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EFFECTS OF USING PEA PROTEIN AND WHEAT GLUTEN INSTEAD OF FISH MEAL ON GROWTH PERFORMANCE AND FEED UTILIZATION OF EUROPEAN SEA BASS (*Dicentrarchus labrax*)

ABSTRACT

This study evaluated the effects of replacing fishmeal with increasing levels of pea protein and wheat gluten in European sea bass diets over a 20-week feeding trial. Experimental diets contained 45% crude protein and 20% crude lipid. Pea protein (7%–28.3%) and wheat gluten (6.5%–26.2%) were tested at four inclusion levels each. Among pea protein treatments, PEA7 and PEA14 showed the highest survival, whereas CON generally exhibited the best growth performance. Similarly, WG13 and WG19 had the highest survival, while CON showed the highest specific growth rate (SGR), weight gain, and carcass yield. Increasing plant protein inclusion generally impaired growth performance and altered body composition. Whole-body moisture, protein, and ash contents were highest in CON, while lipid content peaked in PEA28 and WG26. Liver composition varied among treatments. Overall, increasing fishmeal replacement with pea protein and wheat gluten negatively affected growth performance and nutrient composition in European sea bass.

Keywords: Alternative Protein Sources, *Dicentrarchus labrax*, Pea Protein, Plant Protein, Wheat Gluten

1. INTRODUCTION

The aquaculture sector is one of the fastest-growing food production sectors worldwide [1]. This growth is largely driven by the increasing demand for seafood and its growing popularity as a nutritious food source [2]. The cultivation of fish constitutes a major segment of the aquaculture sector. The primary objective of fish production is to achieve market size in the shortest possible time while maintaining optimal production efficiency [3]. To ensure survival, growth, reproduction, and resistance to diseases and environmental stressors, cultured fish must be provided with nutritionally balanced, high-quality feeds. Adequate nutrition not only improves fish performance but also enhances the economic viability and sustainability of aquaculture operations [4]. Therefore, the main objective of feed manufacturing is to produce highly digestible, nutritionally balanced, and cost-effective feeds. To achieve this goal, the feed industry continuously seeks to utilize a wide range of feed ingredients in the most economical manner while maximizing production efficiency and feed quality [5]. Feed conversion ratio (FCR), which represents the amount of feed required to produce one kilogram of fish biomass, is one of the most important indicators used to evaluate feed efficiency and production performance in aquaculture [6].

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In aquaculture, feed is the most important production input, accounting for approximately 40–60% of total production costs. Consequently, the feed sector is a key contributor to the sustainability and economic success of aquaculture. To meet the increasing demand of this rapidly growing sector, feed manufacturing plants began producing compound feeds in the 1980s. Initially, production was limited to powdered and pelleted feeds; however, with the adoption of technological advancements in feed processing, the industry gradually transitioned to the production of extruded fish feeds [7].

The increasing production capacity of aquaculture enterprises, together with the growing demand for feed, has contributed directly to the development of the aquaculture feed industry [8]. Fish feeds are formulated using a variety of ingredients, including fish meal, fish oil, soybean products, hydrolyzed fish protein, wheat and wheat by-products, corn gluten, yeast and yeast derivatives, lecithin, amino acids, vitamins, and minerals [9]. Among these ingredients, fish meal and fish oil remain the major components used within aquaculture diets. However, the high cost and limited availability of these raw materials significantly affect feed production costs and, consequently, the market price of aquafeeds [10].

Depending on the fish species and stage of development, fish meal is typically included in aquafeeds at levels ranging from 40% to 60% [11]. Fish growth and performance are closely associated with feed digestibility. Fish meal is considered a valuable feed ingredient due to its high protein quality, excellent digestibility, and balanced amino acid profile [12 and 13]. Feed Conversion Ratio (FCR) is one of the most widely used indicators for evaluating feed efficiency in aquaculture and is commonly employed by both researchers and producers [14]. Although several factors, including fish species and water temperature, influence FCR, feed quality particularly ingredient composition and digestibility is among the most important determinants of feed utilization efficiency [15]. With the rapid expansion of aquaculture, the demand for aquafeeds has increased steadily, resulting in a greater demand for fishmeal [16]. At the same time, declines in fish catches caused by various environmental and ecological factors have led to substantial increases in fishmeal prices [17, 18 and 19]. Fishmeal is an animal-derived protein source widely used in aquafeed formulations [20]. It is generally produced from small pelagic fish that are not commonly used for direct human consumption or from by-products generated by seafood processing industries [21]. Fishmeal is highly valued because it contains high levels of protein, essential amino acids, minerals, phospholipids, and fatty acids, making it one of the most important ingredients in aquafeed production [16].

Fishmeal is produced from various fish species, and this varies from country to country. Peru obtains fishmeal from anchovies (*Engraulis spp.*), Chile from anchovies (*Engraulis spp.*) and horse mackerel (Trachurus spp.), Norway and Iceland from herring (*Clupea harengus*) and capelin (*Mallotus villosus*), Japan and South Africa from sardines (*Sardina pilchardus*), and Canada from herring (*Clupea harengus*)[22]. The rapid developments in the aquaculture sector have also led to the development of the fish feed industry. In recent years, the supply of fishmeal has gradually declined, while its cost has continued to rise. The investigation of alternative plant-based sources instead of fishmeal is of great importance [23 and 24]. Marine fish farming represents an important segment of the aquaculture industry and continues to increase its share of global aquaculture production [25]. Among marine fish species, the European sea bass (*Dicentrarchus labrax* Linnaeus, 1758) is one of the most widely cultured species. Its high economic value, favorable flesh quality, strong consumer demand, and well-established

hatchery production techniques have made it an increasingly important species in marine aquaculture [26 and 27]. Worldwide, European sea bass are predominantly reared using commercially formulated extruded or pelleted feeds. Increasing proportions of pea protein and wheat gluten were incorporated into diets as substitutes for fish meal, and their effects on the growth performance and feed utilization efficiency of European sea bass (*Dicentrarchus labrax*) were subsequently evaluated. The findings of this study may support the development of more sustainable and cost-effective aquafeed formulations.

2. RESEARCH SIGNIFICANCE

This study provides valuable insights into the potential use of pea protein and wheat gluten as alternative protein sources to fishmeal in European sea bass diets. By evaluating different inclusion levels, it identifies their effects on growth performance, feed utilization, survival, and body composition. The findings contribute to understanding the limitations of plant-based protein replacement and provide useful information for developing nutritionally balanced and sustainable aquafeeds while maintaining fish growth and production efficiency.

Highlights

- Fishmeal replacement with pea protein and wheat gluten was evaluated in sea bass.
- Diets were tested over 20 weeks with graded inclusion levels of both proteins.
- Growth, feed utilization, and survival varied depending on inclusion level.
- High substitution levels generally reduced growth performance and nutrient quality.

3. MATERIALS AND METHODS

A 20-week feeding experiment was undertaken at the Experimental Research Unit of İzmir Katip Çelebi University. Two polyester tanks, each with a volume of 5m³ and a diameter of 2m, were used in the experiment. Nine net cages (50×50×50cm; 125 L volume; 10mm mesh size) were placed in each tank. Each experimental treatment was conducted in duplicate.

3.1. Preparation of Experimental Diets

The feed ingredients used in the experimental diets were supplied by Agromey Gıda ve Yem A.Ş. and Farin Kimya İnşaat Sanayi ve Tic. A.Ş. Proximate composition analyses of the ingredients were performed at the laboratories of the Faculty (Table 1). All test diets contained 45% crude protein and 20% crude lipid. Four diets containing different levels of pea protein (PEA) and wheat gluten (WG) were prepared. In addition, a control diet (CON) without pea protein or wheat gluten supplementation was formulated (Table 2).

Table 1. Nutritional characteristics of the feed ıngredients (%) İncorporated ınto the experimental diets

Ingredients	Proximate Analysis (%)			
	Moisture	Protein	Lipid	Ash
Moroccan Fish Meal	8.8	63.1	12.6	17.3
Wheat Gluten	7.7	81.4	2.6	0.6
Pea Protein	8.6	75.4	6.2	8.3
Feather Meal	7.4	86.2	2.7	1.9
Chicken Meal	9.6	71.3	10.6	7.3
Soybean Meal	11.2	49.8	1.7	7.7
Corn Gluten	10.5	67.2	1.3	6.2
Bakery Waste Meal	11.2	20.4	4.1	2.9

Table 2. Ingredient proportions used in the experimental diets (g/kg)

Ingredients	Experimental Diets (g/kg)								
	CON	PEA 7	PEA 14	PEA 21	PEA 28	WG 6	WG 13	WG 19	WG 26
Fish meal	310	232	155	78	0	232	155	78	0
Methionine	0	1	2	3	4	1	2	2	3
Lysine	0	0	0	0	0	1	3	5	8
Pea protein **	0	70	141	211	283	0	0	0	0
Wheat gluten **	0	0	0	0	0	65	130	196	262
Corn gluten	110	110	110	110	110	110	110	110	110
Feather Meal	20	20	20	20	20	20	20	20	20
Chicken Mea	100	100	100	100	100	100	100	100	100
Soybean Meal	170	170	170	170	170	170	170	170	170
Bakery Waste Mea	145	145	142	140	137	151	154	158	160
Salmon Oil	142	149	157	165	173	147	153	158	164
Mineral Premix	1	1	1	1	1	1	1	1	1
Vitamin Premix	2	2	2	2	2	2	2	2	2
TOTAL	1000	1000	1000	1000	1000	1000	1000	1000	1000

* Supplied by Agromey Food and Feed Inc.
** Supplied by Farin Chemistry Construction Industry and Trade Inc.

3.2. Nutritional Composition of the Formulated Diets

The moisture, crude protein, crude lipid, and crude ash contents determined for each formulated diet are summarized in Table 3.

Table 3. Nutritional composition of experimental feeds (%)

Proximate Analysis (%)	Experimental Diets								
	CON	PEA 7	PEA 14	PEA 21	PEA 28	WG 6	WG 13	WG 19	WG 26
Moisture	2.5	2.9	3.5	4.0	4.5	3.1	4.2	3.5	4.0
Protein	45.7	46.1	45.7	46.0	46.0	45.5	45.4	46.0	46.0
Lipid	19.5	19.4	19.7	19.9	19.0	19.0	18.9	18.8	18.5
Ash	8.5	7.5	6.7	5.5	4.6	7.1	6.2	5.0	4.0

To prepare diets containing 45% crude protein and 20% crude lipid, all feed ingredients were first ground to a particle size of 0.5 mm and then weighed according to their designated inclusion levels. A 10-min mixing step was first applied to the dry ingredients. Fish oil was then incorporated into the mixture, followed by an additional 5 min of mixing to ensure uniform distribution. Water was gradually added until a dough-like texture was achieved. The prepared dough was processed through a pellet mill, resulting in pellets with a diameter of 3 mm. Freshly produced diets were placed in a forced-convection oven and dried at 40 °C for a period of 24 h. Following the drying process, the pellets were sieved, packaged and frozen at −20 °C for subsequent use.

3.3. Experimental Fish

In this study, 270 European sea bass (*Dicentrarchus labrax* Linnaeus, 1758) with an average initial body weight of 86.48±3.14 g were stocked, with 15 fish per net cage. During the experimental period of 20 weeks, feeding was carried out twice daily, from 09:00 to 09:30 and from 16:00 to 16:30 until apparent satiation. Weighing was conducted at 4-week intervals in the morning prior to feeding. Prior to weighing, the fish were exposed to 2-phenoxyethanol (0.5 mL L^{-1}) to induce anesthesia. At each sampling, the positions of the cages within the tanks were randomly rearranged to minimize positional effects. The tanks were siphoned daily to remove feces and waste materials.

3.4. Water Parameters

Water quality parameters in the system were measured using a Hach multiparameter meter. Throughout the 141-day feeding trial, daily measurements of water quality was maintained at 20–24 °C for temperature, 8–9 mg L^{-1} for dissolved oxygen, and a pH of 7.5–8.0

3.5. Growth Performance and Somatic Indices

Specific growth rate (SGR) (% day ^{1})=100*(ln FBW- ln IBW) / days (where FBW represent the final body weight and IBW and the initial body weights) [28].

Weight gain (WG) (%)= (FBW (g) – IBW (g)) / IBW (g) × 100 [28].

Feed conversion ratio (FCR)=feed intake / weight gain (g) [29].

Survival rate (SR) (%)= (Final Number of Fish / Initial Number of Fish) × 100 [30].

Protein efficiency ratio (PER)= (FBW – IBW) / protein intake [29].

VSI=Viscerosomatic index (%)=100*(viscera weight/FBW) [31].

HSI=Hepatosomatic index (%) HSI=100*(liver weight/FBW) [31]

CF=condition factor=100 ×(body weight, g)/(body length, cm) [32]

CY= Carcass yield(%)= (eviscerated fish weight / Body weight) × 100 [33].

3.6. Proximate Chemical Composition

Analyses were conducted on the formulated diets as well as whole-body samples collected from the fish. The samples underwent drying at 105°C until no further change in weight was observed before moisture content was determined. Crude protein content was calculated from total nitrogen using the Kjeldahl method (N×6.25) in accordance with AOAC International procedures [34]. Samples underwent ashing in a muffle furnace set at 450°C until no further change in weight was observed to determine ash content. Total lipid content was measured using the extraction method described by Bligh and Dyer [35].

3.7. Statistical Analyses

The experimental data were analyzed in SPSS (version 22.0). A one-way analysis of variance was employed to determine the effect of dietary treatments on the measured parameters. Significant ANOVA results were followed by Duncan's multiple range test to identify differences among treatment groups. Statistical significance was accepted at $P<0.05$, and the findings are reported as mean values ± standard deviation (SD).

4. RESULTS

4.1. Growth Performance of the Pea Protein–Supplemented Groups

After a 20-week feeding trial, final body weight values in the pea protein–supplemented groups ranged from 140.40±0.00 to 187.15±14.92 g. No statistically significant differences were detected among the control, PEA7, and PEA14 diets ($p>0.05$), while significant pairwise differences ($p<0.05$) were detected across the remaining treatments. The highest survival rate (96.7%) was obtained from the the PEA14 and PEA21 treatments, whereas the PEA28 treatment resulted in the lowest survival rate (86.7%) (Table 4). The PEA28 group exhibited the highest feed conversion ratio (FCR) value (2.01±0.00). In terms of FCR, PEA7, and PEA14 treatments remained statistically comparable ($p>0.05$), while significantly different values were obtained for the PEA21 and PEA28 treatments ($p<0.05$). Regarding SGR, the control group achieved the highest value (0.58±0.01), while the lowest SGR was recorded in the PEA28 group (0.3 ±0.00). All experimental groups differed significantly from each other with respect to SGR ($p<0.05$). The highest live weight gain (%) was obtained from the control treatment (124.7%). This was followed by the PEA7 (115.0%) and PEA14 (109.7%) groups, while a lower value was recorded in the PEA21 group (88.3%). The minimum weight gain was observed in the PEA28 group, and the evaluated treatments exhibited significant statistical variation ($p<0.05$). PER values varied between 0.08 and 0.11. The lowest PER values were determined in the PEA21 and PEA28 groups (0.08), whereas the control and PEA14 groups showed the highest values (0.11). An intermediate value was recorded for the PEA7

group (0.10), with all experimental treatments exhibited statistically significant variation ($p<0.05$).

Table 4. Growth performance and feed utilization parameters in groups fed diets with partial replacement of fish meal by pea protein

Growth Performance and Feed Utilization	Experimental Diets Containing Pea Protein				
	CON	PEA7	PEA14	PEA21	PEA28
Initial body weight(g)	83.3	84.4	87.7	85.5	82.9
Final body weight (g)	187.15±14.92^{a}	181.50±2.40^{a}	183.90±0.00^{a}	161.00±0.00^{b}	140.40±0.00^{c}
Survival (%)	93.3	93.3	96.7	96.7	86.7
FCR	1.27±0.10^{c}	1.30±0.01^{c}	1.31±0.00^{c}	1.80±0.00^{b}	2.01±0.00^{a}
SGR (%/day^{-1})	0.58±0.01^{a}	0.55±0.02^{b}	0.53±0.00^{b}	0.45±0.00^{c}	0.37±0.00^{d}
Weight gain (%)	124.7^{a}	115.0^{b}	109.7^{b}	88.3^{c}	69.4^{d}
PER	0.11^{a}	0.10^{a}	0.11^{a}	0.08^{b}	0.08^{b}
Each value corresponds to the mean±SD (n=3). Differences between treatment groups (P≤0.05) are indicated when row-wise values carry distinct superscript letters					

4.2. Growth Performance of the Wheat Gluten–Supplemented Groups

The final body weight of fish fed wheat gluten–supplemented diets was highest in the control group (187.15±14.9), whereas among all treatments, the WG26 treatment produced the lowest value (155.10±0.00) (Table 5). All experimental treatments could be statistically distinguished from one another ($p<0.05$). Survival rate reached its maximum in the WG13 and WG19 groups (96.7%), while the lowest survival (93.3%) was observed in the control, WG6, and WG26 treatments. Concerning feed conversion ratio (FCR), the highest value was obtained in the WG26 group (1.71±0.03), with significant differences detected among the dietary treatments ($p<0.05$). For SGR, the control group exhibited the highest value (0.58±0.01), whereas the lowest SGR was recorded in the WG26 group (0.43±0.00), and all groups differed significantly from each other ($p<0.05$). The highest weight gain was recorded in the control group (124.7%), whereas the lowest value was observed in the WG26 group (82.9%). Differences among the experimental treatments were statistically significant ($p<0.05$).

Table 5. Growth performance and feed utilization parameters in groups fed diets with partial replacement of fish meal by wheat gluten

Growth Performance and Feed Utilization	Experimental Diets Containing Wheat Gluten				
	CON	WG6	WG13	WG19	WG26
Initial body weight(g)	83.3	84.0	84.1	84.8	84.8
Final body weight (g)	187.15±14.9^{a}	183.10±1.70^{ab}	174.30±1.70^{ab}	168.55±1.48^{bc}	155.10±0.00^{c}
Survival (%)	93.3	93.3	96.7	96.7	93.3
FCR	1.27±0.10^{d}	1.35±0.08^{db}	1.47±0.01^{bc}	1ç63±0.08^{ab}	1.71±0.03^{a}
SGR (% /day^{-1})	0.58±0.01^{a}	0.54±0.02^{b}	0.52±0.02^{bc}	0.49±0.01^{c}	0.43±0.00^{d}
Weight gain (%)	124.7^{a}	118.0^{a}	107.3^{bc}	98.8^{cd}	82.9^{d}
PER	0.11^{a}	0.11^{a}	0.10^{a}	0.10^{a}	0.10^{a}
Each value corresponds to the mean±SD (n=3). Differences between treatment groups (P≤0.05) are indicated when row-wise values carry distinct superscript letters					

PER, an indicator of dietary protein utilization efficiency, showed comparable values among groups, with 0.11 recorded in both the control and WG6 groups, and 0.10 in the WG13, WG19, and WG26 groups. No significant differences were detected among treatments ($p>0.05$).

4.3. Somatic Indices of the Pea Protein–Supplemented Groups

At the end of the trial, 10 fish were randomly sampled from each group. Mean values of viscerosomatic index (VSI, %), hepatosomatic index (HSI, %), carcass yield (CY, %), and condition factor (CF) are presented in Tables 6 and 7.

Table 6. Somatic indices (%) of sampled fish in the pea protein-supplemented groups

Somatic Indices	Experimental Groups Containing Pea Protein				
	CON	PEA 7	PEA 14	PEA 21	PEA 28
VSI	13.82±1.24^{c}	14.73±1.31^{bc}	14.95±1.10^{bc}	15.53±1.35^{b}	16.82±1.66^{a}
HSI	1.27±0.36^{a}	1.22±0.20^{a}	1.27±0.13^{a}	1.37±0.28^{a}	1.38±0.21^{a}
CY	86.17±1.24^{a}	85.26±1.31^{ab}	85.04±1.10^{ab}	84.47±1.35^{b}	83.17±1.66^{c}
CF	1.21±0.07^{ab}	1.24±0.08^{ab}	1.20±0.08^{b}	1.28±0.07^{a}	1.22±0.06^{ab}
Each value corresponds to the mean ± SD (n=3). Differences between treatment groups (P≤0.05) are indicated when row-wise values carry distinct superscript letters					

Differences in viscerosomatic index (VSI, %) were found to be significant across the diet treatments ($p<0.05$). Fish in the PEA28 group reached the highest VSI value (16.82±1.66), whereas the lowest value was measured in the CON group (13.82±1.24). Hepatosomatic index (HSI, %) remained unaffected by dietary treatments, remained statistically comparable ($p>0.05$). Following the completion of the study, HSI values changed narrowly between 1.22±0.20 and 1.38±0.21. Carcass yield (%) responded significantly to the nutritional interventions ($p<0.05$), whereas the control group ranked highest for this parameter (86.17±1.24) and the PEA28 group the lowest (83.17±1.66). As for CF, statistical similarity was observed in CON, PEA7, and PEA28 treatments ($p>0.05$), while the remaining diets exhibited statistically significant disparities from these groups ($p>0.05$). The greatest CF was recorded in PEA21 treatment (1.28±0.07), and the lowest was found in the PEA14 group (1.20±0.08)(Table 6).

4.4. Somatic Indices of the Wheat Gluten-Supplemented Groups

When the viscerosomatic index (VSI, %) values were examined among the groups fed wheat gluten-supplemented diets, the CON, WG6, WG13, and WG19 treatments showed similar values ($p>0.05$), in contrast, the WG26 group differed significantly compared with all other treatments ($p<0.05$). The dietary treatments produced no significant differences in HSI ($p>0.05$), with values ranging from 1.25±0.50 to 1.37±0.24. Carcass yield showed comparable values in the CON, WG6, WG13, and WG19 groups ($p>0.05$), whereas the WG26 treatment differed significantly from all other groups ($p<0.05$). By the conclusion of the experiment, CF displayed no significant variation significantly among the control, WG6, and WG19 treatments ($p>0.05$). The greatest CF value was detected in the WG26 group (1.31±0.21),while the lowest value was recorded in the WG13 meal (1.18±0.08)(Table 7).

Table 7. Somatic indices (%) of sampled fish in the wheat gluten-supplemented groups

Somatic Indices	Experimental Groups Containing Wheat Gluten				
	CON	WG6	WG13	WG19	WG26
VSI	13.82±1.24^{b}	15.04±1.02^{b}	14.58±1.18^{b}	15.60±1.47^{b}	20.7±10.89^{a}
HSI	1.27±0.36^{a}	1.25±0.25^{a}	1.26±0.20^{a}	1.37±0.24^{a}	1.36±0.40^{a}
CY	86.18±1.24^{a}	84.96±1.02^{a}	85.42±1.18^{a}	84.40±1.47^{a}	79.30±10.89^{b}
CF	1.21±0.07^{ab}	1.29±0.08^{ab}	1.18±0.08^{b}	1.25±0.09^{ab}	1.31±0.21^{a}
Each value corresponds to the mean±SD (n=3). Differences between treatment groups (P≤0.05) are indicated when row-wise values carry distinct superscript letters					

4.5. Proximate Composition of Fish

4.5.1. Body Composition of the Pea Protein-Supplemented Groups

Analysis of moisture content in fish fed pea protein-supplemented diets revealed that the treatments produced similar values ($p<0.05$), with measurements varying from 62.55±1.20 to 64.40±0.99. Protein content of the whole body did not differ significantly in the CON, PEA7, PEA14, and PEA21 treatments ($p>0.05$), whereas the PEA28 group exhibited a statistically different response ($p<0.05$). Body lipid levels varied

significantly across the experimental groups ($p<0.05$), although the PEA21 and PEA28 treatments were statistically comparable ($p>0.05$). At the conclusion of the feeding trial, ash content showed a consistent pattern among all dietary groups, with no significant differences observed (control, PEA7, PEA14, PEA21, and PEA28; $p>0.05$)(Table8).

Table 8. Body composition (%) on a dry matter basis of groups fed diets supplemented with pea protein

Proximate Analysis (%)	Experimental Groups Containing Pea Protein				
	CON	PEA7	PEA14	PEA21	PEA28
Moisture	64.40±0.99^{a}	62.55±1.20^{a}	62.65±2.05^{a}	63.05±2.62^{a}	62.60±2.12^{a}
Protein	45.15±0.21^{a}	43.95±0.49^{a}	44.00±0.14^{a}	44.15±0.49^{a}	40.15±0.92^{b}
Lipid	26.15±0.49^{b}	27.05±0.21^{ab}	27.00±0.57^{ab}	27.90±0.14^{a}	27.95±1.06^{a}
Ash	13.75±0.07^{a}	13.25±0.92^{a}	12.75±0.92^{a}	12.75±1.20^{a}	13.05±0.21^{a}
Each value corresponds to the mean±SD (n=3). Differences between treatment groups (P≤0.05) are indicated when row-wise values carry distinct superscript letters					

4.5.2. Body Composition of the Wheat Gluten–Supplemented Groups

Evaluation of moisture content in fish fed wheat gluten–supplemented diets revealed that the treatments were statistically similar ($p>0.05$), as values fell within the interval of 60.10±0.90 to 64.40±0.99. Body protein content differed significantly across the feeding regimens ($p<0.05$), with the maximum value was attained in the CON treatment (45.90±0.49) and the lowest in the WG26 group (44.15±0.42). The treatments resulted in significant variation in whole-body lipid content. ($p<0.05$), as all wheat gluten–supplemented groups (WG6, WG13, WG19, and WG26) exhibited higher lipid levels than the control group. Similarly, the evaluated groups exhibited statistically distinct ash content values ($p<0.05$), with the control group presenting higher values compared with all WG-treated groups (Table 9).

Table 9. Body composition (%) on a dry matter basis of groups fed diets supplemented with wheat gluten

Proximate Analysis (%)	Experimental Groups Containing Wheat Gluten				
	CON	WG6	WG13	WG19	WG26
Moisture	64.40±0.99^{a}	62.15±0.21^{a}	61.85±2.62^{a}	64.15±0.54^{a}	60.10±0.99^{a}
Protein	45.90±0.49^{a}	45.60±0.35^{ab}	45.07±1.13^{ab}	45.10±0.14^{ab}	44.15±0.42^{b}
Lipid	26.15±0.49^{c}	27.30±0.57^{c}	31.15±0.07^{b}	30.75±0.07^{b}	33.70±1.27^{a}
Ash	13.05±0.07^{a}	11.90±0.00^{ab}	10.55±1.34^{b}	12.05±0.21^{a}	10.40±0.14^{b}
Each value corresponds to the mean±SD (n=3). Differences between treatment groups (P≤0.05) are indicated when row-wise values carry distinct superscript letters					

4.6. Liver Proximate Composition of Fish

4.6.1. Liver Nutrient Composition of the Groups Supplemented with Pea Protein

Statistical analysis indicated similar liver moisture content in the control and PEA7 groups of fish receiving pea protein–supplemented diets ($p>0.05$), whereas the remaining treatments differed significantly from these groups ($p<0.05$). Values for liver moisture varied between 54.43±0.33 and 63.80±0.14. Statistical analysis demonstrated that liver protein content varied across all experimental treatments ($p<0.05$). Liver lipid content also differed significantly across treatments ($p<0.05$). The PEA21 and PEA28 groups were statistically comparable ($p>0.05$). For liver ash content, the control and PEA7 groups were statistically similar, as were the PEA14, PEA21, and PEA28 groups ($p>0.05$)(Table 10).

Table 10. Liver nutrient composition (%) of groups fed diets supplemented with pea protein (on a dry matter basis)

Proximate Analysis (%)	Experimental Groups Containing Pea Protein				
	CON	PEA7	PEA14	PEA21	PEA28
Moisture	54.47±0.10^{d}	54.43±0.33^{d}	58.74±0.08^{b}	57.15±0.07^{c}	63.80±0.14^{a}
Protein	31.60±0.00^{a}	24.80±0.14^{cd}	26.10±0.00^{b}	25.00±0.00^{c}	24.35±0.49^{d}
Lipid	44.75±1.20^{ab}	43.65±2.19^{b}	42.0±0.00^{c}	45.00±0.00^{a}	45.02±3.25^{a}
Ash	4.10±0.07^{b}	3.90±0.28^{b}	5.25±0.49^{a}	5.15±0.07^{a}	5.50±0.00^{a}
Each value corresponds to the mean±SD (n=3). Differences between treatment groups (P≤0.05) are indicated when row-wise values carry distinct superscript letters					

4.6.2. Liver Nutrient Composition of the Groups Supplemented with Wheat Gluten

Comparable liver moisture content was observed in fish fed the control and WG6 diets ($p>0.05$), whereas the remaining treatments differed significantly from these groups ($p<0.05$). Moisture values ranged from 36.24±0.34 to 55.84±0.09. Protein content in the liver exhibited a similar pattern between the WG13 and WG26 groups ($p>0.05$), while the remaining treatment groups showed statistically distinct values ($p<0.05$). The control, WG6, and WG13 treatments did not differ significantly with respect to liver lipid content ($p>0.05$); significant differences were observed between the WG19 and WG26 groups and all other treatments ($p<0.05$). A statistically significant variation was also recorded for liver ash content among all experimental treatments ($p<0.05$)(Table 11).

Table 11. Liver nutrient composition (%) of groups fed diets supplemented with wheat gluten (on a dry matter basis)

Proximate Analysis (%)	Experimental Groups Containing Wheat Gluten				
	CON	WG6	WG13	WG19	WG26
Moisture	54.43±0.1^{b}	54.69±0.26^{b}	36.24±0.34^{d}	51.85±0.22^{c}	55.84±0.09^{a}
Protein	24.35±0.49^{a}	23.00±0.14^{b}	21.05±0.49^{c}	19.10±0.14^{d}	21.50±0.00^{c}
Lipid	44.75±1.20^{a}	44.25±2.76^{a}	44.90±0.99^{a}	46.50±0.42^{b}	46.70±0.00^{b}
Ash	4.10±0.00^{b}	3.50±0.28^{c}	4.40±0.00^{a}	3.10±0.00^{d}	3.45±0.07^{c}
Each value corresponds to the mean±SD (n=3). Differences between treatment groups (P≤0.05) are indicated when row-wise values carry distinct superscript letters					

5. DISCUSSION

This research examined the influence of replacing fish meal with different levels of pea protein concentrate and wheat gluten in feed formulated for *Dicentrarchus labrax* during the 20-week feeding trial. Pea protein and wheat gluten, two plant-based protein sources with high protein content, were evaluated as alternative fish meal replacers to determine their suitability in aquafeed formulations. Experimental feed formulations were adjusted to achieve 45% crude protein and 20% crude lipid levels. Both alternative protein sources were tested at four inclusion levels by replacing a fraction of the dietary fish meal. The results were discussed in comparison with relevant literature. In their study on Dicentrarchus labrax, Tibaldi et al. [36], reported that pea protein concentrate could replace up to 60% of fish meal without adversely affecting feed intake, acceptance, or palatability. While their study indicated that a 60% inclusion level of pea protein concentrate was acceptable based on feed intake, in the present study, a 28% inclusion level was not suitable for optimal growth performance. Considering all performance parameters, incorporating 14.1% pea protein into the diet did not result in measurable changes in growth performance or feed utilization compared with the control treatment. The evaluated protein inclusion levels produced growth performance and feed utilization similar to those of the control treatment. Since pea protein concentrate did not cause any noticeable palatability problems, it may be inferred that 14.1% is the most suitable inclusion level under the

experimental conditions applied. Higher inclusion levels resulted in significant reductions in growth and feed utilization.

In work carried out on *Dicentrarchus labrax*, Gouveia and Davies [37], used pea seed meal at inclusion levels of 10%, 20%, and 30%, corresponding to fish meal protein replacement levels of 5%, 10%, and 15%, respectively. Although their 11-week trial reported no significant negative effects at inclusion levels up to 30% pea seed meal, in the present 20-week growth trial, the maximum inclusion level was limited to 14.1% pea protein concentrate replacing 10.5% fish meal protein.

Higher inclusion levels resulted in significant variations in FCR, SGR, and body weight gain. It is suggested that the differences between the raw materials used in the two studies (pea seed meal versus pea protein concentrate) may explain the observed differences in performance results.

In a study on 10 g juvenile European sea bass (*Dicentrarchus labrax*), Gouveia and Davies [37], reported that a 25% pea meal inclusion level over an 84-day period did not negatively affect weight gain, feed intake, or specific growth rate. In contrast, in the present study, significant negative effects on growth performance were observed at pea protein inclusion levels of 21.1%. Although their study indicated that carbohydrate digestibility was affected by pea starch in the diet matrix and that digestible energy decreased, no adverse effects on growth performance were reported over the 84-day trial period. In the present study, negative effects on performance parameters were observed at inclusion levels exceeding 14.1% pea protein.

In another study on juvenile *Dicentrarchus labrax*, Ganzon-Naret [38], reported that diets containing 20% hulled green peas or autoclaved green peas resulted in similar feed conversion ratio (FCR) values compared with the control group. The findings of the current investigation demonstrated comparable FCR values across all experimental treatments receiving diets containing 7% and 14.1% pea protein concentrate. However, a significant difference in FCR was observed in the group fed 21.1% pea protein concentrate, a level comparable to that reported by Ganzon-Naret [38]. At similar inclusion levels, the present study indicated significantly adverse effects on FCR.

Tibaldi et al. [36], evaluated wheat gluten as a fish meal replacer at inclusion levels of 70% and 50% in a 96-day feeding trial with European sea bass (*Dicentrarchus labrax*). They reported no treatment-related variation was evident in FCR across the dietary treatments; however, fish fed the 70% wheat gluten meal exhibited reduced growth compared with the other treatments. Results obtained in this experiment showed that the 6.5% wheat gluten diet supported growth performance comparable with the control treatment; however, significant differences were observed in the groups fed 13%, 19.6%, and 26.2% wheat glüten. These results differ from those of Tibaldi et al. [36], who reported no adverse effects even at a 50% wheat gluten inclusion level.

Robaina et al. [39], reported that in a study on 200 g European sea bass(*Dicentrarchus labrax*), extruded diets containing up to 30% wheat gluten could replace fish meal without causing significant differences in performance. In the present study, the maximum wheat gluten inclusion level was limited to 6.5%. This variation may be explained by differences in fish size between the studies.

It is suggested that higher inclusion levels (up to 30%) may be suitable for larger fish (≥200 g), whereas lower inclusion levels are more appropriate for smaller fish. In the present study, a 6.5% wheat gluten inclusion level was found to be suitable for fish with an initial weight of approximately 84 g.

A 96-day feeding trial was conducted to evaluate the effects of diets containing wheat gluten, soybean meal, and pea meal on growth

performance and lipid metabolism in juvenile European sea bass (*Dicentrarchus labrax*) with an initial body weight of 23.9±0.1 g. No adverse effects on final body weight were observed in any of the experimental groups, except for the group fed the diet containing 70% wheat gluten [40]. In the present study, final body weight values were similar between the control group and the group receiving 6.5% wheat gluten in the diet, whereas the group fed 26.2% wheat gluten exhibited the lowest final body weight. Significant differences in liver lipid content among dietary treatments have previously been reported, and hepatic steatosis was suggested to be reflected in the hepatosomatic index (HSI) [40]. In contrast, in the present study, hepatosomatic index (HSI) did not differ significantly among the wheat gluten treatment groups.

In a 48-day feeding trial with Lates calcarifer, hydrolyzed wheat gluten was evaluated as a fish meal replacer. No significant differences in feed conversion ratio (FCR), specific growth rate (SGR), final body weight, or daily feed intake were observed in the group fed a diet containing 6% wheat gluten [41]. Although L. calcarifer is a different species from *Dicentrarchus labrax*, it is a closely related marine fish species, and the findings of the present study were generally consistent with those reported by Apper et al. [41]. Similarly, no significant adverse effects on growth performance were observed at the lowest wheat gluten inclusion level (6.5%), suggesting that this inclusion level may be suitable for use in European sea bass diets.

Pereira and Oliva-Teles [42], evaluated the use of pea meal as a fish meal alternative in gilthead sea bream (*Sparus aurata*) diets at dietary inclusion levels of 10% and 20%. Following a 12-week feeding trial, they reported that incorporating 20% pea meal did not compromise growth performance, FCR, or body composition. The ability to use similar inclusion levels in gilthead sea bream suggests that pea-based ingredients may serve as promising alternative protein sources in aquafeeds. These results further indicate that plant-based protein sources may serve as suitable partial alternatives to fish meal, especially considering the rising costs and limited supply of fish meal resources.

The effects of amino acid supplementation on the utilization of pea protein concentrate in diets for *Sparus aurata* were investigated using diets containing 0, 162, 325, and 487 g kg^{-1} pea protein concentrate [43]. The authors found that incorporating pea protein concentrate at inclusion levels up to 325 g kg^{-1} (32.5%) had no negative impact on weight gain, FCR, or PER. Similarly, Sanchez-Lozano et al. [43], found that replacing up to 60% of fish meal with pea protein concentrate did not negatively affect growth performance in gilthead sea bream. In contrast, the current study demonstrated that the maximum acceptable inclusion level of pea protein concentrate in European sea bass diets was approximately 14.1%, beyond which significant reductions in growth performance and feed utilization were identified. The marked differences between studies suggest that the tolerance and utilization of pea protein concentrate may be species-specific. These findings highlight the importance of considering species-related nutritional requirements when formulating feeds and suggest that diets developed for one species may not necessarily be directly applicable to another.

In a study evaluating the effects of various pea-derived products, the authors reported that juvenile rainbow trout (*Oncorhynchus mykiss*) received diets including the tested ingredient exhibited comparable feed intake, final body weight, and SGR relative to the control group. The authors concluded that pea products could be included in diets at levels of up to 20% without negatively impacting growth performance [44]. In a similar study on the same species, Collins et al. [45] evaluated the

effects of soybean meal, soybean protein concentrate, pea meal, pea protein concentrate, canola meal, and canola protein concentrate on growth performance. They reported that pea protein concentrate was considered suitable for dietary incorporation at levels as high as 30% while maintaining normal growth performance or FCR in rainbow trout. In the present study, pea protein concentrate was included at a maximum level of 14.1% without leading to any significant negative effects on growth performance or feed conversion ratio (FCR) in European sea bass. These results indicate that the optimal inclusion level of pea protein concentrate may differ depending on the fish species.

Overland et al. [46], assessed the potential of pea protein concentrate to replace fish meal and soybean meal in diets for *Salmo salar* and examined its effects on growth performance, nutrient digestibility, carcass composition, and intestinal health over a 12-week feeding trial. The authors reported that pea protein concentrate may serve as a potential alternative to fish meal at levels of up to 50% without adversely affecting feed conversion ratio (FCR). Furthermore, based on carcass composition results, they suggested that substitution levels of up to 20% could be achieved while maintaining carcass quality comparable to that obtained with high-quality fish meal.

In a study evaluating the effects of replacing fish meal with wheat gluten in diets for Atlantic salmon (*Salmo salar*), Storebakken et al. [47], reported that the inclusion of wheat gluten at a level of 35% did not adversely affect growth performance during an 18-week feeding trial. The authors also concluded that this inclusion level could be used without additional lysine supplementation, as the lysine supplied by fish meal was adequate to provide the nutrients required by the fish. Similarly, in the present study, the inclusion of 6.5% wheat gluten in place of fish meal did not negatively affect growth performance during the 20-week feeding period. However, unlike the findings of Storebakken et al. [47], the successful use of wheat gluten at this inclusion level was achieved with dietary lysine supplementation, highlighting the importance of amino acid balance in diet formulation.

In a study evaluating wheat gluten as a fish meal replacer in Atlantic halibut (*Hippoglossus hippoglossus*), diets containing 0%, 10%, 30%, and 60% wheat gluten were tested. Following a 56-day feeding trial, the authors concluded that up to 30% wheat gluten could replace fish meal when the diets were supplemented with 2.6% lysine [48]. In the present study, the highest wheat gluten inclusion level (26.2%) was associated with the highest level of dietary lysine supplementation. However, this group showed the highest FCR along with the lowest SGR and weight gain. These findings indicate that increasing lysine supplementation alone was not sufficient to compensate for the negative effects associated with higher wheat gluten inclusion levels. Based on the growth performance results, a wheat gluten inclusion level of 6.5% was found to be suitable for European sea bass without adversely affecting growth performance.

6. CONCLUSION

Based on the present findings, the inclusion of 14.1% pea protein concentrate or 6.5% wheat gluten as partial fish meal replacements supported normal growth performance, feed conversion efficiency and specific growth rate in sea bass. Accordingly, both ingredients can be regarded as viable alternatives to fish meal at the tested inclusion levels. However, elevated inclusion levels of pea protein concentrate and wheat gluten negatively affected growth performance and feed utilization. Overall, the findings suggest that the high protein content of pea protein concentrate and wheat gluten enables their use as partial substitutes for fish meal in European sea bass diets. Given the

increasing demand for sustainable aquafeed ingredients and the limited availability of fish meal resources, the identification and evaluation of alternative protein sources remain essential for the long-term the expansion and intensification of aquaculture. Additional research efforts are needed to optimize inclusion levels and improve the utilization of these alternative protein ingredients in aquafeeds.

AUTHOR DECLARATION

The author(s) declare that this study was not generated by artificial intelligence, that its scientific content and intellectual responsibility belong entirely to the authors, and that they accept full responsibility for all scientific, ethical, material, moral, and legal consequences arising from the work

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DATA AVAILABILITY

The datasets generated and/or analyzed during the current study can be obtained from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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