Valorization of Northern shrimp shells meal of *Pandalus borealis* (Krøyer, 1838) as partial substitution for fish meal in diet for European seabass *Dicentrarchus labrax*: effects on growth and feed efficiency

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Abstract. To contribute to aquaculture industry sustainability, limited by the stagnation in Fish Meal (FM) production from fisheries, it was necessary to search for operational alternatives based on FM reduction in aquaculture feed formulation. In this regard, the present study focused on fish feeds formulation using locally available raw material (shrimp shells meal (SSM)) as partial substitute for FM while maintaining nutritional quality of the fish feed. The purpose is double: find an alternative to FM by developing a new formulation using shrimp shells generated by processing plants and to find a valorization solution to the problem of waste from shrimp husking factories which causes an environmental problem compromising environmental protection and sustainable development. Experiment using European seabass (*Dicentrarchus labrax*) was conducted with four diets: control diet with 45% FM content (D1-SM0) and three experimental diets based on control diet with three levels of FM substitution: 11% (D2-SM5; 50 g SSM/kg diet), 22% (D3-SM10; 100 g SSM/kg diet) and 33% (SSM15; 150 g SSM/kg diet). This experiment lasted six months and was done in duplicates. The biochemical composition of these feeds did not differ significantly ($p>0.05$). Results showed no significant difference ($p>0.05$) in weight and size of seabass juveniles fed on these different feeds for six months. No significant difference was recorded in growth and feed efficiency of juveniles fed on SM0, SM5 or SM10 while those fed on SM15 have reduced growth and feed utilization. As final output, SSM could be used as a substitute up to 22% for FM in feed of European sea bass, with economic benefits while maintaining feed quality altogether. So, these results profile SSM as a potential ingredient in European sea bass fed a low FM and oil diets.

Key Words: aquaculture sustainability, byproducts, feed formulation, fish feed, marine ingredients.

Introduction. By 2030, aquaculture production is expected to reach 109 million tons, which represent an increase of 32% compared to 2018. Over the past decade, the levels of fish meal (FM) and fish oil (FO) in aquaculture feed formulations have declined significantly with the reduction peaking at more than 30 million tons in 1994 and then falling to less than 18 million tons in 2018 (FAO 2020). For the sustainability of this sector, it is necessary to develop new fish formula diets that include nutritious and eco-friendly feed ingredients as an alternative to the use of large amounts of protein and lipids directly obtained from marine fisheries with more sustainable sources such as plants, single-cell biomass, animal by-products, insects, and krill (Naylor et al 2021).

FM can be partially substituted in the diet of many fish species. Generally, a higher or total replacement of terrestrial components will adversely affect the performance and health of the fish (Conde-Sieira et al 2018), particularly in European seabass (*Dicentrarchus labrax*), a marine specie widely farmed in the Mediterranean region (FAO

2019) and recently farmed out in the southern Atlantic coast of Morocco (Izzabaha et al 2020). Multiple studies have shown that it is possible to substitute up to 50% of FM by plant-based meals without affecting growth performance (Torrecillas et al 2017).

Shrimp peeling and processing industry is one of the activities that generate a large amount of organic waste (co-products) every year. These co-products include shrimp heads, shells, and tails. Many studies have shown that shrimp processing waste can be used for animal feed production due to its nutritional and bioactive compounds such as protein, carotenoids, chitin, lipids etc. (Pattanaik et al 2020). In addition, they can improve the growth performance and immunity of farmed fish (Gisbert et al 2018).

Shrimp shell meal (SSM) diet improved growth performance and protein utilization of many fish species with different inclusion levels, such as red porgy (*Pagrus pagrus*) with 16% SSM dietary inclusion for 105 days (Kalinowski et al 2007), humpback grouper (*Cromileptes altivelis*) with 10% SSM (Rachmansyah et al 2004), large yellow croaker (*Larimichthys croceus*) with 12% SSM for 9 weeks (Yi et al 2015), juvenile of cobia (*Rachycentron canadum*) fed with different percentages of SSM (0–25%) for six weeks, which showed an increased weight gain and had a survival rate of over 87% (Lu & Ku 2013).

On the other hand, shrimp head silage could replace FM for tilapia *Oreochromis niloticus* (Cavalheiro et al 2007). It has been reported that in the case of African catfish (*Clarias gariepinus*) feed, it is profitable to substitute 20% of FM with shrimp heads (Nwanna 2003). However, higher levels compromised growth rate, feed conversion ratio and protein efficiency ratio (Rachmansyah et al 2004).

Furthermore, shrimp protein hydrolysate can be incorporated in aquatic feed with high levels of FM substitution by plant sources without detrimental impact on growth performance of fish (Gisbert et al 2018). In addition, non-specific humoral immunity of seabass and its survival when affected by bacteria *Vibrio pelagius* were positively affected, which indicates that shrimp protein hydrolysate has immunomodulatory effects in promoting health and preventing fish diseases (Gisbert et al 2018).

In Morocco, especially in Tangier, several peeling factories have been installed, processing an average of more than 20,000 tons of shrimp imported from Canada, Denmark, and Netherlands, producing more than 14,000 tons of shrimp waste (Ouaach 2014; Ichibane 2021). They are not reused and completely discarded, causing environmental and economic issues.

Besides, Morocco has a significant aquaculture potential estimated at 380,000 tons/year by National Agency for Aquaculture Development (ANDA). To contribute to the sustainable development of national aquaculture, it is necessary to follow global trends in fish nutrition which support searching for alternative ingredients to FM.

Therefore, the present study aimed at evaluating the potential of SSM as a feed supplement ingredient to substitute partially FM in European seabass juveniles diet and their effects on growth performance and feed utilization.

Material and Method

Raw materials. The ingredient used to substitute FM is shrimp by-products generated after hand peeling process in a factory located in Tangier (Morocco). Shrimp species used is *Pandalus borealis* (Krøyer, 1838) which is a decapod crustacean of Pandalidae family. It has a circumboreal distribution in the northern hemisphere, and it is the most abundant shrimp species in eastern Canada (Bergstron 2000). Other used ingredients such as corn gluten, soybean meal, wheat flour, sunflower oil were purchased from local market. FM flour, FO, mineral, and vitamin premix were supplied by an extruded feed manufacturing plant located in Assilah (Morocco).

Pretreatment of shrimp shell meal. Before use, shrimp by-products were first washed thoroughly with water to eliminate organic residues (tissues) then they were dried by sun exposure for 48 hours. Once dried, they were ground into meal by a domestic grinder, to have homogeneous particle sizes less than 0.5 mm (Figure 1). Then, this obtained meal was stored in a refrigerator.

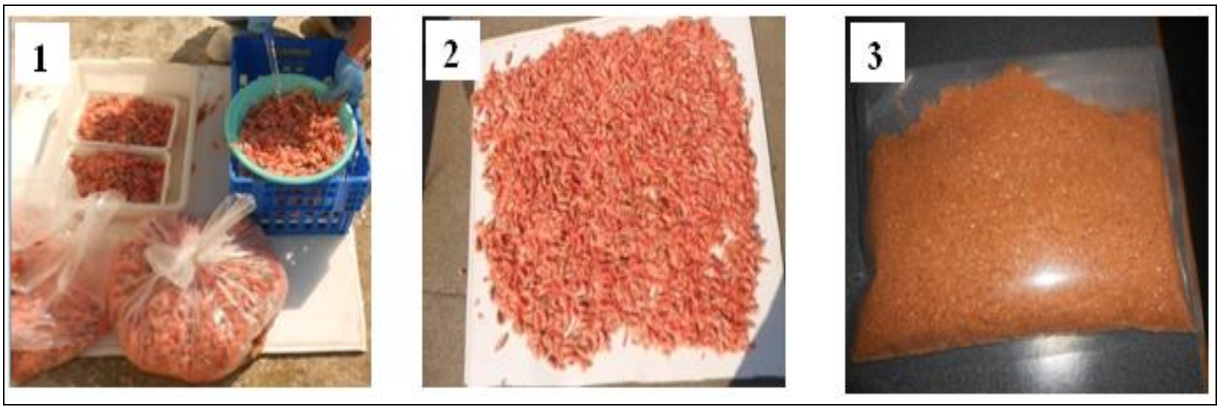

Figure 1. Pretreatment of shrimp shell meal 1: washing, 2: drying, 3: shrimp shells meal.

Formulation of experimental diets. Four feed diets (D1-SM0, D2-SM5, D3-SM10 and D4-SM15) were formulated based on nutritional requirements of seabass and the energy ingredients labelling (Table 1). These feeds were made with different proportions of FM substitution with 0%, 11%, 22%, and 33% respectively. All feeds contain 4% FO and 8% sunflower oil.

Table 1

Formulation of the four experimental feeds

Ingredients %	*D1-SM0*	*D2-SM5*	*D3-SM10*	*D4-SM15*
Fish meal	45.00	40.00	35.00	30.00
Shrimp meal	0.00	5.00	10.00	15.00
Soybean meal	16.00	16.00	16.00	16.00
Corn gluten	16.00	16.00	16.00	16.00
Wheat flour	9.00	9.00	9.00	9.00
Fish oil	4.00	4.00	4.00	4.00
Sunflower oil	8.00	8.00	8.00	8.00
Vitamin Premix	2.00	2.00	2.00	2.00

Diets preparation. Solid ingredients were weighed, ground, and homogenized to distribute them evenly throughout mixture mass. Manufacturing method was based on extrusion using single phase extruder (Henan, China, type DGP-80) owned by local fish farm in Mdiq (Morocco). This device is a small single screw extruder with a feed hopper and a downstream die allowing to obtain granules of 3 mm in diameter. Feed drying was carried out using a small horizontal oven of fan heater FH-241 Denwa 2000w allowing inside temperature homogenization. Depending on formulation, extruded feed was sprayed with oils mixture and stirred vigorously to ensure better pellets absorption of oils (Figure 2). Once coated, feeds were stored in buckets with lids and stored in a cool place.

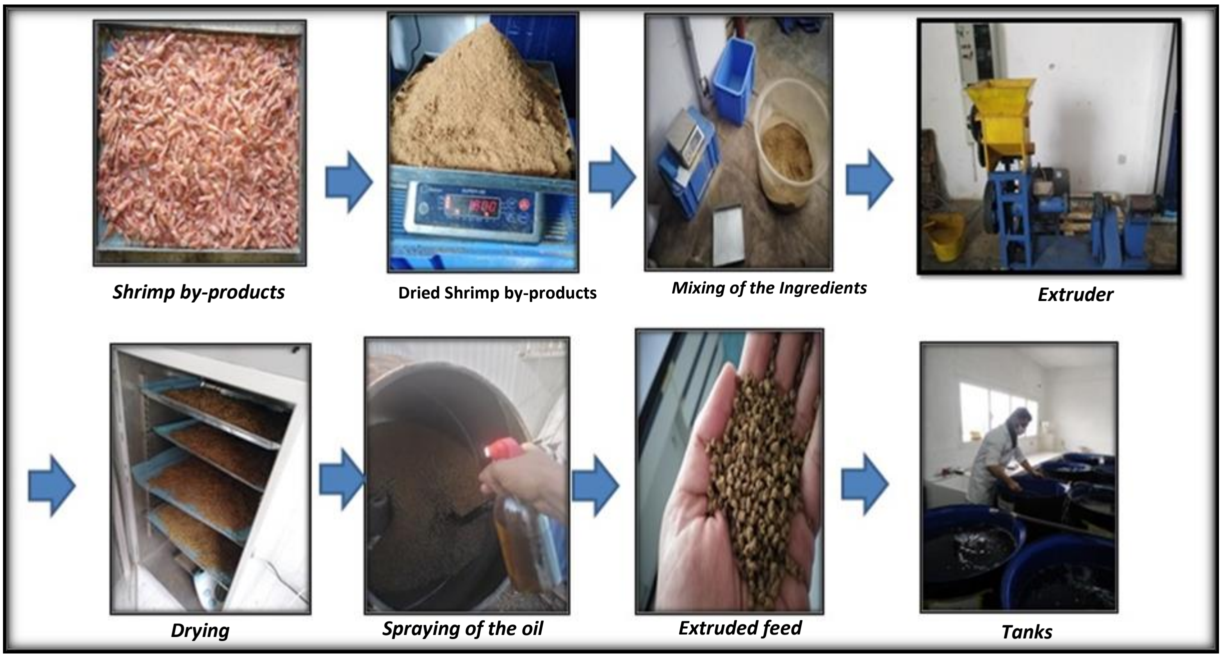

Figure 2. Diets processing.

Biochemical analysis of ingredients and experimental diets. Biochemical composition analysis of ingredients and experimental feeds assessed levels of proteins and lipids in FM, shrimp meal, soybean meal, corn gluten and wheat flour. Protein content was defined by Kjeldahl method based on total nitrogen determination. Lipid levels were assessed using Bligh and Dyer extraction method and ash content was determined by calcination in a muffle furnace at 550°C.

Fish, growth trial and sampling. The experiments started in December 2020 and lasted until June 2021. Seabass juveniles were obtained from local fish farm in M'diq located in the Bay of M'diq. The latter is located on Moroccan Mediterranean west coast. It extends between Ceuta Cape (35°54'N, 5°17'10''W) on the north and Negro Cape (35°40'N, 5°16'40''W) on the south. Seabass juveniles were transferred to Aquaculture Center of the National Fisheries Research Institute based in M'diq (Morocco) where experiment was conducted. Juvenile fishes had an average initial weight of 42±0.7 g and were evenly distributed randomly in eight tanks (200-liter polycarbonate cylindrical conical tanks). They were fed on a mixture of the four feeds for two weeks, twice a day, and six days a week, for stabling purpose and adaptation to the experimental conditions. Seawater renewal rate was 40% h^{-1} during the experiment time. Seawater was filtered and its oxygen level was kept near saturation by using air stone bubblers in each tank. Fishes were exposed to natural light and local ambient temperature. Initial stocking density was 30 fishes per tank, equivalent to 6 kg m^{-3}; the average biomass was 1330±74 g per tank. Two tanks were randomly allocated for each kind of diet. During the six months' period of the experiment, fishes were fed "ad libitum" once a day at 13:30 hours and six days a week. To avoid over-feeding, diets were distributed gradually and carefully. In addition, uneaten pellets were collected systematically at the end of each day and consumed amount of feed by fishes was recorded for each tank. To assess growth performances, individual weight and total length were measured using a precision balance (maximum range 4.100 g and accuracy 0.1) and a fish measuring board. All fishes were sampled and measured monthly. Before any sampling, seabass juveniles were starved for 24 hours. To relieve stress, they were first anesthetized with 2-phenoxyethanol 0.3 ml^{-1}/liter before weighing. At the end of the feeding experiment, following indicators were calculated as follows:

Survival (%) =100 x (Final number of fish / Initial number of fish)

SGRw (specific growth rate) = 100 x [ln final weight (g)–ln initial weight (g)]/trial duration (days)
SGRL (specific growth rate) = 100 x [ln final Length (g)–ln initial Length (g)]/trial duration (days)
Feed conversion ratio (FCR) = Feed consumed (g) / Wet weight gain (g)
Feed Efficiency ratio = 1 / Feed conversion ratio (FCR)
Biomass gain (BG) = Final biomass - Initial biomass
Condition factor (CF) = 100 x body weight (g) / (body length (cm)) ^{3}
Protein efficiency ratio (PER) = Fish wet weight gain (g) / Protein intake (g).
Energy efficiency ratio (EER) = Fish wet weight gain (g) / Energy intake (g).

Statistical analysis. Data was analyzed by using one-way Analysis of Variance (ANOVA) to test effects of the four experimental diets. Tukey and Fisher LSD methods were employed to rank groups when significant differences were found ($p<0.05$). Statistical analyses were made by using JMP 16 Software.

Results

Biochemical analysis of ingredients and experimental diets. The proximate analysis of experimental feed ingredients is shown in Table 2. Protein and fat contents of shrimp meal were 41.11% and 11.10% respectively.

Table 2

Proximate composition of ingredients

Ingredients	*% Proteins*	*% Lipids*
Fish Meal	60.73	11.10
Shrimp meal	41.11	13.00
Wheat flour	14.72	06.80
Soybean meal	46.00	03.00
Corn gluten	53.12	04.10

The proximate composition of experimental feeds is shown in Table 3. Results show statistically similar composition in all experimental feeds with respect to moisture, ash, protein, lipids. As well, the ratio protein/energy is similar for all diets, which is 19 mg/Kcal.

Table 3

Proximate composition of formulated diets (given as percentage of dry weight)

Constituent	*D1-SM0*	*D2-SM5*	*D3-SM10*	*D4-SM15*
Proteins	42.89	41.63	42.6	41.67
Lipids	18.4	17.4	18.5	17.5
Ash	10.67	11.06	10.97	11.03
NFE[1]	28.04	29.91	27.93	29.8
Proteins/lipids (P/L)	2.33	2.39	2.30	2.38
Gross Energy[2] (KJ/100 g)	2,226	2,188	2,221	2,191
P/E (mg/Kcal)	19.27	19.02	19.18	19.02

Note: 1) Nitrogen-free extract=100-(%moisture+%ash+%protein+%lipid+%fiber)
2) Gross Energy (1g protein = 23.7 kJ = 5.66 kcal, 1g lipids = 39.5 kJ = 9.44 kcal, 1g carbohydrate = 17.2 kJ = 4.11 kcal)

Survival rate and condition factor. Survival rate was between 85% and 96.67%; the best value was recorded by fishes fed on D3-SM10. Statistical analysis showed no significant difference in survival rates between all different diets. Since no symptoms or other pathological signs of bacterial diseases were observed, mortality that occurred during the experiment was related to natural mortality and manipulations during sampling and biometric measurements (Figure 3).

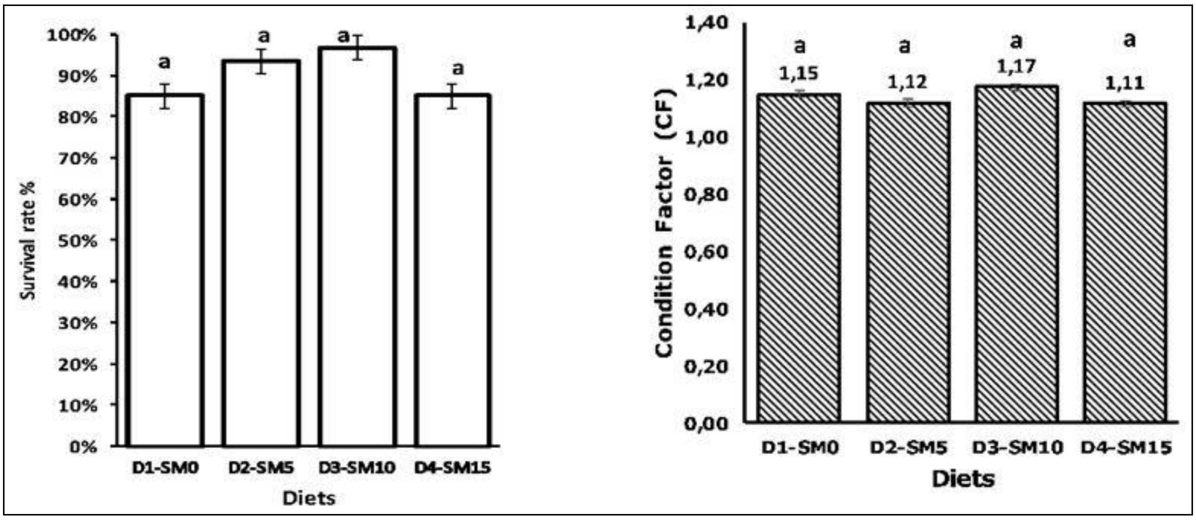

Figure 3. Survival rate and condition factor.

In terms of the condition factor, no statistical differences were observed between the different diets. However, D3-SM10 recorded the highest value (1.17), followed successively by D1-SM0 (1.15), D2-SM5 (1.12) and finally D4-SM15 (1.11) (Figure 3).

Growth and feed utilization. Results of growth parameters are reported in Table 4 and Figure 4. No significant differences were detected between biomass gains for D2-SM5, D3-SM10 and D1-SM0. However, D4-SM15 recorded the lowest biomass gain with 1.24 kg. No significant difference was found in terms of specific growth rate (SGR) between fishes fed on D1-SM0, D2-SM5 and D3-SM10 recording 0.52%, 0.48% and 0.50% respectively, while fishes fed on D4-SM15 showed the lowest value (0.42%).

Table 4 shows average growth performances of fishes fed on the four diets recorded after six months feeding experiment.

Table 4

Weight, growth performance and feed efficiency of European seabass (*Dicentrarchus labrax*) juveniles fed on diets with different % shrimp by-products levels along the experiment

	D1-SM0	*D2-SM5*	*D3-SM10*	*D4-SM15*
Survival Rate %	85.00±5.00^{a}	93.33±0.00^{a}	96.67±0.00^{a}	85%±1.67^{a}
Biomass Gain (Kg)	1.73±0.31^{a}	1.51±0.24^{a}	1.75±0.13^{a}	1.24±0.04^{a}
Initial fish weight (g/fish)	44.76±1.37^{a}	43.68±0.15^{a}	42,42±0.19^{a}	46.51±0.68^{a}
Final fish weight (g/fish)	120.29±6.59^{a}	109.68±0.46^{a}	109.90±2.97^{a}	103.48±1.28^{a}
Initial fish Length (g/fish)	16.5±0.09^{a}	16.36±0.09^{a}	16.22±0.03^{a}	16.65±0.13^{a}
Final fish Length (g/fish)	21.88±0.31^{a}	21.42±0.08^{a}	21.09±0.21^{a}	21.03±0.25^{b}
Specific Growth ratio SGRw (%)	0.52±0.01^{a}	0.48±0.00^{a}	0.50±0.02^{a}	0.42±0.00^{b}
Specific Growth ratio SGRL (%)	0.94±0.03^{a}	0.90±0.03^{ab}	0.87±0.03^{ab}	0.78±0.02^{b}
Condition Factor (CF)	1.15±0.01^{a}	1.12±0.02^{a}	1.17±+0.00^{a}	1.11±0.01^{a}
Feed intake (Kg)	3.24±0.15^{a}	2.86±0.28^{a}	3.28±0.05^{a}	3.19±0.05^{a}

Feed conversion ratio (FCR)	1.91±0.24[a]	1.92±0.12[a]	1.89±0.17[a]	2.57±0.05[a]
Feed Efficiency ratio	0.53±0.07[a]	0.52±0.03[a]	0.53±0.05[a]	0.39±0.01[a]
Protein Efficiency Ratio (PER)	1.24±0.16[a]	1.26±0.08[a]	1.27±0.10[a]	0.93±0.02[a]
Energy Efficiency Ratio (EER)	0.10±0.01[a]	0.10±0.00[a]	0.10±0.01[a]	0.07±0.00[a]

Note: Values expressed in mean ± SD. Different superscript letters are significantly different (P < 0.05) based on Tukey's multiple-range test (one-way ANOVA).

Specific growth rate SGR of fishes fed D1-SM0, D2-SM5, and D3-SM10 diets was significantly higher than that of fishes fed D4-SM15 diets (p=0.38; F=1.31). The highest SGR value was recorded by the control diet with 0.52%/day, while D4-SM15 was the lowest one with only 0.42%/day. Diets can be ranked according to their growth rate as follows: D1-SM0 > D3-SM10 > D2-SM5 > D4-SM15.

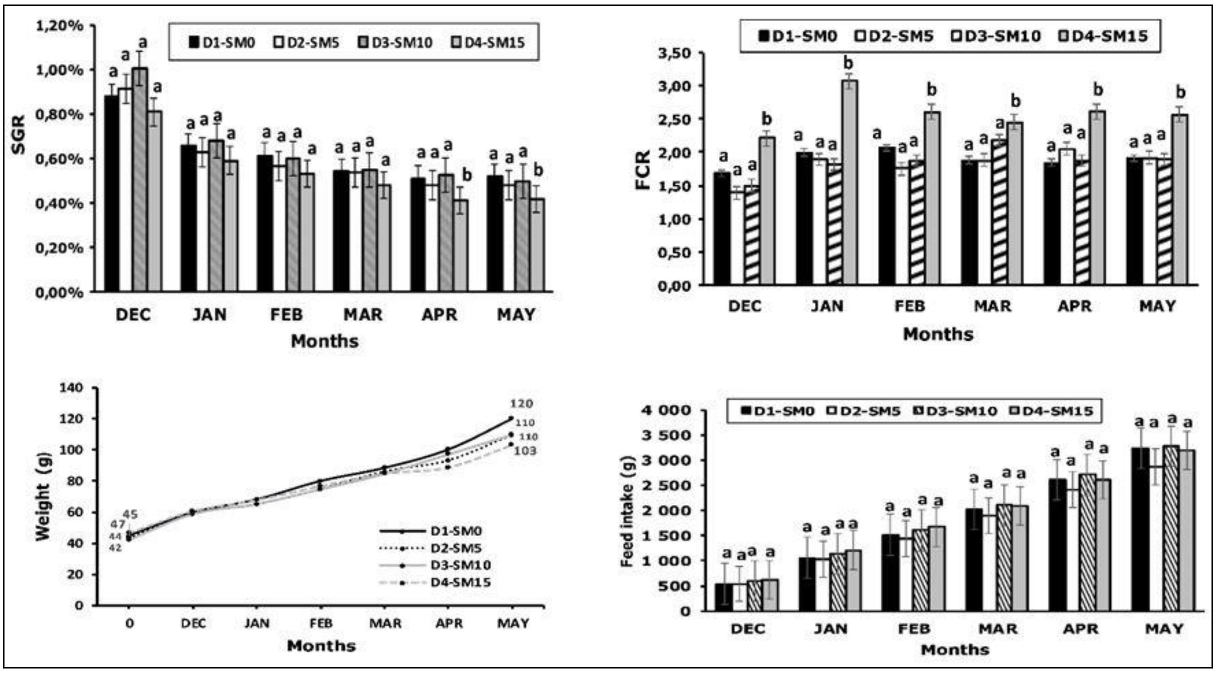

Figure 4. Weight, SGR, feed intake and feed conversion ratio of European seabass (*Dicentrarchus labrax*) juveniles fed on diets with different % shrimp meal levels along experiment. The values expressed in mean ± SD. Different superscript letters are significantly different (P<0.05) based on Tukey's multiple-range test (one-way ANOVA).

No significant differences were recorded between used diets in terms of feed intake and feed conversion ratio (FCR) during the whole experiment. However, a trend of decreased feed conversion ratio was observed because of supplementation of 15% of shrimp meal on fishes fed D4-SM15 compared to those fed other three diets.

It was noticed that, in the first four months, no differences were reported between the four diets in terms of specific growth rates; but after the fourth month, specific growth rate of fishes fed D4-SM15 decreased apparently (Figure 4).

Protein efficiency ratio (PER) and energy efficiency ratio (EER). Results showed no significant differences were found between the four diets in terms of PER and EER. However, values of both recorded ratios trended to decrease with the high level of inclusion of shrimp meal. At the end of the experiment, PER ranged from 0.93 to 1.26. Highest value (1.26) was obtained for fishes D2-SM5 and D3-SM10, followed by those fed on D1-SM0 (1.24); the lowest value was recorded for D4-SM15 (0.93). EER ranged from 0.07 to 0.10, D1-SM0, D2-SM5 and D3-SM10 have had the highest value (0.10) while the lowest value was recorded by Fishes fed D4-SM15 (0.07) (Figure 5).

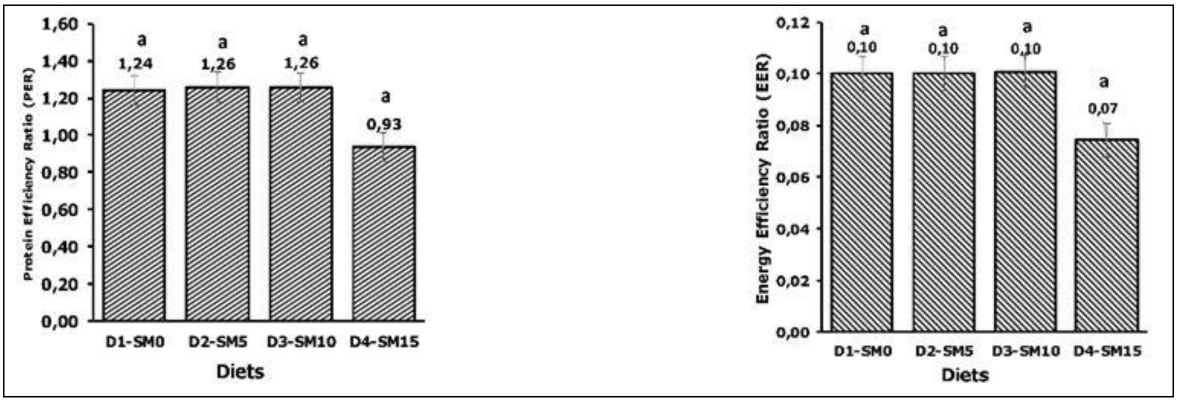

Figure 5. Protein efficiency ratio (PER) and energy efficiency ratio (EER).

Discussion. The present study aimed at investigating the potential of shrimp by-products of *Pandalus borealis* as FM partial alternative in practical European seabass diets during six feeding months with three experimental diets characterized by different substitution level (11%, 22%, and 33%) compared to control diet (0%).

Reported approximate composition of *Pandalus borealis* shells meal (Shahidi & Synowiecki 1991) is composed of 1.1-2.3% in lipid content (wet weight) and 41.90% in protein content (dry weight). Kim et al (2016) pointed out that lyophilised shrimp by-products had contents of 12.19% lipid and 44.50% protein. Dave et al (2020) found that lipid content was 8.12% and protein content was 50.65%.

During the six-month feeding, all fishes accepted well the experimental diets, which had no effect on fish survival, neither on the condition factor. Feed intake of the four diets was similar with no significant difference, indicating that these diets were widely palatable, and in line with nutritional requirements of European seabass (Wilson 2002).

SGR and FCR values, obtained in the present work, fall well within the range of those found in many studies. SGR values are between 0.23 and 1.63%/day (Yilmaz & Eroldoğan 2015; Özşahinoğlu et al 2013; Eroldoğan et al 2012) while those of FCR are between 1.11 and 3.48 (Scerra et al 2016; Yilmaz & Eroldogan 2015; Özşahinoğlu et al 2013).

In the present study, there was no significant difference in growth performance and feed conversion rate of European seabass fed with 10% diet SSM. A similar improvement has been reported in previous studies showing SSM diet improving growth performance and protein utilization of many fish species such as red porgy (*Pagrus pagrus*) with 16% SSM dietary inclusion (Kalinowski et al 2007) and large yellow croaker (*Larimichthys croceus*) with 12% SSM (Yi et al 2015).

The lowest results recorded in growth and feed utilization of European seabass fed D4-SM15 may be due to the low digestibility and utilization of SSM, because compared to fishes fed SM0 and SM10, SM15 showed lower PER and higher FCR (Table 4). Yi et al (2015) found similar results in his study, where adding SSM at 24% to large yellow croaker diet reduce growth and feed utilization. Shahidi and Synowiecki (1991) reported that amino-acid composition of shrimp shell proteins is very balanced, meaning they can be used as excellent ingredients in aquafeeds. However, high levels of chitin (17-23%) and ash (28-34%) are also found in shrimp shells (Rødde et al 2008), which have been shown to cause low protein digestibility (66.7%) (Ibrahim et al 1999) and energy (41.4%) apparent digestibility coefficients in Atlantic cod (*Gadus morhua*) (Tibbetts et al 2006).

The present study shows that substitution of FM up to 22% with SSM has no significant negative effects on survival, growth, and feed utilization of European seabass.

Conclusions. In conclusion, this study showed that shrimp byproducts meal from *Pandalus borealis* could be used as a supplement and partial substitution of fishmeal in the diet of the European seabass (*Dicentrarchus labrax*). We can substitute up to 22% of fishmeal without compromising the nutritional quality of the feed, while being economical, besides it is a valorization solution to the problem of waste from shrimps husking factories compromising environmental protection. These results show a potential ingredient to substitute fish meal by partial inclusion and supplementation to improve growth performance and to enhance the immunity status of farmed fish.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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