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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

# The effect of feeds containing different protein levels on growth and survival rates of European sea bass (*Dicentrarchus labrax* L., 1758) juveniles grown in freshwater

## Farklı protein seviyesi içeren yemlerin tatlısuda yetiştirilen Avrupa levreği (*Dicentrarchus labrax* L., 1758) yavrularının büyüme ve yaşama oranları üzerine etkisi

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**Abstract:** In this study, the effect of the use of feeds containing different protein ratios on the growth performance of juvenile sea bass (*Dicentrarchus labrax* L., 1758) reared in freshwater was investigated. Sea bass fry with an average body weight of 1.0 ± 0.03 g were stocked in 120 liter tanks in triplicate after their adaptation to freshwater. The feeds used in the study had different protein/similar fat content. Accordingly, the experimental groups were named as 45CP (45% CP / 18% CF), 50CP (50% CP / 18% CF) and 55CP (55% CP / 18% CF). At the end of the experiment, the highest body weight gain was 5.84 ± 0.03 g in the 55CP group, while the other groups were 5.73 ± 0.09 g (45CP) and 5.8 ± 0.08 g (50CP). SGR rates were similar for all three groups and there was no statistical difference between the groups (P>0.05). SGR values of the groups were calculated as 1.94 ± 0.04 (45CP), 1.89 ± 0.01 (50CP), 1.91 ± 0.02 (55CP), respectively. There was no statistical difference between the 45CP and 55CP groups, while the 50CP group showed a statistically lower FCR rate than the other groups (P<0.05). FCR values were calculated as 1.36 ± 0.05, 1.29 ± 0.03, 1.37 ± 0.04, respectively. There was no difference between the survival rates of the groups. According to the results of the study, it was concluded that sea bass fish can be raised in freshwater, especially in the fry stage, and that it is more effective than the commercial feeds currently used.

**Keywords:** Sea bass, *Dicentrarchus labrax*, freshwater, protein, growth performance, survival rate

**Öz:** Bu çalışmada tatlı suda yetiştirilen yavru levrek (*Dicentrarchus labrax* L.,1758) balıklarının beslenmesinde farklı protein oranları içeren yemlerin kullanımının büyüme performanslarına üzerine etkisi araştırılmıştır. Ortalama canlı ağırlıkları 1,0 ± 0,03 gram olan levrek yavruları, tatlı suya adaptasyonlarından sonra 120 litre hacimdeki tanklarda üçer tekrarlı olacak şekilde stoklanmıştır. Çalışmada kullanılan yemler farklı protein / benzer yağ içeriğindedir. Buna göre deneme grupları 45CP (%45 HP / %18 HY), 50CP (%50 HP / %18 HY) ve 55CP (%55 HP / %18 HY) olarak isimlendirilmiştir. Deneme sonunda en yüksek canlı ağırlık artışı 5,84 ± 0,03 g ile 55CP grubunda iken diğer gruplarda 5,73 ± 0,09 g (45CP), 5,8 ± 0,08 g (50CP) olarak tespit edilmiştir. SGR oranları her üç grup için benzer oranlarda olup gruplar arasında istatistiki bir farklılık bulunmamaktadır (P>0,05). Grupların SGR değerleri sırası ile 1,94 ± 0,04 (45CP), 1,89 ± 0,01 (50CP), 1,91 ± 0,02 (55CP) olarak hesaplanmıştır. FCR değerlerine bakıldığında 45CP ve 55CP grupları arasında istatistiki bir farklılık tespit edilmemişken, 50CP grubu istatistiki olarak diğer gruplardan daha düşük bir FCR oranı göstermiştir (P<0,05). FCR değerleri sırası ile 1,36 ± 0,05, 1,29 ± 0,03, 1,37 ± 0,04 olarak hesaplanmıştır. Grupların yaşama oranları arasında bir farklılığa rastlanmamıştır. Çalışma sonucuna göre özellikle yavru dönemlerinde levrek balıklarının tatlı suda yetiştirilebileceği, halen kullanılmakta olan ticari yemlere göre daha düşük oranlarda protein içeren yemlerle de balıkların beslenebileceği gözlenmiştir.

**Anahtar kelimeler:** Levrek, *Dicentrarchus labrax*, tatlı su, protein, büyüme performansı, yaşama oranı

## INTRODUCTION

Aquaculture production is increasing rapidly all over the world. World aquaculture production in 2020 was 87.5 million tons in total, including 33.1 million tons of marine production and 54.4 million tons of production in inland waters (FAO, 2022). Türkiye's aquaculture production in 2022 was 514,815 tons, of which 368,742 tons were produced at sea and 146,63 tons were produced in inland waters (Turkish Statistical Institute, 2022). While most of the production in the sea in Türkiye is European sea bass and sea bream species, almost all of the production in inland waters is trout production. The limited production areas in the seas will limit the increase in production capacity in the next years. Therefore, increasing recirculated aquaculture systems in terrestrial areas is an important issue for aquaculture. Water supply is the most important issue in recirculated or open production systems built on land. Even in closed aquaculture systems, 3-10% of the total water volume needs to be replaced with new water daily. This causes additional costs and problems for facilities that are

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far from the sea or have difficult access to salty or brackish water from underground.

The adaptation period of European sea bass to freshwater has been investigated in many studies and it has been reported that direct adaptation of European sea bass to freshwater is possible, but long-term adaptation can reduce stress effects in fish (Nebel et al., 2005; Kokou et al., 2019).

The quantity and quality of protein in feed are very important for somatic growth in fish. Protein sources play a crucial role in providing adequate levels of amino acids needed to synthesize new tissue protein (Jana et al., 2021). Aquaculture with feeds with balanced essential amino acid composition and optimum protein levels will ensure better growth of fish as well as economically viable and environmentally friendly production.

In their natural habitat, the nutrient requirements of European sea bass are 48-52% crude protein and 14-16% crude fat during the fry period and 43-45% crude protein and 18-20% crude fat during the growth period (Oliva-Teles, 2000; Ghisaura et al., 2014). However, there are no studies on the optimum protein values that should be used in the diets of freshwater European sea bass fry. Understanding the interactions between growth performance and food consumption of European sea bass fry kept in freshwater is important for the success of aquaculture. For this purpose, in this study, the effects of using feeds with different protein contents on the survival and growth rates of European sea bass fry adapted to freshwater were investigated.

## MATERIALS AND METHODS

### Fish and experimental plan

Sea bass fry, which are widely cultivated in Türkiye, were used in the study. The study was carried out at Ege University Center for Research on Laboratory Animals, Aquaculture Research Laboratory. The fish used in the experiment had an average live weight of 1.0±0.03 grams and were obtained from a private hatchery (Akvatek, Çandarlı-Izmir) and transferred to the experimental unit with a fish transport tank. Three experimental groups were formed in the study. Accordingly, the fish were stocked in 9 cylindrical conical polyester tanks with a volume of 120 liters, each group was stocked with 35 individuals/tank with 3 replicates for a total of 315 individuals. The salinity in the experimental tanks was gradually reduced from 38 ppt to 0 ppt over 4 days.

The experimental system used in the study was a recirculated aquaculture system (RAS) and a sand filter, protein skimmer, biological filter and UV filter were used for water filtration. The current capacity of the system was maintained by adding freshwater to the system. Water flow rates were set at 2 l/minute so that 100% of the total volume was changed in one hour. Water temperature was kept between 21.4-22°C and dissolved oxygen between 7.1-7.3mg/l. Lighting was set to 12 hours day and 12 hours night. Water parameters (temperature, dissolved oxygen, pH and salinity) and daily mortality rates were measured and recorded every morning before feeding started. YSI P1100 model multi-parameter device was used for the measurements.

The study was initiated with the adaptation of the fish to freshwater. The study lasted 75 days and biometric measurements (body weight and total length) of the fish were measured at the beginning of the experiment, on the 25th, 50th and 75th days. Visceral and hepatosomatic index values were measured and calculated at the end of the experiment.

### Feed and nutrition

The fish adapted to freshwater were fed with 3 different proteins (45, 50, 55%) and 18% fixed lipid ratios. Feed groups and their nutrient contents are given in Table 1.

**Table 1.** Ingredients and nutritional values of the feeds used in the study

| | 45CP (45/18%) | 50CP (50/18%) | 55CP (55/18%) |
|---|---|---|---|
| **Ingredients** | | | |
| Fish Meal (65.5% CP) | 33 | 46.5 | 59 |
| Soybean Meal (48% CP) | 23 | 23 | 15.5 |
| Wheat Meal (15% CP) | 3 | 3 | 0 |
| Corn Gluten Meal (66% CP) | 10 | 10 | 12 |
| Sun Flower Meal (35.18% CP) | 15.5 | 3 | 0 |
| Fish Oil (Anchovy) | 14.5 | 13.5 | 12.5 |
| Vitamin Mix* | 0.4 | 0.4 | 0.4 |
| Mineral Mix* | 0.3 | 0.3 | 0.3 |
| Methionin | 0.1 | 0.1 | 0.1 |
| Binder | 0.2 | 0.2 | 0.2 |
| Total | 100 | 100 | 100 |
| **Chemical Composition (%DM)** | | | |
| CP % (min) | 45.26 | 49.73 | 54.57 |
| CF % (min) | 18.29 | 18.21 | 18.16 |
| CS % (max) | 3.2 | 2.72 | 2.04 |
| DM % (min) | 92.9 | 90.83 | 90.41 |
| Ash % (max) | 12.4 | 10.56 | 10.48 |
| ME Kcal/kg** | 4308.79 | 4568.19 | 4892.73 |
| DE Kcal/kg*** | 4290.05 | 4370.93 | 4400.72 |
| NFE %**** | 13.75 | 9.58 | 5.16 |
| P/E***** (mg protein/Kcal DE) | 105.49 | 113.56 | 123.82 |

*Commercial premixes were used for European sea bass fry feeds. Vitamin A 15000 IU/Kg, Vitamin D3 2500 IU/Kg, Vitamin E 350 mg/Kg, Vitamin K 20 IU/Kg, Vitamin C 350 mg/Kg, P 1.5%, Ca 1-3%, Se 0, 1%.
**Metabolic Energy (ME) = 0.239. [10.(CP.0,9.18.64+HY.0,9.39.57+NFE.0,88.17.17)] (Kcal/kg)
***Digestible Energy (DE) = 0.239. [10.(CP.0,9.23.66+HY.0,9.39.27+NFE.0,88.17.17)] (KCal/kg)
****Nitrogen Free Extractives= 100-(%CP+%HY+%HK+%HS+%Moisture)
*****Protein Energy Ratio=[(%CP/SE(Kcal/100g))X1000]

The raw materials used in feed production were purchased from a company producing commercial fish feed. The feeds were produced as a pellet form by using a meat grinder in the Fish Nutrition and Feed Technology Laboratory. After drying, they were ground to 0.8-2mm in diameter in accordance with the size of the experimental fish.

The experimental groups were named as 45CP group (45% CP/18% HY), 50CP group (50% CP/18% HY) and 55CP

group (55% CP/18% HY). Feeding was made 3 times a day at a rate of 4% of the total body weight at the beginning of the experiment and 3% of the total body weight from the second half of the experiment due to the increase in the body weight of the fish. Feed amounts were recalculated after body weight measurements every 25 days, taking into account survival rates. Feed amounts given to the experimental tanks were recorded daily in order to determine the feed utilization rates.

Depending on the feeds, the growth performance of the fish was evaluated according to the following formulas (Halver, 1976; Metailler, 1986; Hoşsu et al., 2005; Mritunjoy et al., 2022).

Live weight gain(g)=Final weight(g)-Initial weight(g)

Feed conversion ratio (FCR)=(Total feed consumed(g))/(Body weight gain(g))

Specific growth rate (SGR,%day$^{-1}$)=[Ln(Final weight(g))-Ln(Initial weight(g))]/(Trial duration(days))×100

Protein efficiency ratio (PER)=Weight gained(g)/Crude protein consumed

Condition factor (CF)=(Total live weight(g))/(Total height(cm))$^3$x100

Visceral index (VSI)=(Visceral weight(g)/Body weight(g))x100

Hepatosomatic index (HSI)=(Liver weight(g)/Body weight(g))x100

### Statistical analysis

Live weight gain, growth performance (CF, FCR, SGR and Survival), VSI and HSI values of fish fed with diets produced with different protein and fixed fat ratios were statistically tested by analysis of variance (ANOVA). When significant differences among the variables were detected, Tukey's honestly significant difference (HSD) test was used to determine variables were different. Significance levels were set at P<0.05. The SPSS ver. 25 statistical programs were used to evaluate data.

## RESULTS

During the 4-day adaptation period, European sea bass fry showed normal behaviour, swimming and feeding activities. No mortality was observed during the freshwater adaptation period.

Since the experimental tanks operated in a closed-water system, no big changes were observed in water temperatures. Accordingly, the average water temperature was determined as 21.8±0.1˚C. During the experiment, the lowest water temperature was 21˚C and the highest water temperature was 22.4˚C. The average dissolved oxygen value was 7.2mg/l, with a minimum of 6.8 and a maximum of 7.7mg/l. The pH was 7.3 on average, and nitrite, ammonia and phosphorus values were observed at appropriate values.

There was a statistical difference between the 45CP group and the 55CP group in terms of body weight gain, while there was no difference between the 50CP group and other groups (P>0.05). The highest live weight gain was observed in the 55CP group with 5.84±0.03g, while the lowest weight gain was observed in the 45CP group with 5.73±0.09g. In the 50CP group, the weight gain was 5.8±0.08g. SGR rates were similar for all three groups and there was no statistical difference between the groups (P>0.05). While there was no statistical difference between the 45CP and 55CP groups in terms of FCR, the 50CP group showed a statistically lower FCR rate than the other groups (P<0.05). While there was no statistical difference between the 45CP and 50CP groups in PER values, the 55CP group was statistically different from the other 2 groups (P<0.05). It was observed that the PER value was inversely proportional to the protein value in the feed (Table 2).

When survival rates were evaluated, no difference was found between the groups (Table 2).

**Table 2.** Comparison of growth and development performance between groups

| | 45CP | 50CP | 55CP |
|---|---|---|---|
| Initial body weight (g) | 0.97±0.19$^a$ | 1.01±0.19$^a$ | 1.01±0.22$^a$ |
| Final body weight (g) | 6.71±1.24$^a$ | 6.81±0.94$^{ab}$ | 6.86±1.30$^b$ |
| Live weight gain (g) | 5.73±0.09$^a$ | 5.8±0.08$^{ab}$ | 5.84±0.03$^b$ |
| SGR | 1.94±0.04$^a$ | 1.89±0.01$^a$ | 1.91±0.02$^a$ |
| FCR | 1.36±0.05$^a$ | 1.29±0.03$^b$ | 1.37±0.04$^a$ |
| PER | 1.63±0.06$^a$ | 1.55±0.3$^a$ | 1.32±0.08$^b$ |
| Survival % | 97$^a$ | 96$^a$ | 97$^a$ |
| CF | 1.25±0.03$^a$ | 1.23±0.07$^a$ | 1.22±0.02$^a$ |
| VSI | 11.37±0.9$^c$ | 12.33±0.3$^b$ | 13.26±0.5$^a$ |
| HSI | 1.52±0.05$^a$ | 1.91±0.04$^b$ | 1.98±0.1$^b$ |

CF values were similar for all groups (P<0.05). VSI values were statistically different between all groups; (P<0.05). HSI values of the 45CP group were statistically different from the other 2 groups, while there was no difference between the 50CP and 55CP groups (P<0.05).

## DISCUSSION

Survival rates determined during adaptation to freshwater showed that European sea bass can adapt to freshwater without any problems within 4 days. Dendrinos and Thorpe (1985) reported that European sea bass fish can be adapted to a minimum of 0.5 ppt water, death occurs at 0 ppt, and feed intake and growth decreases as salinity decrease. However, in many other studies, in agreement with our study, the direct or gradual transfer of European sea bass fish to freshwater has been reported to be successful (Cataudella et al., 1991; Venturini et al., 1992; Varsamos et al., 2005; Kokou et al., 2019).

Many studies have reported different survival rates in European sea bass fry kept in freshwater, indicating that there are different physiological capacities in adaptation to freshwater within the same species (Dendrinos and Thorpe, 1985; Allegrucci et al., 1994; Jensen et al., 1998; Nebel et al., 2005; Giffard-Mena et al., 2008). Considering the results obtained in the study, the survival rate of all groups is quite high with 97%, but it is lower than the 100% value reported by

Eroldoğan and Kumlu (2002). It has been reported by various researchers that European sea bass in the Mediterranean region have genetic differences and therefore their adaptation to environmental conditions is different (Caccone et al., 1997; Allegrucci et al., 1997). The difference in survival rates may be due to the fact that the experimental fish in our study and the study of Eroldogan and Kumlu (2002) were European sea bass taken from different regions of Türkiye. Thus, more studies should be carried out on this subject.

In general, growth rate is positively correlated with protein levels in feed in many species. However, in some studies, it has been reported that fish change their feed intake due to the effect of salinity and this has an effect on growth. Fish growth is reduced or unaffected when fish are fed lower or higher feed protein than the optimum level (Talukdar et al., 2019; Kim and Lall, 2001). In such a situation, there is no protein synthesis, and there is a decrease in protein conversion efficiency, depending on the percentage of protein catabolized (Deng et al., 2014). In addition, although there is high protein in the feed, an insufficient non-protein energy source in the feed also decreases growth performance. (Winfree and Stickney, 1981). The carbon chains constituting amino acids due to the increase in the protein ratio in the feed are used in energy production. In addition, the amino parts cannot be used and must be excreted from the body. In addition, giving too much protein disrupts the amino acid balance and deamination is observed. This leads to increased nitrogen excretion and energy loss and protein efficiency decreases (Hoşsu et al., 2005). It has been reported that there is a negative correlation between protein efficiency ratio (PER) and feed protein level and feed protein/energy level ratio for almost every growth period of European sea bass. (Kousoulaki et al., 2015). In our study, it was observed that PER values decreased as the protein content of the feed increased. This may be due to the increase in nitrogen excretion in freshwater due to protein content.

Oliva-Teles (2000) reported that the optimum protein/ener gy ratio of European sea bass diets is higher than that of trout and sea bream and that feeds should contain 45 to 50% protein and a minimum of 9-12% lipids. Furthermore, the optimal DP/DE ratio for European sea bass fry was recommended to be 21-22 mg DP/kJ DE (0.525 kcal/kg) (Peres and Oliva-Teles, 1999). Saleem et al. (2022) reported that feeds containing 42% protein and 17.4% protein were suitable for the growth of European sea bass raised in seawater. The protein contents of the feeds used in the study were within the limits given in the above literatures. The fat ratios used in the feed are the ratios used in commercial feeds and are slightly higher than those given in the literatures. In future studies, different fat and energy contents should be tried to increase feed efficiency.

It has been reported that increasing the fish meal ratio in the feed causes an increase in SGR and FCR in European sea bass adapted to freshwater (Shalaby et al., 2023). In addition, it has been reported that decreasing the fish meal ratio and adding salt to the feed increases feed intake, SGR and FCR values (Cnaani et al., 2012). In the study conducted by Rimoldi et al. (2015), the addition of 3% salt to a diet containing only 10% fishmeal resulted in feed intake, SGR and FCR values similar to those of fish fed a diet containing 30% fishmeal. Therefore, in future studies, it will be important to try to reduce the utilization rate of fish meal by using salt in the diet of fish adapted to freshwater. Also, osmoregulation capacity may differ according to fish developmental stages and new studies should be conducted to include the whole production period (Bernardino and Fernandes, 2016).

The value of the viscerosomatic index increases as a result of visceral fat accumulation. Lovell (1989) reported that when fish are fed a high protein diet, excessive fat accumulation in the visceral cavity and tissue is reported due to an imbalance in the digestible energy/crude protein ratio. The highest HSI and VSI values obtained in the group fed low protein diets may be attributed to an imbalance in the P/E ratio due to the conversion of carbohydrates from raw materials into liver glycogen and lipid, thus increasing liver weight, and not as a result of freshwater rearing (Brown et al., 1992). As observed in this study, higher HSI and VSI values indicate poorer growth compared to the other groups. According to the condition factor data obtained in our study, it was observed that the VSI and HSI differences observed in the internal organs and liver did not cause a morphological difference. This shows that the feeds used were suitable in terms of nutrition and feeding rates.

## CONCLUSION

According to the results obtained, at the end of 75 days, there was no significant difference between the groups fed with feed containing 45% protein and feed containing 50% protein. Accordingly, it was observed that European sea bass fish can be adapted to freshwater at the fry stage and can be fed with feeds containing lower protein ratios than the currently used commercial feeds, and this will not have a negative effect on the growth of the fish. This will help to reduce the cost of production.

In addition, the cultivation of European sea bass in freshwater will make a great contribution to the development of aquaculture. The fact that European sea bass is not affected by water temperature increases as much as trout and can live in higher water temperatures will enable the utilization of freshwater resources that cannot be used for trout production during hot periods and remain empty.

## AUTHORSHIP STATEMENT

All authors contributed to the idea and design of the study. Material preparation and research was carried out by (Kutsal Gamsız), (Ali Yıldırım Korkut) and (Aysun Kop). The writing and editing of the manuscript was done by (Kutsal Gamsız), (Ali Yıldırım Korkut) and (Aysun Kop) and all authors have read and approved the manuscript.

## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest or competing interests.

## ETHICS APPROVAL

Approval was obtained from Ege University Animal Experiments Ethics Committee (25.12.2019/2019+-116).

## DATA AVAILABILITY STATEMENT

Data sets generated and/or analyzed during the current study will be provided by the corresponding author upon request of the editor or

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