EFFECTS OF TEMPERATURE AND DIETARY CARBOHYDRATE LEVEL TO GROWTH PERFORMANCE AND FEED DIGESTIBILITY OF EUROPEAN SEA BASS JUVENILES (*DICENTRARCHUS LABRAX*)

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Abstract

ALTAN, O. A. YILDIRIM KORKUT, 2010. Effects of temperature and dietary carbohydrate level to growth performance and feed digestibility of European sea bass juveniles (*Dicentrarchus labrax*).

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In this study, the effect of temperature and dietary starch level on growth performance and nutrient utilisation in European sea bass juveniles was investigated. An experimental diet as the control was formulated to contain 50% crude protein, 11.5% crude lipids and 30% pregelatinized starch (diet 1); two other diets were formulated to include the same levels of all ingredients as diet 1 except for the pregelatinized starch, which was included at 20% (diet 2) or 10% (diet 3). Diets were assigned to triplicate groups of 25 fish (initial weight: 22 g) at two water temperatures (20°C and 27°C) for 10 weeks. The group receiving diet 1 was fed to apparent satiation and groups fed diets 2 and 3 were pair-fed according to group fed diet 1, so that each group ate the same amount of all nutrients except for carbohydrates. At the end of the trial, growth rate, feed efficiency and protein efficiency ratio were higher at 27°C than at 20°C. Dietary starch level did not affect growth or N utilisation. Feed efficiency was inversely related to dietary starch level. ADC of protein was neither affected by temperature nor dietary starch levels.

Key words: starch, carbohydrates; growth performance; digestibility

Introduction

European sea bass (*Dicentrarchus labrax*) is one of the most important aquaculture species in the Mediterranean countries. An adequate knowledge of its nutritional requirements and nutrient utilisation is of importance for a sustainable production, as it will allow formulation of high quality diets that promote optimum growth and diet utilisation, while minimising losses to the environment (Oliva-Teles, 2000). European sea bass has high protein requirement (Peres and Oliva-Teles, 1999). It has a negative impact on dietary costs as protein is the most expensive nutrient in formulated feeds. High dietary protein levels have also negative impacts to environment due to potential of high nitrogenous losses. Therefore, reducing dietary protein levels and maximizing protein utilisation by the animals is priority both from eco-
nomical and environmental perspective. As a result, great deal of research has been conducted on the protein-sparing potential of lipids and carbohydrates in fish diets (Oliva-Tele, 2000; Watanabe, 2002). Carbohydrates are the least expensive energy source; therefore its inclusion in the diets allows a reduction of feed costs. However, in nature fish have limited access to carbohydrates and are better adapted both at digestive and metabolic levels, to utilise proteins and lipids than carbohydrates as energy sources (Wilson, 1994). The protein-sparing effect of lipids is well demonstrated in several fish species (Watanabe, 1982; Sargent and Tacon, 1999) but that of carbohydrates is still controversial (Wilson, 1994; Hemre et al., 2002; Stone, 2003). The recommended inclusion level of digestible carbohydrates in fish diets depends on the fish species. In general, carnivorous species, including salmonids and most marine fish, tolerate dietary carbohydrate levels lower than herbivorous or omnivorous species (Wilson, 1994). Besides dietary inclusion level, the efficiency of dietary carbohydrate utilisation by fish has been associated to factors such as botanical origin, complexity of the molecule, and technological treatments applied (Wilson, 1994; Stone, 2003; Krogdahl et al., 2005).

Data on carbohydrate utilisation by European sea bass is not enough sufficient. A protein-sparing effect of pregelatinized starch incorporated in the diets at 15% was evaluated by Hidalgo and Alliot (1988). Lanari et al. (1999) underlined that the dietary incorporation of digestible carbohydrates such as dextrin or gelatinized starch should not exceed 20% of the diet. Gouveia et al. (1995) concluded that either raw or gelatinized starch could be included in the diets at 25% without significant effects on growth performance and feed utilisation. Similarly, Enes et al. (2006) reported that neither level (10 or 20%) nor nature (raw or waxy) of starch had measurable effects on growth performance and feed utilisation in juveniles. In contrast, Dias et al. (1998) reported improved growth and feed utilisation of juveniles fed gelatinized starch as compared to raw starch at both dietary inclusion levels tested (11% and 23%). Finally, according to Peres and Oliva-Teles (2002) performance of juveniles was better when fed a mixture (12.5% raw starch plus 12.5% gelatinized starch) than either 25% gelatinized or 25% raw starch.

Data on the effect of temperature on carbohydrate utilisation by fish is even scarcer. In carp, increasing water temperature improved starch digestibility (Medale et al., 1999) while enzyme activities were higher at lower temperatures, denoting thermal compensation for acclimation to low temperature (Shikata et al., 1995). In rainbow trout, starch digestibility and utilisation as energy source improved at higher temperature (Medale et al., 1991). Growth and protein retention in rainbow trout were not affected by dietary carbohydrate: lipid ratio at 8°C, but at 18°C weight gain tended to be higher with the lower carbohydrate: lipid ratio (Brauge et al., 1995). In European sea bass, besides growth performance, temperature was shown to affect feed efficiency, protein utilisation, body composition and glycolytic, lipogenic and gluconeogenic capacities (Peres and Oliva-Teles, 1999; Person-Le Ruyet et al., 2004; Enes et al., 2006).

The aim of this study was to evaluate, in a pair-feeding experiment, the effect of three dietary inclusion levels (10%, 20% and 30%) of pregelatinized starch on growth performance, and nutrient utilisation of European sea bass juveniles reared at two water temperatures (20°C and 27°C).

Materials and Methods

The experiment was carried out in Urla Experimental Facilities of the Faculty of Fisheries, in Ege University, and consisted of growth and digestibility trials.

Diets

An experimental diet was formulated to contain 50% crude protein, 11.5% crude lipids and 30% pregelatinized starch (diet 1); two other diets were formulated to include the same levels of all ingredients as diet 1 except for the pregelatinized starch, which was included at 20% (diet 2) or 10% (diet 3). Diets 2 and 3 were not adjusted to 100% by manipulating any ingredient. All dietary ingredients were finely
ground, well mixed and dry pelleted in a laboratory pellet mill (Erigi Pellet Machine) through 3 mm dies. Ingredients and proximate composition of the experimental diets are presented in Table 1.

**Growth trial**

Juvenile European sea bass (*Dicentrarchus labrax*) were obtained from a commercial hatchery and after a quarantine period were introduced in two thermoregulated recirculating water systems equipped with nine fiberglass cylindrical tanks of 1000 L water capacity, supplied with a continuous flow of filtered seawater. During the trial, water temperature was adjusted to 27 ± 0.5°C in one system and to 20 ± 0.5°C in the other, and salinity averaged 36 ± 1‰. After 2 weeks of adaptation to the experimental conditions, groups of 100 fish with an initial mean body weight of 22.0 g were randomly distributed to each tank. Diets were randomly assigned to triplicate groups of these fish. During the trial, fish were fed by hand, three times a day (at 08:00, 12:00 and 17:00), seven days a week, on a pair-feeding scheme as follows: group receiving diet 1 was fed to apparent visual satiation, while the other groups received 90% (diet 2) and 80% (diet 3) of the ration offered to group 1 in order to assure that each group received the same amount of all nutrients except carbohydrates. Utmost care was taken to assure that all feed supplied was

<table>
<thead>
<tr>
<th>Ingredients, %DW</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peruvian Fish Meal</td>
<td>67</td>
<td>66</td>
<td>65</td>
</tr>
<tr>
<td>Cod Liver Oil</td>
<td>67</td>
<td>66</td>
<td>65</td>
</tr>
<tr>
<td>Gelatinized starch</td>
<td>6.8</td>
<td>7.2</td>
<td>7.4</td>
</tr>
<tr>
<td>Vitamin Premix&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.9</td>
<td>21.1</td>
<td>12</td>
</tr>
<tr>
<td>Mineral Premix&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lignin sulphate</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Proximate analyses, %DW

<table>
<thead>
<tr>
<th></th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>98.7</td>
<td>99.2</td>
<td>99.2</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>50</td>
<td>51</td>
<td>49.6</td>
</tr>
<tr>
<td>Crude fat</td>
<td>11.5</td>
<td>12.3</td>
<td>12.5</td>
</tr>
<tr>
<td>Starch</td>
<td>31.2</td>
<td>21.7</td>
<td>12.9</td>
</tr>
<tr>
<td>Ash</td>
<td>9.7</td>
<td>11.3</td>
<td>12.3</td>
</tr>
<tr>
<td>Gross energy, kJ g&lt;sup&gt;-1&lt;/sup&gt; DM</td>
<td>21.4</td>
<td>21.8</td>
<td>21.7</td>
</tr>
</tbody>
</table>

*Vitamin Premix (IU or mg kg<sup>-1</sup> diet): Vitamin A 800000 IU; Vitamin D3 50000 IU; Vitamin E 15000 mg; Vitamin K3 2700 mg; Vitamin B1 5000 mg; Vitamin B2 4000 mg; Vitamin B6 3000 mg; Vitamin B12 3 mg; Folic acid 1 mg*

*Mineral Premix (g or mg/kg diet): Calcium carbonate (40% Ca), 2.15 g; magnesium oxide (60% Mg), 1.24 g; ferric citrate, 0.2 g; potassium iodide (75% I), 0.4 mg; zinc sulphate (36% Zn), 0.4 g; copper sulphate (25% Cu), 0.3 g; manganese sulphate (33% Mn),
consumed. The growth trial lasted 10 weeks, and the fish were bulk weighted every 2 weeks, after one day of feed deprivation.

**Digestibility trial**

The digestibility trial was conducted in another thermoregulated recirculating water system equipped with a battery of 9 fiberglass tanks of 100 L water capacity and designed according to Cho et al. (1982). Nine groups of 40 fish (mean initial body weight 45 g) were distributed to each tank and each diet was assigned to three groups of fish. Fish were fed by hand, three times a day, in a feeding scheme similar to that used in the growth trial. Fecal collection was carried out in two consecutive periods, first at 27°C and then at 20°C. Fish were allowed to adapt to each tempera-

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**Table 2**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>25°C</th>
<th>18°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diet 1</strong></td>
<td><strong>Diet 2</strong></td>
<td><strong>Diet 3</strong></td>
</tr>
<tr>
<td>IBW (g)</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>FBW (g)</td>
<td>44</td>
<td>44.7</td>
</tr>
<tr>
<td>SGR (%)</td>
<td>1.52</td>
<td>1.54</td>
</tr>
<tr>
<td>FE</td>
<td>0.73</td>
<td>0.85</td>
</tr>
<tr>
<td>PER</td>
<td>1.56</td>
<td>1.59</td>
</tr>
<tr>
<td>N Retention %</td>
<td>30</td>
<td>30.3</td>
</tr>
<tr>
<td>E Retention %</td>
<td>31.1</td>
<td>36.4</td>
</tr>
</tbody>
</table>

Two Way Anova

<table>
<thead>
<tr>
<th>Variation Source&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Tukey HSD for Starch level&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>Diet</td>
</tr>
<tr>
<td>IBW (g)</td>
<td>ns</td>
</tr>
<tr>
<td>FBW (g)</td>
<td>***</td>
</tr>
<tr>
<td>SGR (%)</td>
<td>***</td>
</tr>
<tr>
<td>FE</td>
<td>***</td>
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<tr>
<td>PER&lt;sup&gt;c&lt;/sup&gt;</td>
<td>***</td>
</tr>
<tr>
<td>N Retention %</td>
<td>***</td>
</tr>
<tr>
<td>E Retention %</td>
<td>***</td>
</tr>
<tr>
<td>E Intake</td>
<td>ns</td>
</tr>
</tbody>
</table>

SEM: Pooled Standard Error of the Mean, IBW: Initial Body Weight, FBW: Final Body Weight, SGR: Specific Growth Rate, FE: Feed Efficiency, PER: Protein Efficiency Ratio

<sup>a</sup>SGR: ((ln(final body weight)?ln(initial body weight)) / (time in days))×100;  
<sup>b</sup>FE: (wet weight gain / dry feed intake);  
<sup>c</sup>PER: (wet weight gain / crude protein intake);  
<sup>d</sup>*P<0.05, **P<0.01, ***P<0.001, ns: non-significant;  
<sup>e</sup>Significant differences are indicated.
ture during five days. Then, faeces were collected once a day during eight consecutive days. Immediately after collection, faeces from each tank were centrifuged and dried until constant weight. Within each period, all faeces from a tank were pooled for analyses.

Analytical methods

Chemical analysis

Chemical composition of diets and faeces were analyzed using following procedures: dry matter after drying at 105°C until constant weight ash by incineration in a muffle furnace at 550°C for 16 h; protein content (N x 6.25) by the Kjeldahl method after acid digestion using a Kjeltec digestion and distillation units; lipid by petroleum ether extraction (Soxtect HT System) starch according to Thivend et al. (1972); gross energy by direct combustion in an adiabatic bomb calorimeter (PARR, 1261); chromic oxide in the diets and faeces was measured by acid digestion according to Furukawa and Tsukahara (1966).

Statistical analysis

Statistical analysis of data was done by two-way analysis of variance. The probability level of 0.05 was used for rejection of the null hypothesis. Tukey multiple range tests were used for determining significant differences among means.

Results

During the trial, mortality ranged from 1.4% to 4.2% in the tanks and was not different among groups. Final body weight, weight gain and specific growth rate were higher in fish reared at 27°C and within each temperature were not affected by diet composition (Table 2). Feed intake was also higher at 27°C. As fish were fed according to a pair-feeding scheme, feed intake was necessarily different among groups. Feed efficiency was higher at 27°C than at 20°C and decreased with the increase of dietary starch level. Protein efficiency ratio and N retention were higher at 27°C but did not differ among groups within each temperature. As percentage of energy intake, energy retention was not affected by temperature, but was lowest with the highest starch diet.

Apparent digestibility coefficient (ADC) of dry matter was not affected by diet or temperature (Table 3). ADC of protein and energy were not affected by temperature but were inversely related to dietary starch level.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Apparent digestibility coefficients (%) of the experimental diets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>25°C</td>
</tr>
<tr>
<td>Diet 1</td>
<td>Diet 2</td>
</tr>
<tr>
<td>Dry matter</td>
<td>62.5</td>
</tr>
<tr>
<td>Protein</td>
<td>87.3</td>
</tr>
<tr>
<td>Starch</td>
<td>92.2</td>
</tr>
<tr>
<td>Energy</td>
<td>95.3</td>
</tr>
</tbody>
</table>

Two Way Anova

<table>
<thead>
<tr>
<th>Variation Source</th>
<th>Tukey HSD for Starch level</th>
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<tbody>
<tr>
<td>Temperature</td>
<td>Diet</td>
</tr>
<tr>
<td>Dry matter</td>
<td>ns</td>
</tr>
<tr>
<td>Protein</td>
<td>ns</td>
</tr>
<tr>
<td>Starch</td>
<td>*</td>
</tr>
<tr>
<td>Energy</td>
<td>ns</td>
</tr>
</tbody>
</table>

SEM: pooled standard error of the mean.

* P<0.05, **P<0.01, ***P<0.001, ns: non-significant; Significant differences are indicated by different letters (P<0.05)
level. The ADC of starch was higher at 27°C than at 20°C and lower at the highest dietary inclusion level.

**Discussion**

There is a general consideration that carnivorous fish like European sea bass do not digest carbohydrates very efficiently (Wilson, 1994; Krogdahl et al., 2005). It is also known that technological treatments such as gelatinization, may improve starch and energy digestibility (Peres and Oliva-Teles, 2002). Moreover, dietary starch level may negatively affect carbohydrate digestibility and may also interact with the digestibility of other dietary nutrients (Kaushik and Médale, 1994; Wilson, 1994; Stone, 2003). The results of this trial indicate that pregelatinised starch was very well digested by European sea bass juveniles, with ADC ranging from 92 to 96%, although a decrease of ADC of starch with dietary inclusion level occurred. This high digestibility of pregelatinised starch is in line with results previously obtained in this species by Peres and Oliva-Teles (2002). A high ADC of both raw and waxy starch was also observed by Enes et al. (2006). The later authors also observed a decrease of starch digestibility with the increase of dietary inclusion levels. On the other hand, Dias et al. (1998) observed that while for raw starch ADC of starch decreased with dietary inclusion level, the opposite was true for gelatinised starch. Overall, from the results of these different studies it may be concluded that European sea bass digests efficiently starch, independently of its presentation form. Nevertheless, this study showed that gelatinisation improves ADC of starch independently of dietary inclusion level (up to 30%). The effect of temperature on carbohydrate digestibility is scarcely studied in fish and to our knowledge no data was previously available for European sea bass. In agreement to data obtained in other species (Medale et al., 1991, 1999) our results showed an improvement of the ADC of starch with an increase of temperature.

The ADC of protein was also high in the present study, and it was not affected by dietary carbohydrate level or temperature. This is in accordance to previous data on this species regarding the effect of carbohydrate on protein digestibility (Gouveia et al., 1995; Dias et al., 1998; Peres and Oliva-Teles, 2002 and Moreira et al., 2008). However, a decrease of protein digestibility with the increase of the dietary carbohydrate level was recently reported in European sea bass (Enes et al., 2006) as well as in gilthead sea bream (Fountoulaki et al., 2005) and White Sea bream (Sa et al., 2007). Peres and Oliva-Teles (1999) also reported a significant effect of temperature on ADC of protein in European sea bass, although, differences in ADC between the two tested temperatures (20 and 27°C) were very low. We may therefore conclude that temperature has a very minor, if any, effect on protein digestibility in this species.

According to Alliot et al. (1979), incorporation of appropriate levels of carbohydrate in the diets can improve growth performance of European sea bass. However, Peres and Oliva-Teles (2002) did not observe any growth improvement in European sea bass juveniles fed diets including carbohydrates comparatively to fish fed a carbohydrate-free diet. Moreover, in that study feed efficiency decreased with the incorporation of carbohydrates to the diets. Also Enes et al. (2006) observed no effect on growth of European sea bass juveniles when fed starch containing diets comparatively to a starch-free diet. However, in this study feed efficiency improved with the incorporation of starch into the diets. Other studies with European sea bass juveniles also failed to show an effect of dietary starch level on growth improvement (Gouveia et al., 1995; Lanari et al., 1999; Alvarez et al., 1999; Enes et al., 2006). Accordingly, in the present trial, in pair-fed fish and within the starch range tested (10–30%) no growth improvement was also noticed due to dietary starch intake. Moreover, feed efficiency decreased with the increase of dietary carbohydrate intake, suggesting that preglutatinized starch was not efficiently used as energy source, which agrees with the results of Peres and Oliva-Teles (2002). On the contrary, in other studies with sea bass, feed efficiency was not affected with the increase of dietary starch (Gouveia et al., 1995; Lanari et al., 1999; Enes et al., 2006). The inefficient utilisation of preglutatinised
starch as energy source was further confirmed in the present trial by data on PER and N retention. These parameters were not affected by dietary starch intake, indicating that under our experimental conditions dietary starch had no protein-sparing effect. This is also in agreement with the previously reported results in this species (Gouveia et al., 1995; Lanari et al., 1999; Enes et al., 2006).

As expected, temperature improved growth performance. In fact, 27°C is near the optimum temperature range for growth of European sea bass juveniles in cage culture, near Mediterranean costs, while 20°C is a little bit lower than the optimum for that region. Feed efficiency, PER, N and energy retention also improved with temperature but no interaction between temperature and starch utilisation was noticed. According to Person-Le Ruyet et al. (2004) maximum feed efficiency is expected to occur around 24°C while at lower temperatures feed efficiency decreases. Similar effect of temperature on the same performance parameters was also previously observed in this species by Peres and Oliva-Teles (1999). On the contrary Hidalgo et al. (1987) observed that although feed conversion improved with temperature that was not the case of PER. However this last study was carried out at 15 and 20°C, and both temperatures are clearly below the optimum temperature for the species, and this may explain these results.

In conclusion, our data suggest that growth, feed efficiency, protein and energy retention were higher at 27°C than at 20°C. Although high at both temperatures, ADC of starch improved with temperature increase. Increase of dietary starch level did not improve growth rate or protein utilisation, indicating that no protein sparing by dietary starch occurred under this experimental conditions. On the contrary, feed efficiency and energy retention were inversely related to dietary starch level suggesting that starch was not efficiently used as an energy source.

Acknowledgements
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