EVALUATION OF SOYBEAN MEAL AS A PROTEIN SOURCE AND ITS EFFECT ON GROWTH AND NITROGEN UTILIZATION OF BLACK SEA TURBOT (PSETTA MAEOTICA) JUVENILES

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Recommended Citation
DOI: 10.51400/2709-6998.1913
Available at: https://jmstt.ntou.edu.tw/journal/vol18/iss5/8

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This research article is available in Journal of Marine Science and Technology: https://jmstt.ntou.edu.tw/journal/vol18/iss5/8
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Murat Yigit*, Sebahattin Ergün*, Ali Türker**, Burcu Harmantepe***, and Adnan Erteken****

Key words: Black Sea turbot, fish meal, nitrogen retention, soybean meal.

ABSTRACT

Three experimental diets were formulated to be iso-nitrogenous (550 g kg⁻¹ protein) and iso-caloric (20.5 kJ gross energy g⁻¹ diet) by substituting fish meal (FM) for defatted soybean meal (SBM) at levels of 0, 10 and 20% replacement in order to evaluate the effects of replacing FM with defatted SBM in diets for Black Sea turbot. Diets were fed to triplicate groups of young Black Sea turbot (mean initial weight of 30.2 ± 0.2 g) for 60 days. Turbot fed all three diets had no significant difference (P > 0.05) in final weight, specific growth rate, nutrient utilization, Nitrogen loss or retention. Survival rate was 100% in all treatments. The results of this study indicate that fish meal can be replaced with soybean meal up to 20% level in diets for young Black Sea turbot without adverse effects on growth, nutrient utilization or nitrogen balance. This may increase profitability by allowing the production of less expensive feeds, hence supporting the sustainable growth of turbot culture industry with environment-friendly diets.

I. INTRODUCTION

The demand for fish meal (FM) in the world aquaculture industry was 68.2% of the total global fish meal production in year 2006 [39]. A gradual decline of wild fish catch as protein source for fish feed production has been reported as essential for the sustainable development of the aquaculture Industry [29]. Due to the limiting supply of FM in the world, fish nutritionists have intensified their search on replacing FM by less expensive alternative protein sources. Based on the increase of global fish meal costs, Tacon and Metian [39] reported that fish meal usage in compound aquafeeds will decrease in the long term. High protein requirement (500 g kg⁻¹ to 600 g kg⁻¹ of the diet) has been reported for the turbot, an important fish species for the European aquaculture industry [5, 7, 18]. Hence, the partial replacement of the fish meal with protein-rich ingredients is therefore an important and interesting issue to focus on. Previous studies with turbot have focused on some plant protein sources such as corn gluten meal, lupin or soybean meal [6, 10, 14, 32]. Among several protein sources, soybean meal (SBM) is considered as one of the most nutritious because of its favorable protein content and amino acid profile. The use of soy products has been widely studied in a number of fish species. Soybean meal has been tested in diets for Japanese flounder [23, 24, 27], Yellowtail [35-38, 49], Atlantic halibut [4], European sea bass [16, 43], gilthead sea bream [25, 29, 34], and red sea bream [2, 3, 40, 41]. However, little work has been done on the effectiveness of soy products in Atlantic turbot (Psetta maxima) [10], and to our knowledge, so far, there are only few studies available on the evaluation of soybean meal as a dietary protein source for the Black Sea turbot [11, 12, 20]. The objective of this study, therefore, is to evaluate the effects of substituting fish meal (FM) with defatted soybean meal (SBM) in practical diets on growth performance, nutrient utilization and nitrogen balance of young Black Sea turbot.

II. MATERIALS AND METHODS

Three practical diets (iso-nitrogenous and iso-caloric) on a crude protein of 550 g kg⁻¹ and gross energy of 20.5 kJ g⁻¹ diet basis were formulated with commercially available ingredients.
Table 1. Ingredients and nutrient composition of diets used in the experiment.

<table>
<thead>
<tr>
<th>Ingredients (g kg⁻¹)</th>
<th>Replacement level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>White fish meal (FM)</td>
<td>773</td>
</tr>
<tr>
<td>SBM</td>
<td>0</td>
</tr>
<tr>
<td>Fish oil</td>
<td>79</td>
</tr>
<tr>
<td>b-Corn starch</td>
<td>90</td>
</tr>
<tr>
<td>Vit.-Min. Premixa</td>
<td>35</td>
</tr>
<tr>
<td>Attractant^b</td>
<td>3</td>
</tr>
<tr>
<td>Binder (Guar-Gum)</td>
<td>20</td>
</tr>
</tbody>
</table>

Proximate composition (g kg⁻¹ on dry basis)

- Dry matter: 920.3, 913.6, 923.4
- Crude Lipid: 152.6, 161.9, 160.4
- Crude Ash: 130.9, 132.1, 129.4
- Crude Protein: 549.3, 546.3, 547.9
- Nitrogen free extracts: 87.5, 73.3, 85.7

Table 2. Essential amino acid contents in the experimental diets.

<table>
<thead>
<tr>
<th>Amino acid content</th>
<th>Experimental diets</th>
<th>Turbot</th>
<th>Requir.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(g kg⁻¹ DM)</td>
<td>FM100</td>
<td>SBM10</td>
<td>SBM20</td>
</tr>
<tr>
<td>Arg</td>
<td>34.1</td>
<td>35.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Lys</td>
<td>38.3</td>
<td>37.8</td>
<td>37.8</td>
</tr>
<tr>
<td>His</td>
<td>11.4</td>
<td>11.6</td>
<td>11.9</td>
</tr>
<tr>
<td>Ile</td>
<td>23.0</td>
<td>24.0</td>
<td>24.9</td>
</tr>
<tr>
<td>Leu</td>
<td>36.9</td>
<td>37.8</td>
<td>38.5</td>
</tr>
<tr>
<td>Val</td>
<td>25.6</td>
<td>25.9</td>
<td>26.2</td>
</tr>
<tr>
<td>Met + Cys</td>
<td>20.6</td>
<td>20.1</td>
<td>19.7</td>
</tr>
<tr>
<td>Phe + Tyr</td>
<td>34.8</td>
<td>35.9</td>
<td>37.1</td>
</tr>
<tr>
<td>Thr</td>
<td>21.8</td>
<td>21.8</td>
<td>21.8</td>
</tr>
<tr>
<td>Trp</td>
<td>5.6</td>
<td>5.8</td>
<td>6.0</td>
</tr>
</tbody>
</table>

^aKadai, Riken Vitamin, Tokyo, Japan
^bGlutamic acid, 0.9; Inosine, 0.1 (Sigma, Germany)
^cCalculated by difference
^dGross Energy calculated according to 23.6 kJ g⁻¹ protein, 39.5 kJ g⁻¹ lipid, 17 kJ g⁻¹ nitrogen-free extract.
^eProtein to Energy ratio

Table 3. Proximate composition of white fish meal, soybean meal, and turbot.

<table>
<thead>
<tr>
<th>Proximate analyses (g kg⁻¹)</th>
<th>Turbot</th>
<th>WFM</th>
<th>SBM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>80</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>714</td>
<td>480</td>
<td></td>
</tr>
<tr>
<td>Lipid</td>
<td>55</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>120</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

Essential amino acid (g kg⁻¹)*
- Arg: 65.3, 44.1, 34.1
- Lys: 62.2, 49.6, 31.0
- His: 26.7, 14.7, 12.6
- Ile: 33.5, 29.8, 29.2
- Leu: 59.1, 47.8, 40.2
- Val: 38.4, 33.1, 25.3
- Met: N/A, 18.4, 7.2
- Cys: N/A, 8.2, 6.3
- Met + Cys: 41.2, 26.6, 13.5
- Phe: N/A, 25.0, 24.5
- Tyr: N/A, 20.0, 17.2
- Phe + Tyr: 70.9, 45.0, 41.7
- Thr: 38.6, 28.2, 19.2
- Trp: N/A, 7.3, 6.8

N/A = not available. *Data on amino acid content of white fishmeal and soybean meal are from Halver [19].

Total n-3 HUFA in diet, g kg⁻¹ = (total fish oil in diet, g kg⁻¹) × (% n-3 HUFA in fish oil used)

(1)

All dry ingredients were mixed together with oil in a food mixer for 15 min. Thereafter tap water was blended into the mixture to attain a consistency appropriate for passing through a meat grinder with a 3 mm holes die. After pelleting, the diets were dried to a moisture content of 80-100 g kg⁻¹ and cool stored in a refrigerator until the start of the experiment.

Hatchery reared Black Sea turbot (*Psetta maeotica*) with mean weight of 30.2 ± 0.2 g were obtained from the Central Fisheries Research Institute (CFRI) in Trabzon, Turkey. Prior to the experiment, the fish were fed a commercial fish meal-based diet (550 g kg⁻¹ crude protein, 160 g kg⁻¹ crude lipid, 90 g kg⁻¹ nitrogen-free extract (NFE), 21 kJ gross energy g⁻¹ diet and 26.2 mg protein kJ⁻¹ energy) to sat-
Table 4. Growth performance and feed utilization of Black Sea turbot fed test diets for 60 days.

<table>
<thead>
<tr>
<th>Experimental diets</th>
<th>FM100</th>
<th>SBM10</th>
<th>SBM20</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBW (g)</td>
<td>30.22 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.17 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.22 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FBW (g)</td>
<td>71.98 ± 3.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.61 ± 3.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.11 ± 3.45&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SGR (% day&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.45 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.44 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.40 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FI (% day&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.072 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.069 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.065 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FCR</td>
<td>0.86 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.86 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.87 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PER</td>
<td>2.31 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.32 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.27 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values (means ± standard deviation of data for triplicate groups) with different superscripts in the same row are significantly different at 5% level. IBW = Initial body weight, FBW = Final body weight. SGR, Specific growth rate (percent increase in body weight per day) = [(ln final wet weight - ln initial wet weight)/days] × 100. FI, feed intake (percent body weight per day) = [(total feed distributed/(initial weight + final weight)/2)/days] × 100. FCR, Feed conversion ratio = feed/wt gain. PER, Protein efficiency ratio = wt gain/protein intake.

III. RESULTS

Survival was 100% in all experimental groups. Mean final body weights, relative growth rate and feed utilization data are shown in Table 4. Even though the highest growth rate was obtained in turbot fed diet with 100% fish meal, no significant differences (P > 0.05) were found among the experimental groups. Similarly, Specific growth rate (SGR), feed conversion rate (FCR), and protein efficiency rates (PER) did not differ significantly (P > 0.05) among groups (Table 4).

Total nitrogen loss per net gain was not significantly different (P > 0.05) among the experimental groups, even though a slight decline could be seen when dietary SBM increased and FM decreased. In a similar manner, total nitrogen loss as a percent of the nitrogen consumed slightly increased, as the FM replacement levels rose, but no significance was recorded (P > 0.05) (Table 5).

Overall, the muscle tissue moisture, crude protein, crude lipid and crude ash of fish did not show any significant difference among the experimental groups. The muscle tissue moisture of fish fed the diets with SBM inclusion showed a slight increase, when compared to fish fed the reference diet, but no significant difference was recorded (P > 0.05). Conversely to muscle tissue moisture, crude lipid of fish muscle tissue tended to decrease slightly as dietary SBM increased, but no significant difference was observed (P > 0.05). The muscle protein of fish fed diet with SBM inclusion tended to decrease slightly, when compared to fish fed the control diet with FM only, however this decline was also not significant (P > 0.05). Similarly, ash levels of fish muscle tissue of fish did not show any significant difference (P > 0.05) among treatment groups (Table 6).

IV. DISCUSSION

The response to dietary soybean incorporation differs among guidelines as follows: dry matter after drying in an oven at 105°C for 24 h until constant weight, protein (N × 6.25) by the Kjeldahl method after acid digestion, lipids by ethyl ether extraction in a Soxhlet System, ash by incineration in a muffle furnace at 550°C for 12 h, while NFE was calculated by difference.

Calculation of the specific growth rate, feed conversion rate, protein efficiency rate, total nitrogen loss and retention rates were performed as described by Watanabe et al. [47, 48], Burel et al. [6] and Yigit et al. [52].

The Kolmogorov-Smirnov and Levene statistic tests were applied to test normality and homogeneity of variance, respectively. Analysis of variance and the Duncan multiple-range test were used to detect significant differences (P < 0.05) in final body weight, specific growth rate, feed intake, feed conversion rate, protein efficiency rate, nitrogen balance and proximate muscle composition. All statistical analyses were performed using the SPSS Statistical Analysis Software Program for Windows, Version 10.0.1, 1999.

The response to dietary soybean incorporation differs among
Table 5. Nitrogen utilization of Black Sea turbot fed to satiation the experimental diets for 60 days.

<table>
<thead>
<tr>
<th>Experimental diets</th>
<th>FM100</th>
<th>SBM10</th>
<th>SBM20</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNI (mg N g⁻¹ production)</td>
<td>69.32 ± 3.69b</td>
<td>69.04 ± 4.46b</td>
<td>70.66 ± 4.26b</td>
</tr>
<tr>
<td>Nitr. diet (%)</td>
<td>8.79</td>
<td>8.74</td>
<td>8.77</td>
</tr>
<tr>
<td>Nitr. in fish (%)</td>
<td>2.78 ± 0.03a</td>
<td>2.77 ± 0.01a</td>
<td>2.75 ± 0.03a</td>
</tr>
<tr>
<td>TNL (mg g⁻¹)</td>
<td>40.75 ± 1.05a</td>
<td>40.52 ± 2.00a</td>
<td>42.39 ± 1.95a</td>
</tr>
<tr>
<td>TNL (% intake)</td>
<td>58.11 ± 1.01a</td>
<td>58.72 ± 0.89a</td>
<td>60.02 ± 0.84a</td>
</tr>
<tr>
<td>TNR (mg g⁻¹)</td>
<td>28.57 ± 1.74a</td>
<td>28.52 ± 2.46a</td>
<td>28.27 ± 2.31a</td>
</tr>
<tr>
<td>TNR (% intake)</td>
<td>41.19 ± 1.01a</td>
<td>41.28 ± 0.89a</td>
<td>39.98 ± 0.84a</td>
</tr>
</tbody>
</table>

Superscript letters indicate intergroup statistical differences. Means ± standard deviation of data for triplicate groups with different superscripts in the same row differ significantly (One way ANOVA and Duncan’s multiple range test, P < 0.05).

Table 6. Muscle proximate composition of Black Sea turbot fed experimental diets in which fish meal protein was partially replaced by soybean meal protein.

<table>
<thead>
<tr>
<th>% Initial</th>
<th>FM100</th>
<th>SBM10</th>
<th>SBM20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moist (wt wet)</td>
<td>80.6 ± 0.1b</td>
<td>80.1 ± 0.4b</td>
<td>80.1 ± 0.1a</td>
</tr>
<tr>
<td>CP (dry basis)</td>
<td>86.5 ± 0.4a</td>
<td>86.9 ± 0.9a</td>
<td>87.2 ± 0.6a</td>
</tr>
<tr>
<td>CL (dry basis)</td>
<td>6.3 ± 0.1a</td>
<td>6.2 ± 0.4a</td>
<td>6.2 ± 0.1a</td>
</tr>
<tr>
<td>CA (dry basis)</td>
<td>6.2 ± 0.4a</td>
<td>6.1 ± 0.3a</td>
<td>5.8 ± 0.2a</td>
</tr>
<tr>
<td>CP (wet basis)</td>
<td>16.8 ± 0.1a</td>
<td>17.4 ± 0.2b</td>
<td>17.3 ± 0.1b</td>
</tr>
</tbody>
</table>

Means with different superscripts in the same row differ significantly at 5% level. CP, crude protein; CL, crude lipid; CA, crude ash.

Fish meal was replaced with soybean meal up to 20% level in turbot diets without amino acid supplementation and no adverse effects on fish performance or feed utilization have been recorded in the present study. Similar findings were reported for yellowtail [49], gilthead seabream [29, 34], olive flounder [9], and Black Sea turbot [11, 12], while higher soybean meal incorporation of 25% to 50% were reported in Japanese flounder [23, 24], Atlantic halibut [4, 17], Atlantic turbot [10], and in yellow tail [42].

Commercial scale farming of Black sea turbot which is an alternative marine fish species is a new issue for the aquaculture industry, and knowledge about the nutritional requirement of this species is still scarce. To our knowledge so far, there is little information on the use of alternative protein sources in diets for Black Sea turbot [11, 12, 20, 44, 50], and these studies were mainly carried out by our research group. Furthermore, as far as we know, there are also only few studies published on the effectiveness of soy products as a dietary protein source in Atlantic turbot. Day and Plascencia-Gonzalez [10] reported that inclusion of soy protein concentrate up to 25% replacement of fish meal, without amino acid supplementation, did not statistically reduce fish growth and feed utilization, but that supplemental dietary methionine and lysine improved the utilization of soy protein concentrate, but this finding was not statistically significant. In Black Sea turbot, Ergün et al. [11] reported that replacing FM protein by SBM up to 20% did not affect growth performance, nutrient utilization and nitrogen retention. Ergün et al. [11] worked on smaller fish size of about 18 g under winter conditions. In the present study, however, fish was grown from about 30 g to 70 g and differently than the previous study, water temperature was close to optimum and ranged from 14 to 24°C during the course of this study carried out from May to June. Therefore it was interesting to see that fish differing in size and cultured at different water temperatures utilized SBM well up to 20% replacement level. In Ergün et al. [12], where soybean was incorporated with hazel nut meal, brown fish meal (anchovy) was used as the main protein source, however, in the present study high quality white fish meal was used according to Yigit et al. [51], in order to define the results with high quality protein sources and under better water temperature conditions. The growth performance data in the present study appeared better than those in the previous studies with low temperature and with brown fish meal diet.

These results can be attributed to the lower water temperature as could be expected and to the lower quality fish meal (brown fish meal) in the diet for Black sea turbot. Our findings are in agreement with those reported in other studies, in terms of the possible use of SBM as a partial substitute for FM in Black Sea turbot diets. Even though the diets were not supplemented with essential amino acids, almost all amino acid requirements for turbot were covered by the experimental diets, indicating that the sub-optimal amino acid balance of SBM did not lead to negative effects in fish when soybean inclusion in the diet was increased to 20%. Hasimoglu et al. [20], evaluated soybean meal and locally-produced anchovy (Engraulis encrasicolus) meal as protein sources in Black Sea turbot diets, by comparing them with Alaskan fishmeal. They prepared three diets; one contained Alaskan fishmeal and the second anchovy meal. The third diet was prepared by replacing only 5% of the anchovy meal by soybean meal. Their results show that diets with anchovy meal gave poorer growth rate when compared to the diets which used Alaskan fishmeal as a protein source. Even though the partial inclusion level of soybean meal was only 5%, the groups with soybean inclusion showed the worst growth performance. It is well known that the quality of plant protein sources can be improved by thermal treatment and solvent-extraction [6], and the protein quality of anchovy meal can also differ from source to source. Furthermore, Yigit et al. [51] suggested the use of white fishmeal instead of brown fishmeal in turbot diets based on their findings of ammonia-N excretion rates after feeding. Hence these
suggestions were followed in the present study and high-quality white fishmeal (whiting meal, 710 g kg⁻¹ crude protein) was used as the sole protein source in the control diet, whereas the other diets with soybean meal inclusion used defatted soybean meal, which is expected to have a higher digestibility than those without any treatment (heat or solvent-extraction). These conditions might have led to the present findings, different than the previous study by Hasimoglu et al. [20] on Black Sea turbot.

The amounts of lipid contained in diets with up to 20% replacement meet the essential fatty acid requirements of turbot, estimated at 8.0 g kg⁻¹ by Gatesoupe et al. [45].

In the present study, similar nitrogen retention in all turbot nutrition [6, 10-14, 33, 44, 50].

ACKNOWLEDGMENTS

We are very grateful to Prof. Dr. Muammer ERDEM (Dean of Fisheries Faculty, Ondokuz Mayis University, Sinop-Turkey) for his valuable advice and support throughout the study. The authors wish to acknowledge JAPAN International Cooperation Agency (JICA), Trabzon Central Fisheries Research Institute (CFRI), and Assoc. Prof. Dr. Emin ÖZDAMAR from JICA Office in Ankara, Turkey, for supporting the experimental animals. We wish to thank Abalioglu Feed Company in Denizli-Turkey for donating the soybean meal product used in the study.

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