco, PA, USA) was used to separate methyl esters (100 m × 0.25 mm x0.2 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, then increased to 240 °C at a rate of 4 °C/min for another 30 minutes. 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 esters 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: Poly, unsaturated fatty acids; LA: Linoleic acid; LNA: Linolenic acid; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid; ARA: Arachidonic acid.

2.5 Statistical analysis

Growth data, feed utilization, and all data obtained were evaluated using analysis of variance (ANOVA), with the initial live weight as a covariate [26]. The Newman–Keuls test assessed specific diet differences at $p < 0.05$ (Statgraphics, Statistical Graphics System, Version Plus 5.1, Herndon, Virginia, 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. Every day, the fish were examined. In addition, after sedation with clove oil dissolved in water (0.01 mg/L of water) to minimize animal suffering, the health state of the fish was determined through observation. An excess of clove oil (150 mg L^{-1}) was used to euthanize the animals, which were dissected.

3. Results

3.1. Fish growth

Figure 1 shows the average growth of gilthead seabream fed the different experimental diets. Gilthead seabream fed with CONT and ORG diet obtained the highest average weight after the 70 days trial period.

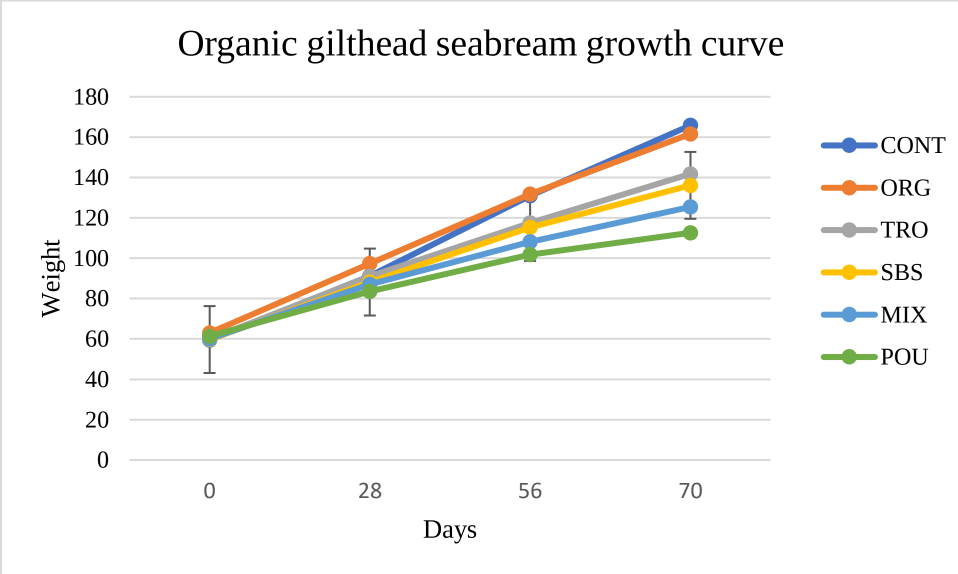

Figure 1. Evolution of the average body weight of gilthead seabream fed on the experimental diets.

Regarding organic diets without fishmeal, the fish fed with the TRO diet showed the highest growth, followed by the SBS MIX diet, and finally, POU with the lowest growth. As reported in Table 4 and Fig. 1, the best growth occurred in the two control diets that contained 30% fishmeal, CONT, and ORG (165.8 and 161.6 g, respectively), regardless of whether the rest of the ingredients were organic or not. Then the fish that grew the best were those fed with organic fish remaining TRO and SBS (141.8 and 136.1, respectively. The worst growth was with diets containing poultry meals, either alone (POU) or in a mixture (MIX) (125.4 and 112.6, respectively). Significant differences were observed in the SGR, showing the highest value in fish fed with the CONT diet (1.46 %), and fish fed with 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)	165.8a	161.6a	141.8b	136b	125.4c	112.6d	3.80
Mortality (%)	3.3b	4.2b	5b	7.5b	8.2b	13.3a	1.44
SGR (% /day)[2]	1.46a	1.35b	1.23c	1.18c	1.05d	0.87e	0.02
FI(g/100gfish/day)[3]	2.25b	2.32b	2.65a	2.50ab	2.46ab	2.57ab	0.07
FCR[4]	1.7d	1.9c	2.3b	2.3b	2.4b	2.9a	0.08
ECR[5]	2.60b	3.36a	2.40b	2.35b	2.57b	3.13a	0.08

Different letters indicate statistical differences between means (n = 3). p-value <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) x price (€/kg) / weight gain (g).

Fish fed the POU diet exhibited the highest mortality (13.33%), while those fed the CONT, ORG, TRO, SBS, and MIX diets presented similar mortality rates (3.33 to 8.25%) without significant differences. Regarding the feed conversion rate, fish fed the POU diet presented the highest value of FCR (2.92%), and fish fed with the CONT and ORG diets showed the lowest (1.72% and 1.91, respectively), the fish fed with diets containing organic fish remains (TRO, SBS, MIX) showed similar FCR without significant differences between them. The lowest feeding rate was observed in the CONT and ORG diets (2.25 and 2.32, respectively) and the highest in the TRO diet (2.65 %). If the economic conversion rate is observed (ECR), the highest values were obtained for the ORG and POU diets (3.36 and 3.13 €/kg r, respectively). The rest of the diets obtained lower values, from 2.35 to 2.60 €/kg.

3.2. *Biometric indices of gilthead seabream fed the experimental diets.*

The biometric indices after the trial period are presented in Table 5. No significant differences 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%).

Table 5. Biometric indexes 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 deviation (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*

Results of 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. The fat was significantly lower in the POU diet (9.39%) compared to the CONT diet (14%).

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}	37.1b^{c}	31.4^{bc}	26.7^{cd}	18.2^{d}	2.64

For each treatment, values are represented as mean ± standard deviation (n = 3). The values that do not share the same letter differ significantly (p <0.05).
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 x initial biomass (g) / fat ingested (kg ingested food ×% crude fat).

Concerning protein efficiency retention (productive protein value, PPV), the highest percentages were shown in the CONT and ORG diets, with 23.13% and 21.00, respectively, without differences between either. On the other hand, fish fed the POU diet obtained the lowest value of PPV (16.11 %). Regarding the EAA Figure 2, it can be seen that almost all the lowest values for the essential amino acids were obtained in the fish fed the POU diet. 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). The CONT diet showed the highest retention efficiency of Lys, Arg, Thr, and Val (29.74, 23.24, 19.21, and 20.05, respectively).

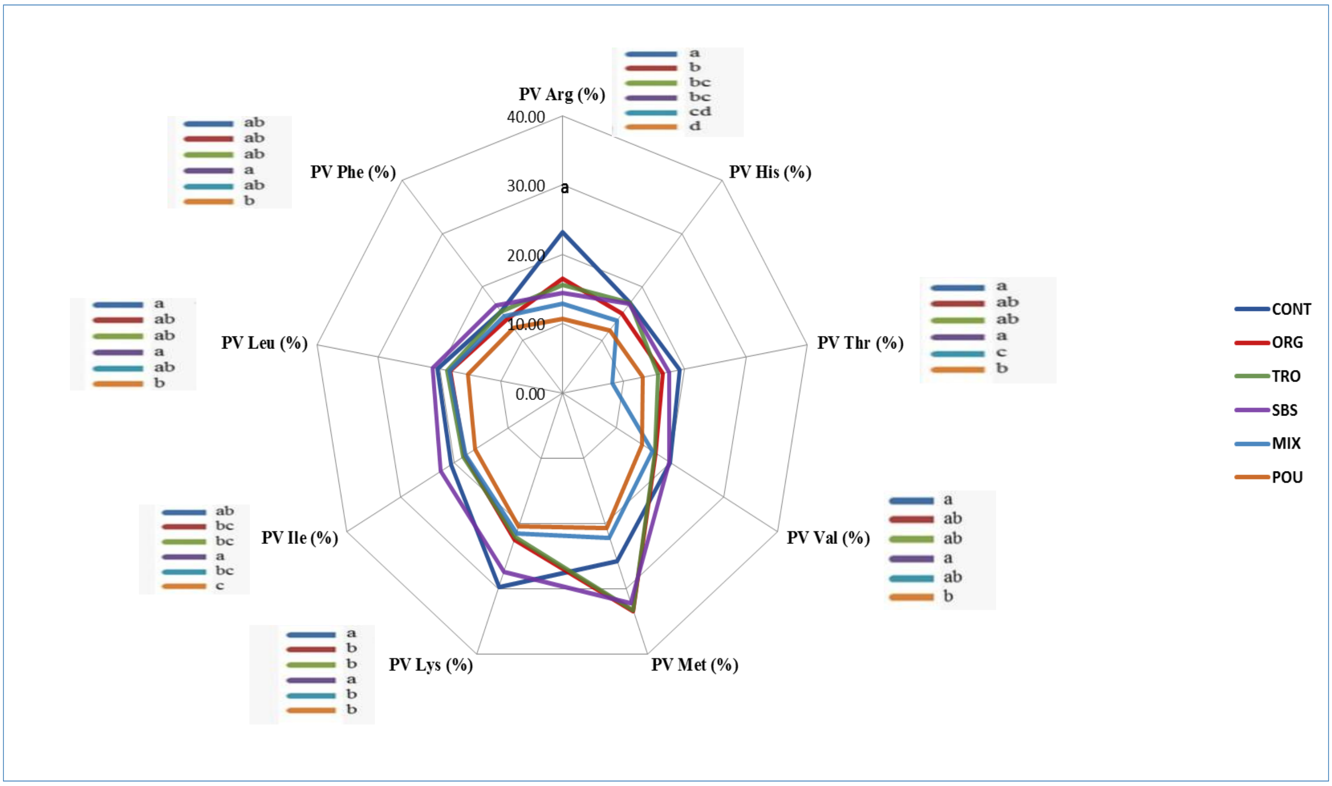

Figure 2. Productive value of essential amino acids (n = 3) for gilthead seabream fed with experimental diets.

NOTE: For each treatment. The values that do not share the same letter differ significantly (p <0.05). 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 with different diets are shown in **Table 7**. The efficiency of retention of the fish fed with the CONT diet was observed to have the highest values in most fatty acids. Different organic ingredients in the diets showed significant changes in retention efficiency between treatments of several saturated fatty acids (SFAs),

w. The efficiency of retention of fish fed with POU was the lowest. The retention efficiency of the monounsaturated fatty acids (MUFAs) showed significant differences 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 highest in fish fed the TRO diet (54.7, 52.1, respectively). The lowest values were in the POU diet. No significant differences were found in eicosapentaenoic acid (EPA. 20:5n3), and docosahexaenoic acid (DHA; 22:6n3).

Table 7. Productive values of fatty acids in gilthead seabream fed with the experimental diets.

	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

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: Poly, unsaturated 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 [27]. 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 questioned [28]. Vegetable protein sources pose challenges, especially for feeding higher-level carnivores like seabream. They contain antinutritional factors and have low biological value due to essential amino acid deficiencies and poor digestibility [29].

This study observed the highest final weight in fish fed the CONT and ORG diets without significant differences. 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 [30,31]. Regarding the amino acid profile, in both cases, CONT and ORG diets, there is a greater quantity of essential amino acids when compared to other diets, which also must impact the final growth results.

For the organic diets without a commercial fish meal, no significant differences were found between the final weight of the fish fed with TRO and SBS, but they had differences with those fed with the MIX and POU diets. However, previous studies of diets produced with the remains of the rest of the seabass and trout cannot be found. However, the growth results can be explained in the same way as in the ORG diet, the nature of the diet since fish protein has an amino acid profile closer to the nutritional needs of the fish. An adequate theory about the differences between the control diet and these two diets, SBS and TRO, can be offered because of are remains of this species and may be the availability of protein. The fish from the MIX treatment obtained a lower final weight than that of the organic ingredients with aquaculture proteins TRO and SBS because of the poultry meal in the MIX that was in its composition since this raw material affected the growth of the gilthead seabream and may be the availability of protein. Nevertheless, the lowest final weight was obtained with the POU treatment.

According to Regulation (EU) 2018/848. Part III. paragraph (e) of section 3.1.3.1 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 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 and statistically different from the rest of the treatments was registered in the fish fed with the POU diet. Because of the above and in agreement with the study by Karapanagiotidis et al. [32] a 100% replacement of fishmeal for poultry meal significantly increased the FCR and reduced the efficiency of feed utilization. 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 is causing 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 [32,33], where no difference in mortality was evident between the treatments. This was possibly a consequence of the lower appetite of these fish and justifying a justification for their worse growth.

Some works on seabream and seabass have been published comparing conventional diets with organic diets, obtaining better growth in organic diets [34,35] 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. [36], where no differences were found. However, it agrees with what was found by other studies where a higher inclusion of poultry meal affected a decrease in body fat [37,38], possibly due to the lower growth obtained with this type of 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 similar to the MIX treatment, which is related to the low growth of the fish fed with these types of diet. The essential amino acid profile in the diet can also explain differences in amino acid retention efficiency. Some authors [39,40] have noticed that the efficiency of retention of protein 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 Fig. 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 amount of amino acids. 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 catabolized. This results in lower retention of EAAs with high concentrations in the organic diets.

The retention efficiency of fatty acids in seabream fed with experimental diets is directly related to the fatty acid profile in different diets, as has been seen in other species such as *Salmo salar* [41,42] or *Dicentrarchus labrax* [43]. 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 would be represented mainly by C16: 0 and C18: 0, and those monounsaturated by C: 18: 1n9 [44]. 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 [45]. The variations of these acids in the analyzed species are probably multiple factors, among them the feeding of the fish, a determining element for their composition [46].

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 favor of n-3 fatty acids [45]. 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* [47] fed 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 have been observed in 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 [48]. These results agree with other studies that show that the dietary fatty acid compositions reflect FA compositions in marine fish. [49]. It is perceived that variations in the fatty acid profile of meals are primarily reflected in the fish composition [50]. The productive values of the FA of the gilthead seabream show that when a 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 with the TRO diet showed the highest growth, followed by the SBS, MIX diets, and finally, POU with the lowest growth. 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 retention efficiency between treatments of several fatty acids. However, no significant differences were found in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

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 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.

Author Contributions:

Conceptualization, Silvia Martínez-Llorens, and Miguel Jover-Cerdá; Data curation, Eslam Tefal, Ana Tomás-Vidal and Silvia Martínez-Llorens; Formal analysis, Eslam Tefal and Ignacio Jauralde; Funding acquisition, Miguel Jover-Cerdá; Investigation, Eslam Tefal and Ignacio Jauralde; Methodology, Silvia Martínez-Lloren,s and Miguel Jover-Cerdá;

Supervision, Ana Tomás-Vidal, and David Peñaranda; Validation, Ana Tomás-Vidal and David Peñaranda; Writing – original draft, Eslam Tefal; Writing – review & editing, Eslam Tefal, Ana Tomás-Vidal and Ignacio Jauralde. All authors have read and agreed to the published version of the manuscript.

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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 minimize the suffering of animals.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

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Conflict of Interest

The authors have no financial or personal conflict of interest to declare. The authors declare no conflict of interest.

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