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# The Effect of Fishmeal Source and Lipid Ratio on Growth and Nutrient Composition of Juvenile Turbot (*Psetta maxima*)*

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**ABSTRACT:** The study investigated how the sources of fish meal and the amount of lipids in the diets of juvenile turbot (*Psetta maxima*) affected growth, feed utilization, body composition, and nutrient balance. The fish were fed with feed produced using two different fish meals for 50 days: white fish meal (WFM) from Alaska cod, *Gadus chalcogrammus* and brown fish meal (BFM) from Black Sea anchovy, *Engraulis encrasicolus*. The diets prepared as BFM-HL (Brown fish meal- high lipid), BFM-LL (Brown fish meal- low lipid), WFM-HL (Whitefish meal-high lipid), WFM LL (Whitefish meal-low lipid) contained 53% protein and 10–14% lipid. Fish meal sources and lipid levels had a substantial (P< 0.05) impact on final weight, specific growth rate (SGR), daily feed intake (FI), feed conversion rate (FCR), protein efficiency rate (PER), nitrogen intake, gain, and loss, and lipid intake, gain, and retention. WFM-HL fed fish showed the best SGR, FCR, and PER results. When compared to the other diets, the WFM-HL diet's better protein utilization led to a considerably higher (P< 0.05) nitrogen retention. The fish-fed WFM-HL and BFM-HL diets had higher lipid retention values. Protein, lipid, and ash levels in the whole fish body were significantly (P< 0.05) influenced by the dietary fish meal source and lipid level. Compared to diets containing BFM, the results suggested that employing WFM in diet would be more promising in terms of higher growth performance and nutrient utilization. Also, with regard to experimental design, dietary lipid levels of 14% could result in greater growth than the diet containing 10% lipid, regardless of fish meal sources and levels.

***Keywords –*** *Fish meal, lipid level, nitrogen and lipid balance, protein quality, Psetta maxima.*

# Kalkan Balığının (*Psetta maxima*) Büyüme ve Besin Madde Kompozisyonu Üzerine Balık Unu Kaynağı ve Lipid Oranının Etkisi[*]

**ÖZET:** Bu çalışmada, yavru kalkan balığı yemlerinde bulunan balık unu kaynaklarının ve lipid düzeylerinin büyüme, yemden yararlanma, vücut kompozisyonu ve besin dengesi üzerine etkileri araştırılmıştır. Balıklara 50 gün boyunca iki farklı balık unu kaynağı (Karadeniz hamsisinden (*Engraulis encrasicolus*) üretilmiş kahverengi balık unu, KBU ve Alaska morina balığından (*Gadus chalcogrammus*) üretilmiş beyaz balık unu, BBU) ve iki farklı lipid seviyesi içeren yemler verilmiştir. KBU-YL (Kahverengi balık unu –yüksek lipid), KBU-DL (Kahverengi balık unu –düşük lipid), BBU-YL (Beyaz balık unu –yüksek lipid), BBU- DL (Beyaz balık unu –düşük lipid) şeklinde hazırlanan diyetler %53 protein ve %10–14 lipid içeriyordu. Deneme sonunda, final ağırlık, spesifik büyüme oranı (SBO), günlük yem tüketimi (GYT), yem dönüşüm oranı (YDO), protein verimlilik oranı (PVO), nitrojen tüketimi, kazanımı, kaybı ve lipid tüketimi, kazanımı, tutulması değerlerinin yemdeki protein kaynağı ve lipid seviyesinden önemli ölçüde (P< 0,05) etkilendiği tespit edilmiştir. En iyi SBO ve YDO, BBU-YL yemi ile beslenen gruptan elde edilmiştir. Nitrojen tutulumu, en iyi protein değerlendirmenin tespit edildiği BBU-YL grubunda diğer gruplara kıyasla önemli derecede P<0,05) yüksek bulunmuştur. BBU-YL ve KBU-YL yemleri ile beslenen gruplarda lipid tutulumu daha yüksek saptanmıştır. Balık vücudundaki protein, lipid ve kül miktarı balık unu ve lipid seviyesinden önemli (P<0,05) ölçüde etkilenmiştir. Sonuç olarak, yemlerde BBU kullanımı, KBU içeren yemlere kıyasla daha iyi büyüme performansı ve besin kullanımı sağlaması açısından daha umut verici olabileceğini göstermiştir. Ayrıca mevcut deneysel koşullar altında balık unu kaynağından bağımsız olarak %14'lük lipid seviyesinin %10'luk lipid seviyesine kıyasla daha iyi bir büyüme sağlayabileceği tespit edilmiştir.

***Anahtar Kelimeler****- Balık unu, lipid düzeyi, nitrojen ve lipit dengesi, protein kalitesi, Psetta maxima*

** This manuscript was produced from Fatma Burcu HARMANTEPE's Ph.D. thesis.*

# 1. Introduction

Fishmeal is the main protein source of fish feeds (Castro et al. 2022; Shepherd and Jackson, 2013; Tacon and Metain, 2008). Today, although it is desired to substitute a fish meal with alternative protein sources, this has not been fully achieved. Because the protein quality of any protein source cannot match it in the the fish meal, for this reason, fishmeal is an indispensable source of fish feeds. The importance of fishmeal increases even more for carnivorous species such as turbot that require high and high-quality protein (Bian et al. 2016; Cho et al. 2005). Different fish meals of varying quality are used in the feed industry, depending on both the production method and the species of fish from which they are obtained. Fish meal is an important factor that determines the quality of the feed. The quality of the feed also affects the growth parameters and meat quality of the fish fed (Anderson et al. 1997; Song et al. 2014). Therefore, it is important to determine the most suitable fish meal source for the fish species to be cultured.

Diet formulations that provide rapid growth, feed evaluation, and protein evaluation generally have high-fat content. For this reason, we come across feeds with high-fat content and even feeds and feed formulations substituted with vegetable oils due to their high price. In addition to being matched with high growth parameters in diets, high-fat content also causes a decrease in ammonia excretion amounts, which is an indicator of protein's use as an energy source (Yiğit et. al., 2003). However, the point to be considered here is to optimize the amount of lipid to be added for the species for rapid growth performance. Feeding the species with feed containing more lipids than needed causes fat in the fish's visceral region and liver tissue (Fan et al. 2021; Yan et al. 2015). For this reason, only weight gain should not be considered when determining the optimum lipid value.

There are limited studies to determine the appropriate protein source for turbot. Yigit et al. (2005, 2003) found that rockfish (*Gobius sp.*), fresh shrimp (*Palaemon adspersus, Palaemon elegans*), and haddock (*Merlangius merlangus*), which are among the natural foods of turbot, have lower ammonia excretion values and better protein quality than anchovy meat. Haşimoğlu et al. (2007), reported that WFM is more suitable for turbot growth, while Sevgi et al. (2015) reported that commercial fish meal sources used in the feed industry have a similar effect on the growth and nutritional performance of turbot.

In this study, it was aimed to determine the effect of meals obtained from white and red meat fish, which are among the natural foods of turbot, on the growth parameters and nutritional balance of juvenile turbot. In addition, the effect of different lipid ratios on growth and nutritional parameters in the feeding of turbot fish, which is known for its low lipid requirement, was investigated.

# 2. Materials and Methods

**Fish and Rearing Conditions:** The Central Fisheries Research Institute in Trabzon, Turkey, provided juvenile turbot, *Psetta maxima*. All experiments were conducted in the Marine Aquaculture Facility in Sinop University, Faculty of Fisheries, Sinop, Turkey. The fish were adapted to the experimental environment for 15 days. The fish were fed a diet with 55% crude protein and 16% crude fat throughout this time. Ten turbot juveniles (average body weight of 40.5±1.98 g) were randomly supplied into 12 60-liters polypropylene rectangular tanks after the acclimation phase. The experiment was planned as 3 replications. The water temperature, salinity, and pH during the 50-day trial were 19.23±1.38 $^{o}$C, 17.21±0.45, and

7.5±0.5 ppt, respectively. Fish were fed twice daily for 50 days, at 08:00 and 17:00, until apparent saturation. Local Ethics Committee principles have complied.

**Preparation of Experimental Diets:** Table 1 displays the nutrient composition and amino acid profiles of BFM, WFM and soybean meal. A total of four experimental diets with isonitrogenous (530 g $kg^{-1}$) and two lipid levels were created (140-100 g $kg^{-1}$). Fish oil was employed as a lipid source, and white fish meal and BFM were the two main protein sources (Table 2). A 500 m mesh was used to grind up ingredients for diets. Fish oil was added after mixing all of the dry feed ingredients. The mixture was run through a meat grinder to create pellets after adding water to make it into a dough-like consistency. The produced pellets were dried at 50 °C and then stored at -18 °C.

**Proximate Composition:** A sample of five fish was initially collected to ascertain the approximate makeup of the entire body. Ten fish from each tank were removed after 50 days; five were used for whole-body composition analysis, five for hepatosomatic index, five for viscerosomatic index, and five for liver lipid content. The AOAC's (1995) standard procedures were used to determine the approximate composition of experimental diets, freeze-dried whole-body samples, and liver samples. The Kjeldahl technique was used to determine the crude protein (Nx6.25). The sample was dried for 12 hours at 105°C to determine the amount of dry matter. Crude ash was determined by incineration at 550 °C for 12 hours in a muffle furnace, and crude lipid was determined using the ether-extraction method with a Soxhlet system. According to Teshima et al. (1996), high-performance liquid chromatography (Shimadzu, Kyoto, Japan) was used to determine the amino acid content of BFM, WFM, and SBM.

Table 1. Proximate analyses of brown fish meal, white fish meal and soybean meal
*Tablo 1. Kahverengi balık unu, beyaz balık unu ve soya fasulyesi küspesinin besin kompozisyonu*

| | Brown fish meal | White fish meal | Soybean meal |
|---|---|---|---|
| Proximate analysis (%) | | | |
| Moisture | 5.9 | 6.3 | 10.5 |
| Protein | 75.2 | 66.1 | 41.4 |
| Lipid | 9.0 | 5.4 | 11.9 |
| Ash | 9.2 | 10.15 | 4.9 |
| Fiber | - | - | 4.6 |
| *Essential amino acids ( g/100 g sample)* | | | |
| Arginine | 4.42 | 5.00 | 3.68 |
| Lysine | 6.32 | 6.32 | 3.09 |
| Histidine | 2.55 | 1.56 | 1.22 |
| Isoleucine | 3.41 | 3.25 | 2.14 |
| Leucine | 5.80 | 5.50 | 3.64 |
| Valine | 3.91 | 3.66 | 2.56 |
| Methionine | 2.29 | 2.38 | 0.68 |

| | | | |
|---|---|---|---|
| Phenylalanine | 3.12 | 3.16 | 2.38 |
| Threonine | 3.43 | 3.09 | 1.90 |
| Tryptophan | 1.1 | 0.81 | 0.69 |
| *Non-essential amino acids (g/100g sample)* | | | |
| Aspartic acid | 7.5 | 5.72 | 4.17 |
| Serine | 3.15 | 1.33 | 1.07 |
| Glutamic acid | 9.89 | 8.31 | 6.6 |
| Proline | 2.79 | 2.81 | 1.43 |
| Glycine | 3.69 | 2.46 | 1.22 |
| Alanine | 4.65 | 3.0 | 1.08 |
| Cystine | 0.11 | 0.39 | 0.75 |
| Tyrosine | 2.31 | 2.31 | 1.83 |
| Taurine | - | 0.31 | - |

Table 2. Ingredient compositions and proximate analysis of the experimental diets
*Tablo 2. Deney yemlerinin bileşen kompozisyonu ve besin maddesi analizleri*

| | *Experimental diets* | | | |
|---|---|---|---|---|
| *Ingredients (g $kg^{-1}$ diet)* | BFM-HL | BFM-LL | WFM-HL | WFM-LL |
| Brown Fish meal[a] | 590 | 590 | - | - |
| White Fish meal[b] | - | - | 685 | 685 |
| Soybean meal | 160 | 160 | 145 | 145 |
| Corn meal | 120 | 160 | 5 | 45 |
| Dextrin | 20 | 20 | 20 | 20 |
| Alfa starch | 20 | 20 | 20 | 20 |
| Fish oil[c] | 60 | 20 | 95 | 55 |
| CMC[d] | 5 | 5 | 5 | 5 |
| Vitamin mix[e] | 10 | 10 | 10 | 10 |
| Mineral mix[f] | 10 | 10 | 10 | 10 |
| Chromium oxide | 5 | 5 | 5 | 5 |
| *Proximate composition ( g $kg^{-1}$ dry diet)* | | | | |
| Moisture | 154 | 146 | 156 | 138 |
| Crude protein | 535 | 538 | 535 | 534 |
| Crude lipid | 144 | 105 | 149 | 109 |
| Crude fiber | 13 | 14 | 11 | 12 |

| | | | | |
|---|---|---|---|---|
| Crude ash | 92 | 93 | 111 | 117 |
| Nitrogen-free extracts[g] | 216 | 250 | 194 | 228 |
| Gross energy (kj/g diet)[h] | 22.0 | 21.1 | 21.8 | 20.8 |
| Protein:energy (mg/kJ) | 24.4 | 25.5 | 24.5 | 25.7 |
| Protein energy:gross energy | 0.57 | 0.60 | 0.58 | 0.61 |
| *Essential amino acids ( g/100g sample)*[i] | | | | |
| Arginine | 3.20 | 3.20 | 3.96 | 3.96 |
| Lysine | 4.22 | 4.22 | 4.78 | 4.78 |
| Histidine | 1.70 | 1.70 | 1.25 | 1.25 |
| Isoleucine | 2.35 | 2.35 | 2.54 | 2.54 |
| Leucine | 4.00 | 4.00 | 4.29 | 4.29 |
| Valine | 2.72 | 2.72 | 2.88 | 2.88 |
| Methionine | 1.46 | 1.46 | 1.73 | 1.73 |
| Phenylalanine | 2.22 | 2.22 | 2.51 | 2.51 |
| Threonine | 2.33 | 2.33 | 2.39 | 2.39 |
| Tryptophan | 0.76 | 0.76 | 1.34 | 1.34 |
| *Non-Essential amino acids( g/100g sample)*[i] | | | | |
| Aspartic acid | 5.09 | 5.09 | 4.52 | 4.52 |
| Serine | 2.03 | 2.03 | 1.07 | 1.07 |
| Glutamic acid | 6.89 | 6.89 | 6.65 | 6.65 |
| Proline | 1.88 | 1.88 | 2.13 | 2.13 |
| Glycine | 2.37 | 2.37 | 1.86 | 1.86 |
| Alanine | 2.92 | 2.92 | 2.21 | 2.21 |
| Cystine | 0.1.9 | 0.19 | 0.38 | 0.38 |
| Tyrosine | 1.6.6 | 1.66 | 1.85 | 1.85 |
| Taurine | - | - | 0.21 | 0.21 |

[a]Anchovy meal (Black Sea)
[b]Cod meal (Alaska)
[c]Anchovy oil (Black Sea)
[d]Carboxymethyl cellulose
[e]Vitamin mixture (%0.8) (mg/100 g feed); β-carotene, vitamin $D_3$, 7.70; menadione $NaHSO_3$-$3H_2O$ ($K_3$), 3.67; DL-α-tochopherol acetate (E), 30.80; thiamine nitrate ($B_1$), 4.62; riboflavin ($B_2$), 15.39; pyridoxine-HCl ($B_6$), 3.67; cyanocobalamine ($B_{12}$), 0.01; ascorbyl-2-phosphate-Mg, 1.2; d-biotine, 0.46; inositol, 307.93, niacine (nicotinic acid), 61.58; Ca panthothenate,

21.56; folic acid, 1.15; choline choloride, 629.54; p-aminobenzoic acid, 30.66 and vitamin C (AMP), 1000**.**
[f]Mineral mix (%1) (mg/100 g feed); NaCl, 89.83; $MgSO_47H_2O$, 316.69; $NaHPO_42\ H_2O$, 201.59; $KH_2PO_4$, 541.83; $Ca(H_2PO_4)_2$-2 $H_2O$, 313.91; Fe citrate, 68.65; Ca lactate, 755.92; $Al(OH)_3$, 0.43;$ZnSO_47\ H_2O$, 8.25; $CuSO_4$, 0.23; $MnSO_45\ H_2O$, 1.85; $Ca(IO_3)_2$, 0.35 and $CaSO_47\ H_2O$, 2.3
[g]Nitrogen free extracts=100-(crude protein+crude lipid+crude fiber+crude ash)
[h]Gross energy is calculated according to 23.6 kj/g protein, 39.5 kj/g lipid and 17 kj/g NFE.
[ı]Essential amino acid contents based on data in Table 1.

**Apparent Digestibility Coefficient (ADC) Measurement:** For digestibility measurement, 5g $kg^{-1}$ chromium oxide ($Cr_2O_3$) was added to the experimental feeds. The fish were adapted for seven days to diets containing chromic oxide. Feeding was carried out at 8:00 and 17:00. Two hours after feeding, the feces samples were collected using the siphon method. Collected feces were stored at -20°C until analysis. Chromic oxide content was determined in diet and feces as described by Furukawa and Tsukahara (1966). The apparent digestibility coefficient (ADC) was calculated using the following equation.
ADC (%) = 100 - {100 x (% $Cr_2O_3$ in diet/% $Cr_2O_3$ in faeces) (% nutrient in faeces/% nutrient in diet)}.
**Statistical Analysis:** In the study, a two-way ANOVA analysis was performed to determine the effects of dietary fishmeal source and lipids. All data were reported as percentages before being converted using arcsine for the ANOVA. The different groups were determined using the Tukey test. Differences were considered significant at $p < 0.05$. The statistical analysis was done with SPSS 10. The data are shown as replicate group means plus standard deviation.

## 3. Results and Discussion

Data on growth performance is shown in Table 3. The fishmeal source and lipid content of the diets were significantly ($P< 0.05$) effective on growth performance and feed consumption. Fish final body weight and SGR were significantly ($P<0.05$) improved by raising the dietary lipid levels for the same supply of fish meal. The final body weight and SGR of the groups fed diets containing white fish meal, however, were considerably ($P< 0.05$) higher than those fed diets containing brown fish. Dietary fish meal source affected DFI in a statistically significant way ($P <0.05$). Additionally, DFI significantly ($P< 0.05$) decreased in diets containing white fish meals as lipid levels increased.

In this study, growth performance was better in turbot juveniles fed WFM diets than in BFM diets. Dietary essential amino acid composition is known to affect growth and protein utilization (Kaushik and Seiliez, 2010). The EAA levels of diets containing WFM were higher than those containing BFM. In particular, the values of arginine, lysine, and tryptophan were high in WFM diets. They are essential amino acids for young fish growth (Fourrier et al,. 2002; Hoseini et al. 2014; Pohlenz et al. 2013; Pohlenz et al. 2014; Tulli et al. 2007; Zhou et al. 2010). It is commonly known that these amino acids play a role in the pituitary (growth hormone, GH), thyroid (T3, T4), pancreatic hormones (insülin, glucagon), calcium-regulating hormones (calcitonin and parathyroid hormone), and antibodies (Kloppel and Post, 1975; Reindl and Sheridan, 2012).

Table 3. Growth performance and feed utilization by turbot juveniles fed with the experimental diets[1]

*Tablo 3. Deney yemleri ile beslenen kalkan yavrularının büyüme performansları ve yemden yararlanmaları[1]*

| | | | | | Two way ANOVA (P<0.05) | | |
|---|---|---|---|---|---|---|---|
| | BFM-HL | BFM-LL | WFM-HL | WFM-LL | Fish meal | Lipid | F x L |
| Initial weight (g) | 40.53±2.06 | 40.53±1.96 | 40.56±1.96 | 40.37±2.03 | - | - | - |
| Final weight (g) | 63.77±7.62$^c$ | 59.73±7.95$^d$ | 90.33±8.07$^a$ | 79.33±7.72$^b$ | 0.000 | 0.000 | 0.001 |
| SGR[2] (%) | 0.91±0.03$^c$ | 0.77±0.08$^d$ | 1.60±0.03$^a$ | 1.35±0.03$^b$ | 0.000 | 0.006 | 0.071 |
| DFI[3] (g/fish) | 0.49±0.01$^a$ | 0.45±0.02$^a$ | 0.71±0.01$^b$ | 0.85±0.01$^c$ | 0.014 | 0.000 | 0.001 |
| FCR[4] | 1.05±0.03$^a$ | 1.17±0.02$^b$ | 0.72±0.00$^c$ | 1.10±0.08$^a$ | 0.000 | 0.000 | 0.690 |
| PER[5] | 1.77±0.05$^a$ | 1.58±0.02$^b$ | 2.61±0.02$^c$ | 1.71±0.01$^a$ | 0.000 | 0.000 | 0.000 |

[1]Values are means of triplicate groups.
[2]SGR, Specific growth rate = [ln (final weight)-ln (initial weight) / 50 days] x 100
[3]DFI, Daily feed intake = (dry feed intake/number of fish) / 50days
[4]FCR, Feed conversion rate = dry feed intake (g) / weight gain (g)
[5]PER, Protein efficiency rate = weight gain (g) / protein intake (g)

However, the point to be noted here is that WFM was used more than BFM to equalize the protein ratio in the trial feeds. Therefore, WFM-containing feeds had higher amino acid content. Haşimoğlu et al. (2007) reported that the best growth performance was obtained from juvenile turbots fed with diets containing Alaskan white fish meal. However, Sevgili et al. (2015) reported that all fish meals showed similar growth and nutritional performance as a result of their studies with five different commercial fish meals, including WFM. Therefore, the difference in the results obtained may be related to the method of obtaining the fish meal, the freshness of the raw material, the type of the raw material, and the protein ratio of the diet. Because Sevgili et al. (2015) used diets with a high protein content of 56% in their experiment, this high protein level may have prevented the difference between fish meals as it contains enough amino acids to meet the needs of the fish.

A significant increase in growth performance of fish fed with diets containing high lipid levels was detected in both fishmeal sources. Similarly, an increase in growth was noted in Atlantic halibut, *Hippoglossus hippoglossus* (Helland and Grisdale-Helland, 1998) and turbot (Cho et al. 2005; Harmantepe et al. 2014). Although Regost et al. (2001) found no differences in weight gain in turbot (weight 660 g) treated with diets containing 10–15% lipid, there has been a drop in weight gain when fed with diets containing 20–25% fat. Similar findings were made by Cacerez-Martinez (1984), who found that young turbot (*Scophthalmus maximus*) fed diets containing 10 to 20% lipid levels experienced slower growth. According to Danielssen and Hjertnes, (1993) and Berge and Storebakken, (1991), lipid levels between 20 and 22% had no adverse effects on the growth of fry flounder (*Platichthys flesus*) and turbot. Studies' varied findings could result from various variables, including fish size and species, food composition (dietary components, lipid content, energy sources, P:E, amino acid composition), and environmental conditions (water temperature and salinity). The SGR values obtained in this study were the same as those reported for turbot of similar sizes (Ergun et al. 2008a; 2008b; Harmantepe et al. 2014; Yigit et al. 2006; Yigit et al. 2010).

While FCR and PER values improved with increasing dietary lipid levels in both protein sources, the best FCR and PER values were obtained from the diets containing WFM-HL. The increase in PER might be pointing to better protein quality in the diet, as has been reported by Gill et al. (2006). Low growth and PER values may also be associated with insufficient dietary amino acid composition for fish and catabolism and excretion of amino acids not used by fish. In this study, whereas the parameters of nitrogen intake, gain, loss, and lipid intake, gain, and retention were significantly (P<0.05) influenced by dietary fish meal and lipid levels, nitrogen retention value was only affected significantly by the source of fishmeal (P<0.05, Table 4). In those fed reduced-fat diets, nitrogen intake, gain, and loss were considerably higher (P<0.05). The high lipid groups showed considerably (P< 0.05) increased lipid intake, gain, and retention. Consequently, the lower protein catabolism led to a lower nitrogen loss in fish fed with diets with WFM as the main protein source. Even if the protein source is sufficient in the diet, the energy level is lower than the fish's needs is another important factor affecting growth and PER.

Table 4. Nitrogen and lipid balances of turbot fed with the experimental diets[1]
*Tablo 4. Deney yemleri ile beslenen kalkan balığının nitrojen ve lipid dengesi[1]*

| | BFM-HL | BFM-LL | WFM-HL | WFM-LL | Two way ANOVA (P<0.05) | | |
|---|---|---|---|---|---|---|---|
| | | | | | Fish meal | Lipid | F x L |
| *Nitrogen* | | | | | | | |
| Intake[2] | 90.52±2.56$^{a}$ | 101.16±1.29$^{b}$ | 61.25±0.35$^{c}$ | 93.48±0.67$^{a}$ | 0.000 | 0.000 | 0.000 |
| Gain[3] | 33.34±0.18$^{a}$ | 37.01±0.65$^{b}$ | 24.42±0.03$^{c}$ | 29.16±0.07$^{d}$ | 0.000 | 0.000 | 0.233 |
| Retention[4] | 36.90±0.90$^{a}$ | 36.58±0.18$^{a}$ | 39.87±0.19$^{b}$ | 31.19±0.21$^{c}$ | 0.000 | 0.728 | 0.000 |
| Loss[5] | 57.18±2.44$^{a}$ | 64.15±0.64$^{b}$ | 36.83±0.33$^{c}$ | 64.33±0.64$^{b}$ | 0.000 | 0.000 | 0.000 |
| *Lipid* | | | | | | | |
| Intake[2] | 150.77±4.26$^{a}$ | 123.15±1.57$^{b}$ | 106.39±0.61$^{c}$ | 120.03±0.85$^{b}$ | 0.040 | 0.000 | 0.000 |
| Gain[3] | 48.04±0.41$^{a}$ | 26.25±0.31$^{b}$ | 102.70±0.50$^{c}$ | 35.82±0.14$^{d}$ | 0.000 | 0.000 | 0.000 |
| Retention[4] | 31.92±0.73$^{a}$ | 21.31±0.02$^{b}$ | 96.54±0.10$^{c}$ | 29.85±0.20$^{d}$ | 0.000 | 0.000 | 0.000 |

[1]Values are means of triplicate groups.
[2]Intake= {(Feed intake (g/fish) x Nitrogen or lipid concentration in diet (%)/ 100)/ (Mean body weight gain (g))}x 1000.
[3]Gain= {Final mean body weight (g) x Final whole body Nitrogen or Lipid concentration (%)/100) – (Initial mean body weight (g) x Initial whole body Nitrogen or Lipid concentration (%)/100)}/(Mean body weight gain (g)) x1000.
[4]Retention per intake in percent= Nitrogen or Lipid Gain (g/kg body weight gain) x 100)/ Nitrogen or Lipid intake (g/kg body weight gain).
[5]Loss= Nitrogen or Lipid intake (g/kg body weight gain)-Nitrogen or Lipid Gain (g/kg body weight gain).

In this study, it was observed that the nitrogen loss values have been affected by the dietary lipid levels. Similarly, McGoogan and Gatlin, (1999) and Harmantepe et al. (2015) reported that ammonia loss decreased with increasing energy levels in the diets. Lower ammonia-nitrogen loss may lead to a cost-effective production and environmentally sound diets. Low nitrogen loss values mean high nitrogen gain value, and the best nitrogen intake and gain values were obtained with the increase in the amount of lipid in the diet in both fishmeal sources.

The best FCR, SGR, and PER values obtained with the increase in lipid levels in the diets show that lipids have a protein-sparing effect. Similar results were obtained in studies conducted with carnivorous fish species, such as turbot, walleye pollock (*Gadus chalcogrammus*), Atlantic cod (*Gadus morhua*), spotted knifejaw (*Oplegnathus punctatus*), rainbow trout (*Oncorhynchus mykiss*) (Cho et al. 2005; Harmantepe et al. 2014; Lee et al. 2021; Morais et al. 2001; Wang etal. 2021; Yigit et al. 2002).

Previous research has suggested that fish fed with high-energy diets consume less feed (Shearer, 1994; Wang et al. 2005; Yamamoto et al. 2000). However, in this study, fish fed with the WFM-LL diet had a higher feed consumption than fish fed with the WFM-HL diet. The fact that the low-lipid diet group consumed more feed can be attributed to the lack of energy it provided for the fish. It was noted that fish-provided diets containing BFM exhibited lower DFI values than fish-fed diets containing WFM. Fish-fed with diets containing WFM achieved higher growth rates than those containing BFM, explaining why feed consumption rises as the fish develop.

The apparent digestibility coefficients (ADCs) of the dry matter, protein, and lipid were generally high and not affected by the dietary fish meals and lipid level (Table 5).

Table 5. Apparent digestibility coefficients (ADC) of dry matter, protein, lipid of turbot fed with the experimental diets[1]

*Tablo 5. Deney yemleri ile beslenen kalkan balığının kuru madde, protein ve lipidin görünür sindirilebilirlik katsayıları (ADC)[1]*

| ADC (%) | BFM-HL | BFM-LL | WFM-HL | WFM-LL | Two way ANOVA (P<0.05) Fish meal | Lipid | F x L |
|---|---|---|---|---|---|---|---|
| Dry matter | 85.76±0.75 | 84.32±0.38 | 84.97±0.47 | 84.69±0.95 | 0.613 | 0.059 | 0.176 |
| Protein | 91.98±0.41 | 91.35±0.63 | 91.95±0.49 | 91.07±1.26 | 0.740 | 0.129 | 0.784 |
| Lipid | 92.89±0.55 | 91.88±0.64 | 92.84±1.08 | 92.33±1.18 | 0.718 | 0.181 | 0.638 |

[1]Values are means of triplicate groups.

Table 6 displays the proximate compositions of the juvenile turbots' whole bodies after the trial. Protein, lipid, and ash levels in the whole body were significantly (P<0.05) influenced by the dietary fish meal source and lipid level. It was that the lipid content was significantly (P< 0.05) higher in groups that were fed high-fat diets. The highest lipid content was obtained from the group fed with WFM-HL diet. The BFM-LL group had the most protein. The lowest protein content was obtained from the group fed WFM-HL diet. Only the dietary lipid level had an impact (P<0.05) on the total body moisture. The highest moisture content was obtained from the group fed with WFM-HL diet. Fish meal and lipid levels had an effect (P<0.05) on liver lipid content. However, the dietary fish meal had an impact on conditional factor. Particularly, fish fed with diets of white fish meal showed greater condition factors. It has been reported in previous studies that high-energy diets increase lipid accumulation in fish. (Craig et al. 1995; Rasmussen et al. 2000). That could indicate poor nutrient utilization, according to Craig et al. (1999). In the present study, the higher lipid diets with both fish meal sources resulted in increased lipid accumulation in the fish body, which has been reported by Andersen and Alsted, **(**1991), Harmantepe et al. (2014) and Saether and Jobling, (2001). Similar results were also found in red drum (*Sciaenops ocellatus*), rainbow trouth (*Oncorhynchus mykiss*), spotted knifejaw (*Oplegnathus punctatus*), ve hybrid puffer fish (*Takifugu obscurus* × *T. rubripes*) (Craig et al. 1999; Meng et al. 2022; Wang et al. 2021; Yoo et al.2022). Furthermore, liver lipid content was significantly decreased by reducing the dietary lipid levels. Previous studies have also suggested that higher dietary energy levels increased the levels of lipids in the liver (McGoogan and Gatlin, 1999; Regost et al. 2001).

Table 6. Proximate composition of whole body, hepatosomatic index, viscerosomatic index, conditional factor of turbot fed with the experimental diets[1]

*Tablo 6. Deney yemleri ile beslenen kalkan balığının toplam vücut kompozisyonu, hepatosomatik indeksi, viserosomatik indeksi, kondisyon faktörü[1]*

| | | | | | | Two way ANOVA ($P<0.05$) | | |
|---|---|---|---|---|---|---|---|---|
| | Initial | BFM-HL | BFM-LL | WFM-HL | WFM-LL | Fish meal | Lipid | F x L |
| Moisture (%) | 80.68 | 76.52±0.34$^{b}$ | 75.95±0.09$^{c}$ | 74.64±0.09$^{d}$ | 77.40±0.15$^{a}$ | 0.062 | 0.000 | 0.000 |
| Crude ash (%) | 4.97 | 5.02±0.08$^{b}$ | 6.06±0.04$^{a}$ | 4.85±0.02$^{d}$ | 4.59±0.04$^{c}$ | 0.000 | 0.000 | 0.000 |
| Crude protein (%) | 12.54 | 15.56±0.15$^{b}$ | 15.92±0.05$^{a}$ | 14.04±0.02$^{c}$ | 15.33±0.05$^{d}$ | 0.000 | 0.000 | 0.000 |
| Crude lipid (%) | 1.81 | 2.90±0.08$^{b}$ | 2.07±0.11$^{d}$ | 6.47±0.11$^{a}$ | 2.68±0.02$^{c}$ | 0.000 | 0.000 | 0.000 |
| Liver lipid (%) | 7.83 | 10.86±0.20$^{b}$ | 8.46±0.29$^{d}$ | 15.58±0.17$^{a}$ | 10.19±0.18$^{c}$ | 0.000 | 0.000 | 0.000 |
| HSI[2] | 0.79 | 1.26±0.29$^{a}$ | 1.09±0.23$^{a}$ | 1.08±0.35$^{a}$ | 1.02±0.13$^{a}$ | 0.067 | 0.087 | 0.450 |
| VSI[3] | 9.09 | 7.31±0.57$^{a}$ | 7.40±0.59$^{a}$ | 7.52±0.55$^{a}$ | 7.39±0.59$^{a}$ | 0.588 | 0.837 | 0.404 |
| CF[4] | 1.21 | 1.30±0.11$^{b}$ | 1.27±0.10$^{b}$ | 1.38±0.09$^{a}$ | 1.39±0.10$^{a}$ | 0.000 | 0.556 | 0.194 |

[1]Values are means of triplicate groups.

[2]Hepatosomatic index = (liver weigh (g)/body weigh (g)) x 100

[3]Viscerosomatic index = (viscera weigh (g) / body weigh (g)) x 100

[4]Conditional factor= {(body weigh (g))/(body length (cm))$^3$} x 100

## 4. Conclusion

The results of this study showed that turbot juveniles were able to evaluate WFM better than BFM as a protein source. In addition, it was determined that the diet containing 14% lipid was more suitable for the turbot juveniles in terms of growth parameters, feed use, protein efficiency and nitrogen, lipid balances. However, equivalent growth parameters may be attained if the essential amino acid levels of diets containing BFM and WFM are equalized. Furthermore, these results are promising in terms of supporting the sustainable growth of the turbot culture industry with nutritionally balanced, environmentally sound, and cost-effective diets.

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