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Effect of Poultry By-product Meal as Replacement for Fish Meal in Diets of Gilthead Seabream (*Sparus aurata*) Juveniles

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Abstract

This study investigated the effects of replacement of fish meal (FM) with poultry by-product meal (PBM) at 55%, 65% and 75% on growth performance and amino acid metabolism of juvenile gilthead seabream (*Sparus aurata*) with an initial weight of 35.01±0.11 g for 120 days. The study also evaluated protein and amino acid digestibility to determine their influence on protein metabolism in juvenile seabream. The results indicated that partial replacement of FM with PBM in diets of juvenile gilthead seabream is feasible up to 65% without compromising their growth performance, feed consumption and digestibility ($P>0.05$). Additionally, hepatosomatic (HSI) showed no significant differences between all groups ($P>0.05$). The viscerosomatic index (VSI) level was at the highest value, whereas the condition factor (K) was at the lowest value in CTRL ($P<0.05$). The highest feed conversion ratio (FCR) value was in the 75PBM group ($P<0.05$). However, there was no statistically significant difference between CTRL and the other PBM groups ($P>0.05$). Similarly, feed intake and whole-body protein content did not significantly differ among dietary groups ($P>0.05$). The protein efficiency ratio (PER) values suggest that replacing FM with PBM (up to 65%) in diets of juvenile gilthead seabream is effective and produces diets with high-quality proteins and digestibility coefficients comparable to those of the control group ($P>0.05$). Additionally, amino acid profiles of juvenile gilthead seabream fed with diets containing up to 75% PBM showed no deficiencies in essential amino acids. Nevertheless, we can suggest that 75% PBM substitution for FM can be used in the diets of gilthead seabream, although it causes a slight decrease in growth, FCR and protein digestibility. These results suggest that partial replacement of FM with PBM can be a viable strategy for feeding juvenile gilthead seabream, offering a sustainable advantageous alternative.

Introduction

The fisheries and aquaculture sectors are increasingly recognized for their essential role in addressing global food security and nutrition challenges in the 21st century (FAO, 2022). According to 2022 data from the Food and Agriculture Organization of the United Nations (FAO), over 157 million tons, representing 89% of animal products from global aquaculture, were designated for human consumption. Meanwhile, 20 million tons were allocated for non-food purposes, including the production of fish meal and fish oil. Fish oil is a valuable energy source, while fish meal is a primary protein component in fish feed. However, in recent years, fish meal (FM) production has not been able to fully meet the growing demand for aquaculture feed. This situation highlights the critical need to explore alternative protein sources for aquafeed to ensure the sustainability and efficiency of aquaculture production. In this context, several scientific researchers have

published results regarding the effectiveness of different alternative proteins of plant and animal origin in different species of farmed fish, such as *Sparus aurata* (Gomez-Requeni et al., 2003, 2004; Yildiz et al., 2006, 2007; Aragao et al., 2019; Sabbagh et al., 2019), *Sparidentex hasta* (Hekmatpour et al., 2018), *Oncorhnchus mykiss* (Amirkolaie at al., 2014; Yürüten Özdemir and Yıldız, 2019). Although the quantity of plant proteins in aquafeed has gradually increased, the presence of a variety of anti-nutritional factors (ANF), such as phytates, tannins, trypsin inhibitors and oligosaccharides (Adeyemo and Onilude, 2013, Engin et al., 2024), deficiency of certain essential amino acids (Hardy, 2006) and reduction in protein digestibility (Santigosa at al., 2008) have limited the use of these resources in fish feed (Francis at al., 2001; Kaushik, 1990). Contrariwise, terrestrial animal proteins classified as processed animal proteins (PAP), such as poultry by-product meal (PBM), have been reintroduced into aquaculture feed by the Union European Commission (EU) since 2013 (Karapanagiotidis at al., 2019; Davies at al., 2018). These ingredients have generated increasing interest as alternatives to FM concentrates. They are cost-effective and sustainable, offering relatively high protein content, more balanced amino acid profile, lack of anti-nutritional factors and a lower carbon footprint compared to most plant-based feeds (Hatlen at al., 2013; Hill at al., 2018). Hence, animal protein sources are generally more effective than plant proteins in the diet, especially for carnivorous species (Hardy, 1998). Poultry by-product meal (PBM) is a highly palatable processed animal product (PAP) made from by-products of poultry slaughterhouses and processing plants. It contains a high proportion of protein (58 to 65%) and an essential amino acid (EAA) profile similar to FM, although it has lower levels of lysine and methionine (Hill at al., 2018; Galkanda-Arachchige at al., 2020; Fontinha at al., 2021). In addition, the fact that PBM is continuously available at certain levels can be considered as another advantage of using this product in carnivorous fish feed. Sabbagh et al. (2019) have shown that by adding methionine and lysine to feed, PBM could ultimately replace dietary FM without compromising growth, digestive enzyme activities and well-being in gilthead seabream. Naylor et al. (2009) noted that terrestrial animal by-products have a favorable nutritional composition for fish feed and are widely available at a low cost in the market. Yıldırım et al. (2009) fed Tilapia zilli fry (2.45 mean weight) with diets containing different amounts of PBM instead of fish meal. They reported that 50% PBM replacement level did not negatively affect whole body proximate composition and feed utilization for the fish. Despite variability in protein digestibility due to raw material differences (Nengas at al., 1999), PBM has demonstrated high protein digestibility in various carnivorous fish species (Hernández at al., 2010; Yu at al., 2013), including gilthead seabream (*Sparus aurata*) (Karapanagiotidis at al., 2019). In the first study, performed before using PBM in Europe, Nengas et al. (1999) concluded that a high-quality PBM could replace fish meal in diet. Modern technological processes, used after the EU re-authorization of using PAP in aquafeeds, have guaranteed the production of more stable and high-quality PBM. Nengas et al. (1999) cultured sea bream fry from an average weight of 1 g to approximately 12 g by feeding them with diets containing PBM. Similarly, Karapanagiotidis at al. (2019) cultured sea bream fry from an average weight of 2.5 g to approximately 40 g by feeding them with diets containing PBM. In both studies, it was reported that PBM can replace up to 50% of FM in the diets with no negative effects on the growth performance and feed utilisation. However, when the PBM level in the diets exceeded 50%, reduced fish growth and feed efficiency were reported. Therefore, the aim of this study was to evaluate the effects of replacing FM with increasing quantities (55, 65 and 75%) of PBM in diets with sea bream larger than those used in previous studies on growth performance, survival rate and whole body amino acid composition of juvenile sea bream. In addition, the study evaluated apparent digestibility coefficients (ADC) of the protein fraction obtained from poultry by-product meal, considering potential commercial use in sea bream diets.

Materials and Methods

Experimental Diets

Four isoproteic (≈48 g/kg crude protein) and isolipidic (≈20 g/kg crude lipid) experimental diets were prepared with different dietary proteins at the Mediterranean Fisheries Production Research and Training Institute (MEDFRI). Production and Training Institute, Beymelek, Antalya in Türkiye. The diets were formulated to meet the nutritional requirements of juvenile gilthead seabream (NRC, 2011). The first diet (control diet) contained mostly fish meal (500 g kg^{-1}) as the protein source and very small amounts of wheat gluten (80 g kg^{-1}) and corn gluten (90 g kg^{-1}) to balance the protein in the dietary formulation. In the remaining diets, fish meal was partially (55%, 65% or 75%) replaced with poultry by-product meal (in 55PBM, 65PBM, and 75PBM, respectively). All ingredients, vitamins and mineral premixes were weighed as indicated in the diet's formulation, mixed and pelleted through a 4 mm dye, using an industrial meat grinder in a fish nutrition laboratory. Pellets were dried at an ambient temperature of 30°C for 3 days. Diets were then placed in nylon bags and stored at -20°C until used. Dietary ingredients and proximate compositions are presented in Table 1. Whereas Table 2 outlines the amino acid (AAs) profiles of experimental diets. Identical experimental diets were prepared for the digestibility trial, adding 1% chromium oxide as an inert digestibility marker.

Table 1. Ingredients and proximate composition of the experimental diets.

	Diets			
	CTRL	PBM55	PBM65	PBM75
Feed ingredients (g kg^{-1} dry weight)				
Fish meal[a]	500	225	175	125
Poultry by-product meal[b]	0	275	325	375
Wheat gluten meal[c]	80	110	120	130
Corn gluten meal[a]	90	110	110	110
Dextrin	80	30	20	10
Fish oil[c]	140	140	140	140
Gelatine	50	50	50	50
Mineral premix[d]	30	30	30	30
Vitamin premix[d]	30	30	30	30
Analysed proximate composition (% DM) and metabolizable energy (KJ g^{-1})				
Moisture	8.13	7.87	7.16	7.04
Crude protein	48.94	48.26	48.44	48.86
Crude lipid	18.47	19.43	19.41	19.28
Ash content	7.94	9.15	9.71	9.63
Crude cellulose	0.88	0.75	0.77	0.79
NFE[e]	15.64	14.54	14.51	14.40
ME[f]	15.22	15.36	15.38	15.40
Gross energy (kJ g^{-1})	21.57	21.60	21.62	21.65

[a]Fish meal and corn gluten meal: origin manufactory EMRE-Türkiye. [b]Poultry by-product meal: origin manufactory SOLEVAL-France. [c]Wheat gluten meal and fish oil: origin manufactory KILIÇ-Türkiye. CTRL: control diet. 55PBM: 55%PBM + 45%FM; 65PBM: 65%PBM + 35%FM; 75PBM: 75%PBM + 25%FM; [d] Premix of vitamins and minerals according to NRC (2011) recommendations for fish. [e] NFE: nitrogen-free extracts. [f] ME: metabolizable energy.

Table 2. Amino acid profile of ingredients (fish meal and poultry by-product meal) and experimental diets (g 100g of protein^{-1}).

	Ingredients		Diets[1]			
Amino acids (AAs)	FM	PBM	CTRL	55PBM	65PBM	75PBM
Essential amino acids (EAAs)						
Arginine	6.48	6.70	7.72	7.5	7.46	7.42
Histidine	3.25	2.82	2.37	2.43	2.17	2.10
Isoleucine	4.24	4.14	6.03	3.60	3.51	3.35
Leucine	7.64	6.86	7.45	7.47	7.40	6.56
Lysine	9.26	6.55	6.76	6.31	6.10	5.12
Methionine	3.06	2.98	3.70	2.83	2.75	2.53
Phenylalanine	4.02	3.60	4.89	3.67	3.69	3.89
Tryptophan	0.89	0.50	0.91	0.56	0.41	0.39
Threonine	7.37	7.45	4.36	5.66	5.36	5.29
Valine	5.15	4.16	5.21	3.92	3.73	3.99
Non-essential amino acids (NEAAs)						
Alanine	4.52	3.50	5.2	5.68	5.71	5.95
Aspartic acid	11.26	10.62	8.64	9.03	9.15	8.98
Glycine	6.26	7.69	5.69	5.80	6.17	6.29
Glutamic acid	12.28	13.53	15.19	19.03	19.28	20.22
Serine	4.37	4.70	3.89	4.51	4.84	4.75
Cysteine	1.14	0.94	0.94	0.88	0.88	0.76
Tyrosine	2.70	3.86	3.38	2.73	2.88	2.83
Proline	4.03	6.82	6.00	5.82	5.91	6.42
∑EAAs	51.36	45.76	49.41	43.97	42.18	40.61
∑NEAAs	46.56	51.66	48.99	53.48	54.82	56.20
∑AAs	97.92	97.42	98.40	97.45	97.00	96.81

[1]FM: fish meal; PBM: CTRL: control diet; poultry by-product meal; 55PBM: 55%PBM + 45%FM; 65PBM: 65%PBM + 35%FM; 75PBM: 75%PBM + 25%FM.

Growth Trial

The Institutional Animal Care Committee at the MEDFRI provided prior approval for all procedures used in the present study under Protocol number 68385072-325.04-0967, encompassing a series of experiments. The present study reports results from one of these experiments. Juvenile gilthead seabream with an average weight of 35.21±4.11 g were randomly assigned to a flow-through system at 50 fish per tank. This system consisted of 12 cylindrical fibreglass tanks, each with a 500 L capacity. The system was supplied with continuously aerated filtered seawater at a rate of 7-8 L/min, a photoperiod of 12-hr light: 12-hr dark and an ambient temperature of 26.7±1.5°C. Fish were acclimatized to experimental conditions 2 weeks before feeding experimental diets. Each tank was assigned to one of the dietary treatments in triplicates and hand fed to visual satiation twice per day (9:30 a.m. and 5 p.m.) for 120 days.

Daily feed quantities given to fish were recorded, and individual fish weights were noted at the end of the feeding trial. Samples of diets and fish (5 fish per tank) were collected and stored at -80°C for proximate

composition and amino acid analyses. Growth performance was evaluated according to Ricker (1979), with parameters listed below;

Weight gain (%) = [(final body weight – initial body weight) / initial body weight] x 100

Specific growth rate (SGR) = [(ln final body weight – ln initial body weight) / days] x 100

Feed conversion ratio (FCR) = feed intake (g) / wet weight gain (g)

Feed efficiency ratio (FER) = wet weight gain (g) / feed intake (g)

Protein efficiency ratio (PER) = wet weight gain (g) / protein intake (g)

Hepatosomatic index (HSI) = (weight of liver/ weight of fish) x 100

Viscerosomatic index (VSI) = (weight of viscera/ weight of fish) x 100

Condition factor (K) = [(weight)/(total length)^3] x 100

Chemical Analyses

The proximate composition of diets, whole fish and faeces were analyzed according to standard procedures (AOAC, 2006) in triplicates. These analyses were conducted in the Fish Nutrition Laboratory of the Faculty of Aquatic Sciences of Istanbul University. Before analyses, all samples were homogenized and then stored at -20°C. The moisture content of the diet and whole-fish samples was determined by drying them in an oven at 105°C for 24 hours. Ash content was determined by incinerating samples in porcelain crucibles in a Muffle furnace at 500°C for 6 hours. Crude protein (CP) content (N x 6.25) was determined by the Kjeldahl method using a semi-automatic Kjeldahl system (Gerhardt Vapodest. 45s). Crude lipid (CL) from dried samples were extracted by diethyl ether using the Soxhlet method (Velp Scientifica Ser. 148). Nitrogen-free extract (NFE) was calculated by the formula: 100 - the values of the previous analyses (moisture + protein + fat + ash + fiber). Metabolizable energy (ME) was calculed by formula [(3.9 (%CP) + 8.0 (%CL) +1.6 (%NFE)] * 10 /1000 * 4.186 (Arthur and Phillips, 1972).

Amino-acids Analyses

The amino acids were analyzed in a private external laboratory. Analyses were performed using a high-sensitivity, high-speed triple quadrupole mass spectrometer method for the Liquid Chromatography Mass Spectrometer system (LCMS/8050). This method allowed a faster analysis of several components (ultra-fast speed) and a qualitative and quantitative analysis simultaneously. First, a 1 g sample (diet, whole fish, fish faeces) was weighed with a 0.0001g precision balance and poured into a Schott bottle. 10 ml of petroleum ether was added and vortexed for 2 minutes. Then the mixture was centrifuged for 3 minutes and the upper petroleum ether phase was pipetted into a 250 ml Schott bottle. This step was repeated once more. 25 ml of 6NHCl solution was added to the sample, placed into an oven preheated to 110°C, and left open for an hour. The bottles were then closed and sealed to continue hydrolysis for 23 hours. Subsequently, the bottles were removed from the oven, cooled to room temperature, and transferred into 200 ml bottles. Distilled water was then added to reach the 200 ml mark. The samples were filtered using a filter (45 microns). 15 ml of the liquid was transferred to a 15 ml falcon tube and diluted 1500 times before sending them to the LCMS-MS device (Jajic et al., 2012).

Apparent Digestibility

Fish faeces were collected by attaching faecal collecting devices to the outlet pipes of each tank. The faeces were collected 2 hours after feeding the fish. These faeces were then stored in plastic containers in a freezer at a temperature of -80°C for digestibility analyses. The method of Williams et. al. (1962) was used to determine the quantity of chromic oxide (inert marker) in the diet and faecal samples by atomic absorption spectrophotometry analysis. The level of apparent digestibility coefficients (ADCs) was calculated using the standard formula below:

100 [-100 ((%Cr_2O_3 in feed) / (% Cr_2O_3 in faeces)) x ((%amino-acids in faeces) / (% amino-acids in diets))]

Statistical Analysis

Data from the present study were reported as mean ± standard deviation of the mean. Growth performance data, diet and whole-body amino acid composition as well as apparent digestibility data were tested by one-way analysis of variance (ANOVA) to determine the dietary treatments' main effect. Differences between means were determined by Tukey's multiple comparison test. The results of this test were considered statistically significant at the p-value≤0.05. Percentage data were arcsine transformed before analyses. All statistical analyses were performed using the SPSS software package (version 24.0).

Results

Amino Acids Composition of Experimental Diets

The amino acid profiles of the ingredients FM and PBM, as well as the experimental diets, are presented in

Table 2. The essential amino acid (EAA) composition of FM was higher than that of PBM. The total amino acid content in the experimental diets ranged from 96.81 to 98 g/100 g of protein. Compared to the CTRL, the EAA values decreased as the concentration of PBM increased in the diet, . However, non-essential amino acids (NEAA) were significantly higher (P<0.05) in PBM-based diets (ranging from 53.48 to 56.20 g/100 g of protein) than in the CTRL (48.99 g/100 g of protein).

Growth Performance and Morphometric Indices

Table 3 reveals that survival rates were similar among the dietary groups (P>0.05) after 120 days of experimental feeding. Furthermore, final body weight and individual weight gain were similar in both CTRL and PBM fed fish (P>0.05), as were the final lengths and mean specific growth rates (P>0.05). Similary, HSI of the PBM feeding groups did not differ from those of the CTRL (P>0.05). VSI values were similar among PBM groups (P>0.05) and were lower than the CTRL (P<0.05). The lowest level of K was found in the CTRL. At the end of the study, 75PBM had highest FCR value (P<0.05), while CTRL and other experimental diets (55PBM and 65PBM) exhibited similar FCR (P>0.05). Finally, except 75PBM (P<0.05), PBM groups showed similar protein efficiency ratios with CTRL (P>0.05).

Whole Body Composition

The results of the body composition analysis at the beginning and end of the feeding trial are presented in Table 4. Overall, body compositions such as dry matter, crude lipid, and crude protein were respectively higher at the end compared to the beginning of the trial in juvenile gilthead seabream fed with either FM or PBM diets. The whole body ash level of fish showed a gradual decrease with increasing levels of PBM in the diets. By the end of the experiment, levels of crude fat in the liver of fish fed the CTRL and PBM-based diets were higher than at the beginning of the experiment. Specifically, the 65PBM and 75PBM group exhibited the highest level of hepatic fat (P<0.05).

Whole-body Amino Acid Profile

The amino acid profiles of the whole body of the experimental fish at the beginning and end of the trial are presented in Table 5. At the end of the trial, fish fed diet containing 75PBM showed slightly lower levels of total EAA compared to the CTRL and the other PBM groups (P<0.05). In contrast, the NEAA profiles of fish fed 75PBM diet was relatively higher than those of the CTRL group (P<0.05).

Amino Acid Profile in Faeces

The data presented in Table 6 summarizes the concentrations of the 9 EAAs and the 8 NEAAs in the feces of juvenile gilthead seabream. According to these results, it was observed that the amino acid profiles in the feces of the experimental fish reflected the amounts of amino acids eliminated by the fish. The quantity of EAAs excreted in the feces of groups fed with PBM-

Table 3. Growth performance of gilthead seabream fed the experimental diets.

Growth performance	CTRL	55PBM	65PBM	75PBM
IBW (g fish^{-1})	35.10±0.10	34.94±0.04	34.95±0.05	35.04±0.22
FBW (g fish^{-1})	140.78±0.45	139.30±0.92	135.20±0.29	135.46±0.50
Weight gain (g fish^{-1})	105.68±0.44	104.36±0.32	100.25±0.96	100.42±0.38
Feed intake	117.30±0.39^{a}	116.88±0.35^{a}	111.28±0.32^{b}	123.52±0.40^{a}
Survival (%)	100	100	100	100
TL (cm)	18.82±0.28	18.73±0.55	18.62±0.98	18.11±0.56
SGR (%day^{-1})	1.16±0.01	1.13±0.01	1.15±0.01	1.13±0.01
FCR	1.11±0.01^{b}	1.12±0.02^{b}	1.11±0.01^{b}	1.23±0.04^{a}
PER	1.84±0.02^{a}	1.83±0.02^{a}	1.88±0.02^{a}	1.70±0.01^{b}
HSI (%)	1.08±0.23	0.95±0.20	0.95±0.20	1.04±0.24
VSI (%)	7.35±1.17^{a}	6.96±1.22^{b}	6.92±1.32^{b}	7.05±1.18^{b}
K	1.60±0.14^{b}	1.70±0.16^{a}	1.72±0.10^{a}	1.74±0.98^{a}

Data are presented as means ± S. D. (n = 3). Different superscript letters within a row denote significant differences among diets as determined by one-way ANOVA using Tukey's comparison test (P < .05). CTRL: control diet; PBM: poultry by-product meal; 55PBM: 55%PBM + 45%FM; 65PBM: 65%PBM + 35%FM; 75PBM: 75%PBM + 25%FM. IBW: initial body weight; FBW: final body weight; TL: total length; SGR: specific growth rate; FCR: feed conversion ratio; PER: protein efficiency ratio; HSI: hepatosomatic index; VSI: viscerosomatic index; K: condition factor.

Table 4. Whole body proximate composition (%) of juvenile gilthead seabream.

Body composition	Initial	CTRL	55PBM	65PBM	75PBM
Dry matter	33.06±0.04^{b}	34.89±0.52^{a}	33.40±0.04^{ab}	33.45±0.2^{ab}	34.75±0.14^{a}
Crude protein	15.04±0.24^{b}	16.14±0.19^{a}	16.19±0.02^{a}	16.09±0.70^{a}	16.05±0.19^{a}
Crude fat	10.86±0.05^{c}	12.40±0.09^{b}	12.25±0.96^{b}	12.87±0.52^{a}	12.97±1.09^{a}
Ash	3.81±0.03^{a}	3.89±0.03^{a}	3.81±0.05^{a}	3.71±0.04^{ab}	3.24±0.09^{b}
Liver fat	11.77±0.53^{c}	15.38±0.34^{b}	15.29±0.57^{b}	16.57±0.23^{a}	16.44±2.47^{a}

Data are presented as means ± S. D. (n = 3). Different superscript letters within a row denote significant differences among diets as determined by one-way ANOVA using Tukey's comparison test (P < .05). CTRL: control diet; PBM: poultry by-product meal; 55PBM: 55%PBM + 45%FM; 65PBM: 65%PBM + 35%FM; 75PBM: 75%PBM + 25%FM.

based diets was similar to that of the control diet. Similarly, these groups of fish exhibited negligible losses in NEAAs, which were comparable to those of the control group (P<0.05).

Apparent Digestibility Coefficients (%) of Amino Acids in Diets

Table 7 presents the apparent digestibility coefficient (ADC) of each experimental diet. According to these results, EAA and NEAA digestibility in fish fed with CTRL diet ranged from 86% to 92% and 82% to 93%, respectively. Similar results were obtained in the groups fed with PBM-based diets (P>0.05).

Discussion

The present study examined the effects of incorporating PBM at concentrations of 55%, 65%, and 75% in the diet of *Sparus aurata* juveniles on growth performance and amino acid metabolism. In this study, replacing up to 65% of FM with PBM did not negatively affect growth performance, while fish fed the 75% (75PBM) showedsligthly reduced growth performance. The reason for this is likely due to the EAA composition of PBM, which was similar to that of the control group (Table 2). Yigit et al. (2006) reported the essential amino acid ratios of fish meal and PBM, and reported that the EAA levels in PBM were lower than those in fish meal. Contrariwise, the EAA levels in FM and PBM were found to be similar in our study as stated above. This may be an indication that the quality of PBM used in the present study was higher than that used by Yigit et al. (2006). Yildiz et al. (2006) emphasized the importance of balancing energy and proteins in the diet of *Sparus aurata* to prevent protein degradation into energy. Nengas et al. (1999) reported that replacing 50% of FM with PBM had no negative effect on the growth performance of *Sparus aurata*, although diets containing 75% PBM produced low growth performance. Hekmatpour et al. (2018) reported that PBM could replace up to 55% of fish meal proteins in diets formulated for Sparidentex juveniles without negatively affecting growth performance. Fontinha et al. (2021) suggested that partial replacement (37.5%) of fish meal with poultry by-product meal does not negatively affect the growth performance of *Sparus aurata*. Karapanagiotidis et al. (2019) reported that PBM can successfully replace up to 50% of FM in the diet of *Sparus aurata* without adverse effects on survival, feed intake, and growth performance, although total replacement resulted in reduced growth performance. However, Sabbagh et al. (2019) reported that total replacement of FM with PBM in the diet of gilthead seabream was possible with supplementation with lysine and methionine. Yigit et al. (2006) reported that the growth of turbot (*Psetta maeotica*) fed diets containing 25, 50, and 75% PBM instead of fish meal decreased gradually despite the increasing PBM in the diets. These results indicate that turbot couldn't utulise PBM effectively.

Table 5. Whole-body amino acid profile of fish (g/100 g protein)

		Diets			
Amino acids (AAs)	Initial	CTRL	55PBM	65PBM	75PBM
Essential amino acids (EAAs)					
Arginine	5.56±0.52^{b}	5.78±0.16^{b}	5.75±0.12^{b}	6.04±0.46^{a}	5.11±0.83^{c}
Histidine	2.75±0.10^{b}	3.09±0.13^{a}	3.06±0.06^{a}	3.07±0.18^{a}	2.72±0.33^{b}
Isoleucine	3.99±0.70^{b}	5.27±0.45^{a}	5.08±0.42^{a}	4.89±0.41^{a}	5.11±0.33^{a}
Leucine	8.51±0.07^{b}	9.77±0.36^{a}	9.73±0.18^{a}	9.45±0.07^{a}	9.22±0.49^{ab}
Lysine	7.27±0.10^{b}	8.37±0.04^{a}	8.33±0.13^{a}	8.63±0.36^{a}	7.82±0.68^{ab}
Methionine	2.33±0.20^{b}	2.46±0.08^{ab}	2.52±0.16^{a}	2.60±0.09^{a}	2.39±0.33^{b}
Phenylalanine	3.72±0.14^{b}	4.25±0.13^{a}	4.28±0.08^{a}	4.21±0.13^{a}	4.00±0.14^{a}
Tryptophan	4.67±0.28^{b}	4.93±0.24^{a}	4.83±0.44^{ab}	5.05±0.44^{a}	4.39±0.81^{c}
Threonine	4.63±0.38^{b}	5.34±0.13^{a}	5.34±0.15^{a}	5.26±0.29^{a}	5.06±0.43^{ab}
Non-essential amino acids (NEAAs)					
Alanine	6.85±0.65^{a}	6.80±0.77^{a}	6.70±0.74^{a}	6.30±0.67^{b}	6.66±0.46^{a}
Aspartic acid	9.17±0.33^{ab}	9.20±0.73^{ab}	9.40±0.52^{a}	9.00±0.83^{b}	9.57±0.89^{a}
Glycine	1.29±0.21^{a}	0.61±0.31^{c}	0.61±0.28^{c}	0.84±0.31^{b}	0.61±0.29^{c}
Glutamic acid	14.21±0.33^{b}	13.99±0.27^{bc}	13.81±0.12^{bc}	13.67±0.33^{c}	15.00±0.03^{a}
Serine	7.74±0.10^{a}	6.58±0.13^{c}	7.02±0.38^{b}	7.07±0.69^{b}	6.73±0.24^{c}
Cysteine	4.32±0.20^{a}	4.22±0.38^{a}	4.22±0.23^{a}	4.04±0.12^{b}	4.15±0.30^{ab}
Tyrosine	4.99±0.18^{a}	3.35±1.05^{c}	3.41±1.11^{c}	3.89±0.98^{b}	3.48±1.22^{c}
Proline	3.57±0.33^{a}	3.30±0.18^{ab}	3.30±0.19^{ab}	3.13±0.09^{b}	3.43±0.10^{a}
∑EAAs	43.22±0.31^{c}	49.26±0.76^{a}	48.92±0.05^{a}	49.20±0.07^{a}	45.82±3.84^{b}
∑NEAAs	52.13±1.36^{a}	48.04±0.85^{b}	48.47±0.04^{b}	47.94±0.02^{b}	49.64±0.03^{ab}
∑AAs	95.34±1.63^{b}	97.30±1.60^{a}	97.39±0.09^{a}	97.13±0.06^{a}	95.46±3.85^{b}

Values are presented as means ± S. D. (n = 3). Different superscript letters within a row denote significant differences among diets as determined by one-way ANOVA using Tukey's comparison test (P< .05). CTRL: control diet; PBM: poultry by-product meal; 55PBM: 55%PBM + 45%FM; 65PBM: 65%PBM + 35%FM; 75PBM: 75%PBM + 25%FM.

In the present study, the partial replacement of FM with PBM did not affect the survival rate of fish. Similar results were obtained by Hekmatpour et al. (2018) and Fontinha et al. (2021). Palatability is a significant concern when fish meal is replaced by an alternative ingredient in fish feed, as poor palatability leads to a decrease in voluntary feed intake, thereby negatively affecting fish growth (Gomez-Requeni at al., 2003; Hu at al., 2013; Hill at al., 2018; Fontinha at al., 2021). According to Oliva-Teles et al. (2015), animal by-products, including PBM, are highly palatable to fish. In the present study, feed intake was similar among the dietary groups. This could mean that diets containing up to 75% PBM were as palatable as the CTRL diet. Evaluation of diet quality, represented by feed intake (FI), feed conversion ratio (FCR), feed efficiency (FE), and protein efficiency ratio (PER), showed that young gilthead seabream effectively converted feed with high levels of PBM (55PBM, 65PBM and 75PBM) into somatic tissues. The results of the protein efficiency factor (PEF)

Table 6. Amino acid profile in faeces (g/100 g protein) [1].

	Diets			
Amino acids (AAs)	CTRL	55PBM	65PBM	75PBM
Essential amino acids (EAAs)				
Arginine	0.83±0.05^{b}	0.85±0.04^{b}	0.94±0.03^{a}	0.97±0.08^{a}
Histidine	0.46±0.05^{a}	0.48±0.05^{a}	0.38±0.03^{ab}	0.24±0.03^{b}
Isoleucine	0.72±0.09	0.75±0.01	0.69±0.04	0.69±0.14
Leucine	0.93±0.05^{b}	1.03±0.09^{ab}	1.02±0.03^{ab}	1.16±0.02^{a}
Lysine	0.82±0.09^{a}	0.85±0.04^{a}	0.76±0.06^{ab}	0.61±0.06^{b}
Methionine	0.49±0.04^{a}	0.52±0.01^{a}	0.40±0.01^{b}	0.24±0.06^{c}
Phenylalanine	0.96±0.02	1.04±0.01	0.91±0.08	1.08±0.06
Tryptophan	0.79±0.10^{b}	0.43±0.02^{c}	0.84±0.03^{ab}	0.97±0.07^{a}
Threonine	0.84±0.08^{ab}	1.01±0.23^{a}	0.77±0.04^{b}	0.98±0.07^{a}
Non-essential amino acids (NEAAs)				
Alanine	1.23±0.02^{ab}	1.11±0.06^{b}	1.23±0.05^{ab}	1.30±0.04^{a}
Aspartic acid	2.13±0.02^{b}	2.35±0.01^{a}	2.05±0.07^{b}	2.07±0.08^{b}
Glycine	0.19±0.04^{ab}	0.17±0.01^{b}	0.19±0.00^{ab}	0.24±0.01^{a}
Glutamic acid	2.80±0.08^{a}	2.24±0.02^{c}	2.70±0.01^{a}	2.49±0.34^{b}
Serine	0.89±0.02^{b}	0.98±0.16^{a}	0.95±0.06^{ab}	0.94±0.06^{ab}
Cysteine	0.80±0.05^{b}	0.93±0.03^{a}	0.78±0.06^{b}	0.86±0.08^{ab}
Tyrosine	0.79±0.18^{b}	0.86±0.02^{a}	0.73±0.05^{b}	0.85±0.08^{a}
Proline	0.55±0.03^{b}	0.72±0.03^{a}	0.56±0.03^{b}	0.57±0.01^{b}
∑EAAs	6.84±0.06	6.96±0.03	6.83±0.03	6.95±0.08
∑NEAAs	9.37±0.14^{a}	9.35±0.20^{a}	9.19±0.02^{b}	9.31±0.19^{a}
∑AAs	16.20±0.20^{ab}	16.31±0.10^{a}	15.92±0.01^{b}	16.27±0.27^{a}

Values are presented as means ± S. D. (n = 3). Different superscript letters within a row denote significant differences among diets as determined by one-way ANOVA using Tukey's comparison test (P < .05). CTRL: control diet; PBM: poultry by-product meal; 55PBM: 55%PBM + 45%FM; 65PBM: 65%PBM + 35%FM; 75PBM: 75%PBM + 25%FM.

Table 7. Apparent digestibility coefficients (%) of protein and amino acids in diets.

	Diets			
	CTRL	55PBM	65PBM	75PBM
Crude protein	75.38±0.68^{a}	74.25±0.15^{a}	74.93±0.54^{a}	72.15±0.78^{b}
Essential amino acids (EAAs)				
Arginine	92.34±0.10	92.03±0.49	91.48±0.22	92.06±0.86
Histidine	86.04±1.30^{b}	85.80±3.67^{b}	88.10±0.46^{a}	83.03±0.85^{c}
Isoleucine	91.29±1.98^{a}	85.24±1.07^{c}	86.71±1.32^{bc}	87.51±1.44^{b}
Leucine	90.77±2.76^{a}	90.23±0.74^{a}	90.68±0.33^{a}	89.10±1.87^{b}
Lysine	90.94±3.25	90.49±0.69	91.65±0.36	90.79±0.55
Methionine	90.59±1.00^{a}	86.89±0.62^{b}	89.84±2.45^{a}	90.11±1.85^{a}
Phenylalanine	85.77±1.94^{a}	79.95±0.54^{b}	83.35±1.49^{ab}	83.24±1.22^{ab}
Tryptophan	86.91±2.14^{b}	94.68±0.38^{a}	89.46±0.76^{ab}	88.91±1.03^{ab}
Threonine	88.51±0.28^{a}	81.83±3.73^{c}	86.10±0.34^{b}	85.09±0.90^{b}
Non-essential amino acids (NEAAs)				
Alanine	83.23±0.20^{b}	86.22±0.53^{a}	85.45±0.07^{a}	86.78±0.32^{a}
Aspartic acid	82.36±0.01^{b}	81.62±0.10^{b}	84.91±0.57^{a}	86.09±0.43^{a}
Glycine	85.59±2.34^{a}	86.17±0.32^{a}	85.44±0.04^{a}	80.36±2.26^{b}
Glutamic acid	86.81±0.01^{b}	91.64±0.35^{a}	90.56±0.11^{a}	92.56±1.24^{a}
Serine	92.27±5.21^{a}	88.09±2.17^{c}	89.65±0.44^{b}	90.96±0.53^{b}
Cysteine	85.35±0.53^{b}	85.43±1.11^{b}	89.12±1.01^{a}	89.08±0.69^{a}
Tyrosine	83.41±2.18^{a}	77.66±0.57^{c}	83.04±0.66^{a}	81.91±2.34^{b}
Proline	93.49±0.53^{a}	91.27±0.18^{b}	93.60±0.38^{a}	94.59±0.09^{a}

Values are presented as means ± S. D. (n = 3). Different superscript letters within a row denote significant differences among diets as determined by one-way ANOVA using Tukey's comparison test (P < .05). CTRL: control diet; PBM: poultry by-product meal; 55PBM: 55%PBM + 45%FM; 65PBM: 65%PBM + 35%FM; 75PBM: 75%PBM

in the present study confirmed the excellent potential for gilthead seabream in utilizing PBM proteins up to 65%, as the FCR and PER were similar to those of the CTRL group. It was observed that the 75PBM group was a usable diet for juvenile sea bream, with FCR and PER slightly lower than those of the CTRL and the other PBM groups. Yıldırım et al. (2009) reported that no negative effects were observed on the FCR level of *Tilapia zilli* fingerlings (2.45 mean weight) fed diets containing 50% PBM instead of fish meal, but FCR increased significantly when the PBM substitution rate was increased to 100%.

The hepatosomatic index of fish showed no relationship with increased levels of PBM in the diet in the present study. In contrast, VSI values in PBM groups were lower than the CTRL group. However, K values in PBM groups were higher than the CTRL group. Fontinha et al. (2021) and Sabbagh et al. (2019) reported that HSI, VSI, and K in gilthead seabream were unaffected by the dietary content of PBM, and were similar to those found in fish fed FM diet diet. We think that the main reason why the VSI and K values in our study are different from Sabbagh et al. (2019) is that the fish we used in the present study were bigger than the fish in the mentioned studies.

The level of fish ash content in the whole body was lowest in 75PBM group. This observation likely indicates an increased availability of minerals such as calcium and phosphorus in FM compared to those derived from PBM-based meals. Nengas et al. (1999) reported that the inclusion of PBM in diets of juvenile sea bream had no significant effect on whole body ash content. On the other hand, Karapanagiotidis et al. (2019) and Fontinha et al. (2021) reported that whole body ash levels increased with increasing levels of PBM in the diets of juvenile sea bream, attributing this increase to the calcium and phosphorus levels in PBM. Compared to the CTRL group, whole body ash content was reduced in fish fed PBM-based diets in spite of the increasing levels of PBM in the diets. It is seen that the differences between calcium and phosphorus content in fish meal (Anderson et al., 1997) and PBM (Pesti et al., 1986) are not significant. We are of the view that the differences in ash content between the groups in our study is related to the digestibility of the diets by the fish (Table 7).

According to the literature, various researchers (Sabaut and Luquert, 1973; Vergara and Jancey, 1993; Santinha at al., 1996; Vergara at al., 1996a.b; Santinha at al., 1999) advocate for a high protein content in the diet of farmed gilthead seabream, a species known for its carnivorous nature. Thus, for optimal growth and development, farmed gilthead seabream requires a dietary composition containing 40 to 55% protein (Sabaut and Luquert, 1973; Santinha at al., 1996; Vergara at al., 1996a.b) and 15 to 22% lipids (Vergara and Jancey, 1993; Santinha at al., 1999). The formulated diets used for gilthead seabream juveniles in the present study contained 47.44% protein and 19.25% lipids, falling within the recommended range of protein and lipid content for farmed *Sparus aurata* juveniles as stipulated in the NRC (2011). PBM does not contain n-3 highly unsaturated fatty acids (HUFA) such as EPA and DHA that sea bream fish need. The fatty acids in PBM oil are mostly of 18:1n-9 (oleic acid, approximately 35%), C16:0 (palmitic acid, approximately 23%) and C18:2n-6 (linoleic acid, approximately 22%). Of the n-3 polyunsaturated fatty acids (PUFA) that sea bream can use, only C18:3n-3 (linolenic acid, approximately 1.45%) is found in low level in PBM (Peña-Saldarriaga et al., 2020). Juvenile sea bream actively use EPA and DHA, which are among the n-3 HUFAs found high in amounts in fish oils, for their normal growth and development. Fish store the dietary lipids in their body and in the liver that they cannot utilise (Wassef et al., 2015; Ofori-Mensah et al., 2020). Therefore, in this study, whole-body and hepatic lipid levels in fish showed a progressive increase with increasing substitution of PBM.

The results of the analysis of the whole-body amino acid composition of juvenile gilthead seabream indicated a slight decrease in levels of essential amino acids (EAAs), particularly arginine, lysine, and tryptophan, in fish fed 75PBM ($P<0.05$). These findings are consistent with those of Hekmatpour et al. (2018) and Sabbagh et al. (2019). Additionally, Hekmatpour et al. (2018) noted that EAAs levels in the whole fish body are naturally lower than those in fillets, as the whole body encompasses all parts of the fish, including skin, bones, gills, and head, while the fillet contains only pure fish flesh.

The results of the present study reveal a significantly higher retention of essential amino acids (EAAs) and non-essential amino acids (NEAAs) in the proteins provided in the experimental diets compared to the CTRL diet (Table 6). These findings suggest that dietary supplementation with PBM up to 65% could enhance the digestibility of dietary proteins in juvenile gilthead seabream. Although slightly reduced digestibility was recorded in fish fed 75PBM diet, this diet can be recommended for juvenile sea bream fish. Hernández et al. (2014) suggested that, PBM could potentially replace up to 50% of FM without altering fish performance, while higher substitutions might affect nutrient digestibility. Conversely, the results of our study demonstrate a positive correlation between dietary levels of EAAs and NEAAs and the protein ADC of experimental diets containing FM and those containing up to 75% PBM. The low estimates of protein digestibility in PBM diets observed in the present study and previous experiments are likely related to the methods used for faecal collection. In the present study, faeces were collected in a small reservoir connected to the tank two hours after feeding. This method, as well as the method of fecal collection by pipetting from the bottom of the tank are often associated with the disintegration/separation of fecal matter and the leaching of nutrients, leading to lower digestibility estimates. Austreng (1978), Windell et al. (1978), and Henken et al. (1985) demonstrated that techniques

preventing nutrient leaching involve removing the fish from the water and collecting fecal samples directly from the distal intestinal region, for example, by dissection. Dietary intake was virtually unaffected by dietary treatments, suggesting a major role of the digestion-absorption process in growth impact. These findings could be attributed to improved nutrient absorption or beneficial effects on fish gastrointestinal microflora (Merrifield et al., 2010). The higher nutrient digestibility and consequently increased availability of nutrients and amino acids in groups fed with PBM likely contributed to improved growth performance.

In conclusion, our study has highlighted the potential for improvement in growth, feed utilization, flesh quality and nutritional apparent digestibility coefficient (ADC) using PBM as an alternative protein source in juvenile gilthead seabream. Our results showed that 65% PBM substitution for FM can be included in the diets of sea bream without any negative effects on growth, flesh quality, FCR and protein digestibility. However, we can suggest that 75% PBM substitution for FM can be used in the diets of gilthead seabream, although it causes a slight decrease in growth, FCR and protein digestibility. Overall, high-quality poultry by-product meal appears to be a promising option as a viable substitute for fish meal in the diet of juvenile gilthead seabream, due to its digestibility and favorable EAA profile. In this context, proteins derived from poultry by-products emerge as valuable ingredients offering significant nutritional benefits for intensive fish-based diets. Moreover, their use may have a positive impact on environmental sustainability by promoting the utilization of by-products and reducing dependence on fish meal in aquaculture feed production.

Ethical Statement

Not applicable.

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Author Contribution

All authors contributed to the design and conception of this study. Material preparation. methodology data collection and analysis were performed by Naoual Damir, Mustafa Yıldız, Samuel Ofori-Mensah and Isa Aydın. The first draft of the manuscript was written by Naoual Damir and all authors provided comments on previous versions of the manuscript, Mustafa Yıldız: Supervision. Conceptualization, funding acquisition, investigation, writing - review and editing this study. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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