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Evaluation of Protein Levels in Diets for Salema Porgy (*Sarpa salpa*) Juveniles, a New Candidate Species for the Mediterranean Aquaculture

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Abstract: In the present study, the effects of different dietary protein levels on salema porgy, *Sarpa salpa* (Linnaeus, 1758) juveniles were investigated. Six iso-caloric (20 kJ/g diet) diets with increasing protein levels (30, 37, 40, 47, 50, and 57%) were formulated. Each test diet was randomly fed to triplicate groups of 13 juvenile fish (initial mean weight 19.28±0.13 g) to satiety over 90 days. Growth performance and feed utilization were best with low dietary protein levels of 30 and 37%, but decreased with diets containing protein levels over 40%. Ammonia nitrogen excretion showed an increasing trend as dietary protein levels gradually increased, whereas retention rates of ammonia nitrogen per intake were highest in the low protein groups of 30 or 37%. The analyses of specific growth rate by broken-line regression indicated that the optimal dietary level of protein for salema porgy juvenile were 33.6% under the conditions applied in this study. As a result, *S. salpa* demonstrated better growth with low protein diets, showing that this marine fish could be a promising candidate for a sustainable and environment friendly aquaculture industry.

Keywords: Salema *Sarpa salpa*, Protein Requirement, Growth Performance, Feed Efficiency, Nitrogen Retention

1. Introduction

The growing trend of marine aquaculture in southern European seas has doubled its production in the last ten years and reached about 276.000 tons with a total income of 1.783.000.000 US dollars in year 2014 [1]. It is estimated that the world population might reach 8.5 billion in year 2030 [2], where the need for human food will increase drastically. The aquaculture industry, with its increasing trend seems to be capable to supply an important amount of the food demand for human consumption. Nevertheless, seabream and seabass are the two main fish species in the Mediterranean with a production around 200.000 tons in Greece and Turkey [1], and the sales value of these two species are in pressure due to the high production rate and limited product diversity in the market. The introduction of new fish species in the market may trigger the demand and expand product diversity with new market opportunities. Salema porgy (*S. salpa*) is a member of the Sparidae family, known as a herbivorous fish species, feeding on plants, distributed around seagrass such as *Posedonia sp*. or *Cymodocea sp*. near the shore on sandy, or rocky sea bottom [3, 4]. Salema porgy can be found in a wide range of area from shallow waters to 70 m deep water layers in the eastern part of the Atlantic (from the North Sea to Cape of Good Hope, the Canaries and Cape Verde Islands, in the Mediterranean and the Black sea), and in the western part of the Indian Ocean (from Mozambique to Cape of Good Hope) [5, 6]). Schooling behaviour of salema porgy around cage farms in the Mediterranean has been reported in an earlier study [7], feeding on uneaten pellets that disperse from the fish pens, which is an indication that salema porgy can easily adapt to artificial pellet diets. From this point of view, salema porgy might be potential marine fish species for the Mediterranean aquaculture industry.

In contrast to gilthead seabream, or other sparid fishes, lower dietary protein requirements of salema porgy could be expected due to its herbivorous nature. Considering the rapid expansion of the world aquaculture industry and the disorganized or irregular state of the global capture fisheries which supplies the ingredients for aqua-diets, the demand for fishmeal and fish oil is likely to increase significantly. During the 2010-2030 period prices are expected to increase by 90% for fishmeal and 70% for fish oil according to [8]. For a sustainable development of the aquaculture industry, a gradual decline of capture fisheries as protein supply for fish feed production has been reported as essential [9]. Based on the increase of global fishmeal costs, an expected decrease of fish meal usage in aqua-diets in the long term has been reported by [10]. Due, nowadays researchers have intensified their studies on the replacement of fishmeal or fish oil by less expensive alternative sources [11]. Besides, Msangi et al. [8] suggested that in the face of higher fishmeal and fish oil prices, the substitution of fish species with less fishmeal requirements should also be considered and preferred for the marine aquaculture industry. Salema porgy (*S. salpa*) is a sparid fish, frequently seen around aquaculture cage farms in the Mediterranean and the Aegean Seas. Neofitou [12] reported that, salema together with striped mullet (*Mugil sephalus*) and white trevally (*Pseudocaranx dentex*) captured around fish cages in the Mediterranean had a stomach with pellets in great quantities. Furthermore, schooling behaviour and feeding on uneaten pellets around floating cage farms for salema in the Mediterranean has also been reported by [7]. These observations strengthens the potential of salema porgy as a candidate marine fish species for the Mediterranean aquaculture industry.

Several reports on the ecology, reproductive biology, age-growth variation or geographic distribution of the wild populations of salema porgy [3, 13-16] are available, however, information on their nutritional requirements relative to their feeding habits is still lacking and needs to be provided. Hence, this is the first attempt to assess the protein requirements and fed utilization of salema porgy with reference to growth performance, fish body bio-chemical composition and nitrogen budget under controlled culture conditions.

2. Materials and Methods

2.1. Experimental Fish and Rearing Conditions

The feeding trial was conducted in a marine recirculating aquaculture system. Initial and final fish were weighed individually (precision 0.01 g). At intervals of 30 and 60 days during the course of the feeding trial however, fish were mass weighed in buckets filled with seawater in order to avoid handling and netting stress. Before weighing, fish were deprived of feed for one day. Experimental fish with initial mean weight of 19.28±0.13 g were placed into 18 circular polyethylene tanks with a water volume of 200 L. A factorial design of 6x3 was applied and a total of 234 fish were randomly stocked in six groups of tanks with 13 fish per tank, and 3 replicates per treatment. Experimental fish were adapted for a period of 1 month to the culture conditions prior to start of feeding trial, which was initiated when all fish accepted pellets. Seawater was supplied to the tanks at a flow rate of 28 L/min. Aeration was continuously supplied by air-stones and the photoperiod regime was a natural light course (40° 04' 37.47" N - 26° 21' 39.04" E). Throughout the feeding trial, ambient water parameters such as temperature, salinity, dissolved oxygen, pH were measured periodically using a YSI multi-probe water analyser. Total ammonia nitrogen (NH_3-N) was determined by the Nessler method using a portable spectrophotometer.

2.2. Experimental Diets and Feeding

Practical diets were formulated and produced with commercially available ingredients. All the test diets were formulated to be iso-caloric on a gross energy (20.0 kJ/g diet) basis and to contain increasing levels of protein (30, 37, 40, 47, 50 and 57%). Total n-3 highly unsaturated fatty acid (HUFA) contents averaged 3.6 g/kg for all test diets. Brown fish meal (anchovy, Blacksea origin) was used as a sole protein source. Ingredients and chemical composition of test diets are given in Table 1, and the amino acid profiles of the experimental diets are presented in Table 2.

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

Experimental diet / Protein level						
Ingredient (g/kg DM)	**D1/30**	**D2/37**	**D3/40**	**D4/47**	**D5/50**	**D6/57**
Fish meal[1]	410	490	565	647	730	810
Corn starch	50	50	50	50	50	50
Dextrin	405	335	265	190	115	43
Fish oil (FO)	90	80	75	68	60	52
Vit-min mix[2]	40	40	40	40	40	40
Cholin chloride	5	5	5	5	5	5
Total	1000	1000	1000	1000	1000	1000
Proximate composition (% dry matter, except for moisture)						
Moisture	8.40	9.00	8.74	8.91	8.41	8.51
Crude protein	29.4	36.7	41.4	46.2	52.1	57.2
Crude lipid	16.0	17.9	17.1	18.3	18.4	19.7
Crude ash	4.46	5.07	6.67	7.53	8.64	9.14
NFE[3]	41.8	31.3	26.1	19.0	12.5	5.41
GE (kJ/g diet)[4]	20.1	20.7	20.6	21.0	21.3	21.8
GE (kcal/g diet)	4.80	4.96	4.93	5.02	5.08	5.20
P:E (mg/kJ)[5]	14.6	17.7	20.1	22.0	24.5	26.3
PE/TE	0.35	0.42	0.47	0.52	0.58	0.62
Crude lipid in FM (%)	8.50	8.50	8.50	8.50	8.50	8.50
Lipid from FM (%)	3.49	4.17	4.80	5.50	6.21	6.89
Σ FO in diet (%)	12.5	12.2	12.3	12.3	12.2	12.1
n-3 HUFA in FO (%)[a]	29.8	29.8	29.8	29.8	29.8	29.8
Σ n-3 HUFA in diet (%)	3.72	3.62	3.66	3.66	3.63	3.60

[1]Anchovy meal, Blacksea-Turkey
[2]Vitamin mixture (per 1 mg): Vit.A 65.000 IU, Vit.D3 45.000 IU, Vit.E 25 IU; Vit.K3 5 mg, Vit.B1 12.5 mg, Vit.B2 12.5 mg, Vit.B6 15 mg, Vit.B12 0.025 mg and ascorbic acid 120 mg; Mineral mixture (per 1 mg): Ca 100 mg, P 50 mg, K 30 mg, Na 20 mg, Mg 10 mg, Fe 22 mg, Zn 3 mg, Mn 3 mg, Cu 1.8 mg, Co 0.15 mg, I 0.12 mg, Se 0.05 mg, DL-calcium pantothenate 40 mg, niacin 50 mg, folic acid 2.5 mg, biotin 0.08 mg and inositol 75 mg.
[3]Nitrogen free extract = 100 - (crude oil + crude ash + crude protein)
[4]Gross energy; calculated based on energy fuels of 23.6 kJ/g protein, 39.5 kJ/g lipid and 17.2 kJ/g NFE.
[5]Protein-enerji ratio = mg protein / kJ energy
[6]PE/TE = energy from protein / total energy
Σ n-3 HUFA in diet (g/kg) = (Σ fish oil in diet, g/kg) x (% n-3 HUFA in fish oil used)

***Table 2.** Amino acid profiles of test diets with increasing levels of protein (g/16 g N).*

Amino acid	Experimental diet / Protein level						
(%/dry matter)	Fish meal[a]	30	37	40	47	50	57
Arginine	4.11	1.69	2.01	2.32	2.66	3.00	3.33
Lysine	5.49	2.25	2.69	3.10	3.55	4.01	4.45
Histidine	1.76	0.72	0.86	0.99	1.14	1.28	1.43
Isoleucine	3.38	1.39	1.66	1.91	2.19	2.47	2.74
Leucine	5.43	2.23	2.66	3.07	3.51	3.96	4.40
Valine	3.81	1.56	1.87	2.15	2.47	2.78	3.09
Methionine	2.16	1.16	1.38	1.59	1.82	2.06	2.28
Phenylalanine+Tyrocine	5.47	2.24	2.68	3.09	3.54	3.99	4.43
Threonine	3.00	1.23	1.47	1.70	1.94	2.19	2.43
Triptophan	0.82	0.34	0.40	0.46	0.53	0.60	0.66
ΣEAA	35.4	14.8	17.7	20.4	23.4	26.3	29.2

[a] according to Halver (1991)
N/A = not available

Initially, all ingredients including oil were mixed with a food mixer for 20 min, then tap water was added in order to prepare a suitable pulp, that was made into a 2 mm sized pellets with a meat grinder. The pelleted diets were then dried to a moisture content of 80-90 g/kg at 40°C in a drying chamber. The test diets were then stored in a freezer (-25°C) until use. Experimental fish were hand fed until satiation twice a day at 09:00 and 16:30 hours for a total of 90 days. Special attention was given to be certain of the even distribution of pellets by all fish in the tanks, and feeding lasted for about 15-20 min. When fish refused feeding, it was accepted as a sign of satiety and feeding was stopped in order to avoid overfeeding. In all tanks, the feed intake was recorded daily by subtracting the feed distributed from the initial weight of feed.

2.3. Sampling and Analytical Methods

Prior to the start of the experiment, 10 fish from the initial pool were anesthetized in a high dose MS-222 (100 mg/L) and stored in polyethylene bags in a freezer (-25°C) for subsequent analysis. At the end of the experiment, the same protocol of sampling was followed for each tank. Five fish per tank (15 fish per treatment) were randomly withdrawn for comparative analysis of fish whole body (dry matter, protein, lipid, ash) and calculation of nutrient retention rates and nitrogen budget. All analyses were performed in triplicate and samples were prepared by homogenizing fish whole body in a kitchen blender. Chemical analyses of test diets and fish whole body were conducted according to [17] guidelines as follows: for dry matter, drying in an oven at 105°C for 24 h until constant weight were obtained; for protein (Nx6.25) by Kjeldahl method after acid digestion; for lipids by ethylether extraction in a Soxhlet System; for ash by incineration in a muffle furnace at 550°C for 12 h. The NFEs were calculated by substracting the sum of protein, lipid and ash from hundred.

2.4. Statistical Analysis

The results were given as mean ± standard deviation (SD) and differences of group means were compared by one-way ANOVA. Significance level of $p < 0.05$ was applied for all data. In order to figure out the optimum dietary protein level that matches with the maximum growth rate, a third order polynomial regression between dietary protein and growth rate values was applied [18].

3. Results and Discussion

At the end of the 90 days growth experiment, survival rates were over 85% for all treatment groups, indicating that dietary protein levels did not affect fish survival. Best growth performance in salema porgy juveniles were obtained when fed a diet with 37% protein. This was followed by the 30% and 40% diet groups, respectively. No significant difference ($p>0.05$) was found between final body weight of fish fed the 37% protein diet and those fed diets with 30% or 40% protein levels. However, dietary protein levels over 40% significantly ($p<0.05$) reduced the growth rates (Figure 1). The best specific growth rates (SGR) were obtained in fish fed the 37% protein diet, which demonstrated significantly better ($p<0.05$) performance compared to the higher protein diet. Eventhough there was no significant difference in SGRs between the 30% and 37% protein diets, the latter performed about 15% better than the 30% dietary protein group. A gradual decline was observed in percent feed intakes whith the decrease in dietary protein levels. The highest feeding rate of 0.69% ($p<0.05$) was recorded in fish fed diets containing 37% protein. Based on the polynomial regression analyses [18] used for the relation between dietary protein levels and the SGRs, it was recorded that the optimum protein requirement for juvenile salema porgy was about 33.6% of the diet under the conditions applied in this study (Figure 2). The values for protein efficiency rates (PER) followed the same trend, with higher rates ($p<0.05$) for the best performing diet groups of 30% and 37%, which demonstrated significantly lower ($p<0.05$) feed conversion rates (FCR) compared to the higher protein diets (Table 3).

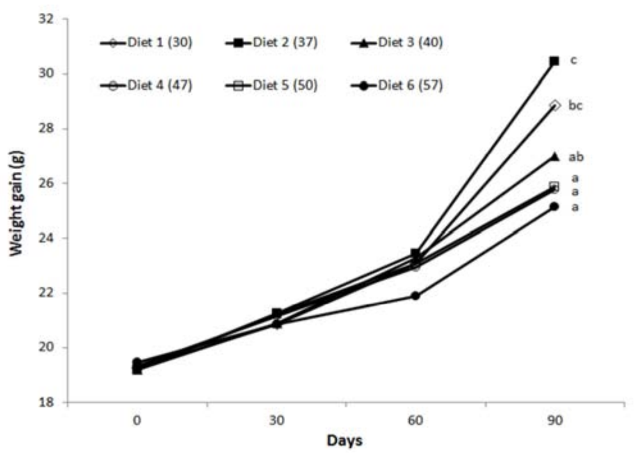

***Figure 1.** Growth of salema progy fed experimental diets. Values with different letters are significantly different (p<0.05).*

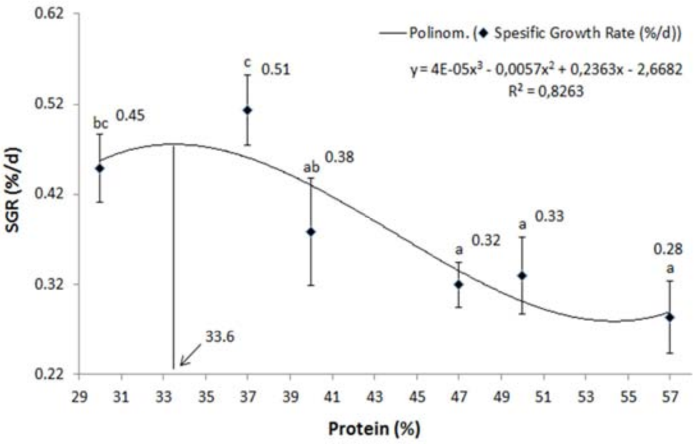

Figure 2. *Optimum dietary protein requirement of salema porgy juvenile by polynomial regression between dietary protein levels and specific growth rates (SGR). Values with different letters are significantly different (p<0.05).*

Table 3. *Growth and feed utilization of salema porgy fed the experimental diets for 90 days (means ± SD)*[*].

Experimental diet / Protein level						
D1/30	**D2/37**	**D3/40**	**D4/47**	**D5/50**	**D6/57**	
IBW	19.3±0.08	19.2±0.04	19.2±0.04	19.3±0.10	19.2±0.07	19.5±0.14
FBW	28.9±1.03^{bc}	30.5±1.12^{c}	27.0±1.45^{ab}	25.8±0.44^{a}	25.9±1.09^{a}	25.2±1.04^{a}
SGR	0.45±0.04^{bc}	0.51±0.04^{c}	0.38±0.06^{ab}	0.32±0.02^{a}	0.33±0.04^{a}	0.28±0.04^{a}
FI	0.59±0.03^{b}	0.69±0.02^{c}	0.61±0.04^{b}	0.51±0.02^{a}	0.55±0.02^{ab}	0.52±0.03^{a}
FCR	1.35±0.18^{ab}	1.24±0.12^{a}	1.66±0.35^{ab}	1.89±0.63^{ab}	1.71±0.26^{b}	1.88±0.37^{b}
PER	2.56±0.33^{c}	2.22±0.23^{c}	1.50±0.32^{b}	1.22±0.35^{ab}	1.14±0.18^{ab}	0.96±0.19^{a}
SR	87.18	92.31	89.74	84.62	84.62	87.18

[*] Values with different superscript letters in the same line are significantly different at p<0.05 level
IBW: initial body weight (g); FBW: final body weight (g)
SGR (specific growth rate,% growth per day) = [(lnW2 - lnW1) / (t2-t1)] x 100
FI (percent feed intake,% per day) = (total feed intake / ((W1+W2) / 2) / day) × 100
FCR (feed conversion rate) = feed intake (g) / weight gain (g)
PER (protein efficiency rate) = weight gain (g) / protein intake (g)
SR (survival rate,%) = (number of remaining fish / number of initial fish) x 100

During the first 2 month of the trial, growth of salema porgy was relatively low, however after the 60 days of the feeding trial, the growth showed an increasing trend compared to the initial performance. Since the culture conditions were the same throughout the study, the acceleration of growth performance in the second month with an increasing trend in the third month of the trial might be attributed to the week adaptation of salema juveniles in tank environment. Eventhough the experimental fish were adapted for a period of 1 month to the culture conditions, and the feeding trial initiated when all fish accepted pellets, it seems that salema juveniles might need a longer acclimatization period to tank conditions of certain sizes. For instance, the best performing group in the present study showed a SGR of 0.5%/day throughout the feeding trial, while fish growth during the last period of 30-days resulted in an increased growth of 0.9%/day. The accelerated increase of growth performance after the 60-days of the feeding trial might be an indication for a better growth performance of salema when a longer adaptation period was applied.

The maximum SGR (0.51%/day) obtained for salema porgy in the present study was higher than an earlier report on axillary seabream (*Pagellus acarne*) (0.23%/day) [19], another candidate sparid fish for aquaculture. Korkut and Balkı [20] reported SGR variation of between 0.32 and 1.04%/day for gilthead seabream under commercial cage farm conditions in the Aegean Sea. Similar to the findings in the present study for salema porgy, Sá et al. [21] and Coutinho [22] also reported other sparid candidates such as white seabream (*Diplodus sargus*) (0.89%/day) and zebra seabream (*Diplodus cervinus*) (0.8%/day) as slow growing marine species, respectively. On the other hand, higher SGRs of 1.22%/day [23] and 1.54%/day [24] were recorded for two-banded seabream (*Diplodus vulgaris*) and sharpsnout seabream (*Diplodus puntazzo*), respectively.

Physico-chemical water parameters recorded in the present study were comparable and within the acceptable limits reported by [25] for a recirculating aquaculture system (Table 4). Water temperature during the course of the present study (12.76±2.24°C) was relatively low compared to the data reported in the southern Aegean Sea where the average annual seawater temperature is in a range between 12.4-25°C [26]. Discrepancies between different reports might be due to rearing conditions such as water temperature, dissolved oxygen, salinity, fish stocking rates, feeding methods, or a combination of these factors [27, 28], as well as to fish species and fish size [29].

Table 4. *Acceptable limits for different physico-chemical water quality parameters and recorded values in the present study.*

Parameter	Unit	Acceptable limits*	Present study
pH	-	6.5-7.5	7.55±0.6
Temperature	°C	Species specific	12.76±2.24
Salinity	Ppm	Species specific	25.64±0.84
Oxygen	O_2,% (mg/L)	70-100	88 (7.98±1.3)
Ammonium	NH_3, mg/L	0-2.5 (pH influenced)	0.27±0.06

* Bregnballe, 2015

Dietary protein requirements estimated based on growth performance (percent growth per day) or nitrogen retention (per N-intake) corresponded to 37% and 30%, respectively. Similar to the findings in the present study, protein requirements for two-banded seabream (*Diplodus vulgaris*) were reported between 35-36% in earlier studies [23, 30], while a lower protein level of 27% was recorded for juvenile white seabream (*Diplodus sargus*) [21]. In contrast, higher protein levels for best growth were found for other sparids such as Blackfin seabream (*Acanthopagrus berda*) (42%) [31], zebra seabream (*D. cervinus*) (43.8%) [22], and sharsnout seabream (*Diplodus puntazzo*) (43-47%) [24, 32]. Tacon and Cowey [33] reported that generally fast growing fish species require higher protein levels in their diets compared to the slow growing species. Apart from this, carnivorous fish species such as gilthead seabream (*S. aurata*), European seabass (*Dicentrarchus labrax*), rainbow trout (*Onchorhynchus mykiss*), common dentex (*Dentex dentex*), Japanese flounder (*Paralichthys olivaceus*), or the red porgy (*Pagrus pagrus*) are reported to require higher levels of dietary protein (45–55%) [34-39]. The lower protein requirement of salema porgy compared to other sparids or other marine fishes could be attributed to their herbivorous feeding nature, whereas the others requiring higher protein diets are either carnivorous or omnivorous species. This is in agreement with the statement of [40], indicating that carnivorous fishes have higher protein requirements compared to omnivorous or herbivorous species.

The regulation of feed intake in order to meet energy demand is general condition for fish [41]. This condition has also been reported for white seabream [21], in zebra seabream [22], in sharpsnout seabream [24, 42], and in Blackfin seabream [31]. The finding in the present study for the feed intake in salema porgy is in agreement with earlier reports, in terms of increased feed intake ($p<0.05$) with the decrease of protein levels in the diets. In the present study, the FCRs of salema porgy linearly increased with the declining dietary protein levels. This is in agreement with the findings in white seabream [21], in sharpsnout seabream [24], and in Blackfin seabream [31]. In contrast, increased FCRs in zebra seabream [22] and gilthead seabream [43] were reported with increased dietary protein levels. The FCRs found in this study (1.24-1.89) were comparable with those reported earlier for two banded seabream (*D. vulgaris*) (1.50-1.80, 1.36-2.96, 1.67-1.92) [30, 44, 45], in zebra seabream (1.69-3.33) [22], and in gilthead seabream (0.91-3.06, 1.14-3.73, 1.22-1.74, 1.24-1.48, 1.37-1.53) [20, 27, 28, 43, 46], respectively. Higher FCR of 2.51 was found in axillary seabream (*Pagellus acarne*), introduced as a new candidate species for the Mediterranean aquaculture by [19], while lower FCRs of 1.1-1.2 were reported in gilthead seabream [47]. Similar to the growth performance recorded in the present study, FCRs and PERs followed the same trend with better results in fish fed lower protein diets. These findings in the present study were in agreement with earlier reports on European eel *Anguila anguila* [48], Yellow snapper *Lutjanus argentiventris* [49], and two-banded Seabream *D. vulgaris* [30] in terms of decreasing PERs with increased dietary protein levels.

The nitrogen retention rates per intake in fish fed diets with 30% and 37% protein were significantly ($p<0.05$) higher than those fed the higher protein diets. In contrast, excretion rate of nitrogen per intake were lowest for the fish fed on lower protein diets of 30 and 37% and showed a significant increase when dietary protein levels rose over 40% (Table 5).

Table 5. *Nitrogen (N) balance (mg/g production) of salema porgy fed experimental diets for 90 days (means ± SD)*.*

Experimental diet / protein level						
N balance						
D1/30	**D2/37**	**D3/40**	**D4/47**	**D5/50**	**D6/57**	
NI	73.5±18.4^{a}	83.4±12.1^{a}	110.2±23.5^{ab}	119.8±12.7^{b}	142.2±21.4^{bc}	171.6±33.6^{c}
NR	30.1±2.54^{a}	30.3±1.83^{a}	31.2±5.81^{a}	31.1±2.47^{a}	27.9±2.97^{a}	30.0±5.16^{a}
TNE	43.4±17.3^{a}	53.0±13.9^{ab}	79.0±17.7^{bc}	88.7±10.9^{c}	114.3±18.4^{cd}	141.6±28.6^{d}
NR (%NI)	42.4±8.7^{d}	37.1±7.12^{d}	28.4±0.90^{c}	26.0±0.73^{c}	19.7±0.99^{b}	17.6±0.81^{a}
TNE (%NI)	57.6±8.7^{a}	62.9±7.12^{a}	71.6±0.90^{b}	74.0±1.73^{b}	80.3±0.99^{c}	82.5±0.81^{d}

* Values with different superscript letters in the same line are significantly different at $p<0.05$ level.

NI (nitrogen intake, mg/g production) = (protein intake / 6.25) / (W2 - W1)

NR (nitrogen retention, mg/g production) = (total g protein retained in fish / 6.25) / (W2 - W1)

TNE (nitrogen total excretion, mg/g production) = (nitrogen intake(g)-nitrogen retention(g))/(W2-W1)

NR (%NI, nitrogen retention as percent of nitrogen intake) = 100 x (N retention / N intake)

TNE (%NI, nitrogen total excretion as percent of nitrogen intake) = 100 x (N excretion / N intake)

W2: final fish weight (g), W1= initial fish weight (g)

The excessive supplement of dietary animal proteins may result in increased nitrogen excretion. The incorporation of dietary animal protein or lipids at an optimum level may support the aquaculture industry economically and environmentally [23]. In the present study, dietary protein levels over 40% resulted in a significant increase of nitrogen excretion, which can be explained by the elevated protein catabolism led to higher ammonia excretion rates in fish fed excessive dietary protein. This finding was also supported by the PERs in the present study with better protein utilization when fed diets lower than 40% protein. The findings in this study regarding nitrogen retention rates per intake (37-42%) in best performing protein groups are in close agreement with earlier reports in European seabass fed different ration levels (36-43%) [50], in rainbow trout (18-46%) [51], Atlantic turbot (28-36% and 36-42%) [52, 53], the Black Sea turbot (38-40%, 19-41%, and 29-30%) [54-56]. Lower retention rate of nitrogen per intake have been reported in Blackfin seabream (20-40%) [31], zebra seabream (19-26%) [22], European seabass (23-32% and 16-26%) [57, 58]. Wilson [59] reported that the optimal protein level in fish diet might be affected by the amino acid composition of the test proteins. In earlier studies, it has been reported that feeding fish with diets over the requirement level may result in an increased protein catabolism [60], induced with the increase of hepatic activity of alanine aminotransferase, aspartate aminotransferase, and glutamate dehydrogenase enzyme activities [61-63]. In the present study, eventhough enzyme activities were not investigated, the reason for the higher nitrogen excretion rates in experimental fish fed higher levels of dietary protein might be attributed to the increased protein catabolism due to the excessive protein levels in the diets.

As a new candidate species for the aquaculture sector, there is no data available on the essential amino acid (EAA) requirements of salema porgy. Considering the best performing diet of 37%, and the reduction in fish growth when fed in excess of requirements, might also be linked to an excessive dietary EAAs for the test diets containing protein levels over 40%. Hence, based on the findings in the present study, it might be assumed that the amino acid profile of the best performing diet (37%) is close to ideal EAA profile for salema porgy juveniles. Because, at this level of dietary protein, there were no limitation of amino acids in the test diets, that otherwise could have resulted in growth limitations of fish. Actually, fish diets below ideal protein profile lacking in one or more EAA can lead to reduced feed intake and growth performance, depress protein or amino acid retention, due to higher protein and amino acid catabolism, which in turn lead to increased nitrogen waste and deterioration of environment waters [22, 31, 51, 57, 60, 64].

A slight decrease in fish whole body protein was observed when dietary protein levels increased over 40% level, however no significance (p>0.05) was recorded among the experimental groups. Crude lipid contents of fish body followed the same trend with no significant differences (p>0.05) among test groups. Fish body ash contents tended to increase with increasing levels of dietary protein, however these differences were not significantly (p>0.05) important as well (Table 6). The hepatosomatic index (HSI) in salema porgy juveniles fed diets with 47, 50, and 57% protein were higher than those of the 30, 37, and 40% dietary protein groups. However, the increasing trend of the HSIs observed here was not significantly (p>0.05) important (Table 7).

Table 6. *Whole body proximate composition (dry basis, except for moisture) of salema porgy fed experimental diets for 90 days (means ± SD)*[*].

Diets	Moisture (%)	Crude Protein (%)	Crude lipid (%)	Crude ash (%)
Initial	75.2±0.28	72.2±0.95^a	13.4±1.17	12.2±0.51
D1/30	75.8±0.32	75.1±0.77^b	12.1±0.59	12.1±0.23
D2/37	75.7±0.40	75.3±0.84^b	12.0±0.40	11.9±0.30
D3/40	75.5±0.45	74.9±0.80^b	12.1±0.68	12.2±0.46
D4/47	75.6±0.43	74.6±0.27^b	12.0±0.59	12.3±0.53
D5/50	76.1±0.63	74.6±0.48^b	11.5±1.15	12.8±0.69
D6/57	75.9±0.49	74.3±0.50^b	11.9±1.17	12.8±0.81

[*] Values with different superscript letters in the same row are significantly different at p<0.05 level.

Table 7. *Body morphological indices of salema porgy juveniles fed experimental diets for 90 days (means±SD)*[*].

Diets	HIS	VSI
Initial	1.68±0.66	6.91±1.14
D1/30	1.52±0.27	6.11±0.52
D2/37	1.51±0.50	6.36±0.87
D3/40	1.53±0.21	6.37±0.41
D4/47	1.60±0.64	6.71±0.92
D5/50	1.83±0.39	7.27±0.67
D6/57	1.66±0.16	6.49±0.13

[*] Values with no superscript letters in the same row are not significantly different at p<0.05 level
HSI, hepatosomatic index = (liver weight / total weight) x 100
VSI, viscerasomatic index = (viscera weight / total weight) x 100

The liver is known to have a function as the deposition site for fat and glycogen in fish [65, 66]. Dietary carbohydrates were reported to stimulate glycolysis, glycogenesis and lipogenesis, while reducing protein catabolism and gluconeogenesis [67]. In the present study, protein levels and NFEs (soluble carbohydrate of the feed) of the test diets were negatively correlated, with increasing carbohydrates at decreasing levels of dietary protein. Due, the increased carbohydrate levels in the present test diets with lower dietary protein might have stimulated the lower protein catabolism. It has been reported that HSI is positively correlated with dietary carbohydrate levels, while inversely related to dietary protein [22, 24, 68, 69]. In the present study however, an adverse relation between HSI and carbohydrate level, but positive correlation with dietary protein levels were observed. The VSI showed similar trend as the HSI in the present study.

Considering that the best growth was obtained with the low protein diets (30-40%), which were higher in NFEs (23-38% vs 2-15%) but lower in protein to energy (P:E) ratio (15-20 mg/kJ vs 22-27 mg/kJ), compared to the higher protein diets (47-57%) might be attributed to the herbivorous nature of salema porgy and also linked to a hypothesis that salema porgy might prefere low-protein but high-energy diets for a best growth performance, as also reported in two-banded seabream [30]. However, the experimental diets in the present study were formulated with a single lipid level. Further studies are encouraged to assess dietary lipid and carbohydrate levels for salema porgy with experimentations at different water temperature regimes.

4. Conclusion

In the present study, optimum dietary protein requirement of salema porgy juveniles by polynomial regression between protein levels and growth rates was found as 33.6%, indicating that this low level of dietary protein is optimum for maximum growth and feed conversion ratio in salema porgy juveniles. Increasing the dietary protein over 40% seems to induce a decline on weight gain, and negatively affect the protein efficiency as well as nitrogen retention rates. As a marine fish species with low protein requirements, salema porgy might be a promising candidate for the Mediterranean aquaculture industry, with the less use of fishmeal based protein sources, that in long run might benefit the global aquaculture in terms of economically sustainable and environment friendly way.

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