Effects of Artificial Diets on Biological Performances of *Acanthopagrus latus* Broodstock in the Persian Gulf

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Abstract

Effects of artificial diets on growth, spawning performance, egg and larval biochemical compositions and quality of *Acanthopagrus latus* in the Persian Gulf were studied. Nine diets representing a combination of three protein levels (40, 50 and 60%) and three energy levels (22.5, 23.5 and 24.5 MJ GE/Kg) were tested in triplicates. Each tank was stocked with 10 fish with a sex ratio of 1:1 and with an average weight of 415g and 236g for females and males, respectively. Fish were fed to satiation, twice daily. The growth of *Acanthopagrus latus* broodstock was not significantly affected (P>0.05) by dietary protein and energy levels. Biometry, relative fecundity and survival rate and crude protein and lipid of eggs, hatchling and 3 day post-hatch (3DPH) larvae were significantly affected (P<0.05), but spawning performance and body composition of broodstock, with the exception of body crude protein and lipid were not significantly affected by dietary protein and energy levels. This study revealed the best spawning performances of *A. latus* broodstock were achieved at 40% dietary protein and 23.5 MJ GE/Kg dietary energy.

Keywords: Artificial diets, Growth, Spawning, Egg quality, *Acanthopagrus latus*, Persian Gulf.

1. Introduction

The Sparide is a predominantly marine family frequented in the Indian, Pacific and Atlantic oceans (Nelson, 1994; Platell et al., 2007) and contains many species of commercial and/or recreational importance and some that are used for aquaculture (Ingram et al., 2002; Platell et al., 2007). Sparids typically consume a wide range of benthic prey and occasionally substantial amount of plant material (Sarre et al., 2000; Mariani et al., 2002; Tancioni et al., 2003; Platell et al., 2007). Furthermore, diets of Sparid species often differ markedly with respect to location, reflecting the opportunistic nature of the feeding behavior of the members of this family (Sarre et al., 2000; Mariani et al., 2002; Tancioni et al., 2003; Platell et al., 2007).

Exogenous nutrition of broodfish provides the essential nutrients required for gonadal development of females and the performance of produced seed (Gunasekera et al., 1997; El-Sayed et al., 2008). Thus, egg yolk is considered the major source of nutrition for embryonic development in fish. Therefore,
inadequate food supply for fish broodstock will lead
to poor spawning performance and seed production
(Gunasekera et al., 1997; El-Sayed et al., 2008).
Proteins and energy, the main components of egg
yolk, are considered to play pivotal role in
reproduction (Afzal Khan et al., 2005). Accordingly,
the relationship between dietary protein and energy
levels in fish feeds should be considered for optimum
fish performance. At inadequate energy levels, dietary
protein may be used as an energy source (Garling &
Wilson, 1976; Cho & Kaushik, 1985; El-Sayed et al.,
2008). At adequate energy levels, dietary protein can
be spared for anabolic functions (El-Sayed et al.,
1987, 2008; Satpathy et al., 2003). Therefore, the
proper balance between dietary protein and energy is
essential for optimum use of fish feeds so that the
dietary protein can be spared by dietary energy effects
(Garling & Wilson, 1976; Satpathy et al., 2003; El-
Sayed et al., 2008).

The most study on Sparids remain confined to fry,
fryerling and young fish (Vergara et al., 1996;
Santhinha et al., 1999; Gomez-Requeni et al., 2003;
Venou et al., 2003; Teshima et al., 2004; Sá et al.,
2006; Benedito-palos et al., 2007) and the effects of
the interaction between dietary protein and energy
on spawning performance of yellowfin sea bream
has not been determined. This study was undertaken
to investigate the effects of dietary protein and
energy levels on growth, spawning performance,
egg and larval biochemical composition and quality
of A. latus.

2. Material and Methods

Experimental trial - Nearly 7 months prior to
natural spawning season (late February- early March)
wild broodstock yellowfin sea bream, A. latus, were
captured with hook from the northwest of the Persian
Gulf during August to September (2008) and
transferred immediately to the Mariculture Research
Station of South Iranian Aquaculture Research
Center, Mahshahr, Iran in a aerated fiberglass tank.
The fishes were maintained in concrete tanks (75m³)
both to the commencement of trial. Fourteen days
before commencing the trial, 270 fish were weighed
distributed randomly between the 27
experimental tanks (1139 L.Tank⁻¹).

The experiment consisted of nine treatments with
three replicates each. Each tank was stocked with 10
fish with a sex ratio of 1:1 and an average weight of
415±39.4g and 236±24g for females and males,
respectively. Fish were fed to satiation, twice daily,
at 09:00 and 17:00 h for 129 days prior to first
spawning (from middle of October to late of
February). Broodstock were weighed in each tank at
the end of the spawning period to determine weight
 gain. The experiment lasted 146 days.

Water temperature, salinity, dissolved oxygen and
pH were recorded daily. Salinity ranged between
39‰ and 44‰ (41 ± 0.1) and temperature fluctuated
between 14 to 22°C (15.7 ± 0.2) during the
experimental period. Average values for dissolved
oxygen and pH were 7.5 mgL⁻¹ and 7.9, respectively.

Experimental diets- Nine semi-purified diets
representing a combination of three protein levels
(40, 50 and 60%) and three energy levels (22.5, 23.5
and 24.5 MJ/Kg) were tested. Biochemical contents
of diets were determined according to AOAC (1990).
Formulation and biochemical composition of the test
diets are presented in Table 1.

Spawning, egg and larval collection- Each tank
was checked daily for eggs. Spawning started at late
of February (water temperature>20°C). Spawning
took place at night (between 02:00 to 05:00) and the
eggs were collected the next morning from the tanks
and transferred to beaker (1L). Total number of eggs
was estimated volumetrically by 54 samples of 1 ml
(6 samples of 3 levels of beaker in triplicates) to
assess relative fecundity. Twenty seven samples of 1
ml (3 samples of 3 levels of beaker in triplicates)
were investigated microscopically in order to sort out
fertilized and unfertilized eggs and to calculate
fertilization ratio. All eggs were left to remain in
beaker for 20 minutes to completely separate sunken
and floating eggs and to record respective proportions. After eggs hatching, 50 five ml samples were collected randomly to observe the hatched ones. To determine survival rate of larvae, 50 five ml samples of were collected randomly at 3DPH, as well. Fifty eggs, hatchlings and 3DPH larvae were selected randomly from each tank and days of spawning period and were preserved in 10% formalin solution for biometry using a compound microscope equipped with ocular micrometer. The remaining eggs were labeled and frozen immediately at -20 °C for chemical analyses.

Biochemical analyses- Initial body composition of fish was analyzed from 20 samples of fish (10 females and 10 males) frozen prior to the commencement of trial. After spawning, four broodfish (two females and two males) from each tank were individually weighed, gutted and frozen for biochemical analyses. Biochemical composition of feed ingredients, experimental diets, broodfish whole body, eggs and larvae were analyzed using standard methods (AOAC, 1990). Each analysis was conducted in triplicate.

Statistical analysis- Analysis of variance (ANOVA) (One-way and two-way, 3 × 3 factorial) and descriptive statistics were used to determine differences among protein, energy levels and treatments, using SPSS 11.5 statistical software. and when differences were found a Tukey’s post hoc test was conducted to determine specific differences in protein and energy levels and treatment.

### Table 1- Formulation and biochemical composition of the test diets (Dry-weight basis)

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>22.5</th>
<th>23.5</th>
<th>24.5</th>
<th>22.5</th>
<th>23.5</th>
<th>24.5</th>
<th>22.5</th>
<th>23.5</th>
<th>24.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal (64% CP)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Casein (75% CP)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Gelatin (97% CP)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>28</td>
<td>28</td>
<td>28</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Soybean meal (51%CP)</td>
<td>41</td>
<td>41</td>
<td>41</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Fish oil</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>1</td>
<td>4</td>
<td>6</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>0.5</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Rice bran</td>
<td>13</td>
<td>10</td>
<td>8</td>
<td>9.5</td>
<td>7.5</td>
<td>5.5</td>
<td>3.5</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>13.5</td>
<td>11.5</td>
<td>9.5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Vitamin premix a</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Mineral premix b</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Biochemical composition c (%)</td>
<td>43.21</td>
<td>43.07</td>
<td>42.48</td>
<td>52.85</td>
<td>53.07</td>
<td>50.9</td>
<td>61.42</td>
<td>61.38</td>
<td>60.48</td>
</tr>
<tr>
<td>Ash</td>
<td>8.39</td>
<td>7.56</td>
<td>7.11</td>
<td>8.14</td>
<td>7.36</td>
<td>6.52</td>
<td>7.73</td>
<td>7.29</td>
<td>6.42</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>11.43</td>
<td>9.8</td>
<td>8.62</td>
<td>8.82</td>
<td>7.67</td>
<td>5.58</td>
<td>10.48</td>
<td>3.61</td>
<td>2.43</td>
</tr>
<tr>
<td>NFE d</td>
<td>23.39</td>
<td>20.71</td>
<td>18.02</td>
<td>17.21</td>
<td>12.87</td>
<td>12.61</td>
<td>4.59</td>
<td>6.5</td>
<td>5.28</td>
</tr>
</tbody>
</table>

a Per kilogram: vit. A, 600,000 IU; vit. D₃, 400,000 IU; vit. E, 40,000 mg; vit. K₃, 1,000 mg; vit. B₁ (Thiamin mononitrate), 3,000 mg; vit. B₂ (Riboflavin), 5,000 mg; vit. B₃ (Pyridoxine hydrochloride), 3,000 mg; vit. B₆ (Pyridoxine hydrochloride), 3,000 mg; vit. B₇ (Cyanocobalamin), 8,000 mg; vit. C, 52,000 mg; Nicotinic acid, 30,000 mg; D-calcium pantothenate, 9,000 mg; Folic acid, 1,600 mg; D-biotin, 100 mg; Inositol, 24,000 mg; Antioxidant, 5,000 mg.

b Per kilogram: Manganese, 2,600 mg; Copper, 6,000 mg; Ferrous, 4,000 mg; Zinc, 6,000 mg; Selenium, 500 mg; Iodine, 2,000 mg; Cobalt, 500 mg; Choline chloride, 120,000 mg.

c Value are means of three replicate samples per diet.

d Nitrogen-free extract (calculated by difference).

e Gross energy, calculated based on 0.17, 0.398 and 0.237 MJ/g for carbohydrate, lipid and protein, respectively.

### 3. Results

Growth- Results on growth and biometry of eggs and hatchlings larvae of *A. latus* fed with different dietary protein and energy levels are summarized in table 2. Growth of *A. latus* broodstock was not affected but, biometry of eggs, hatchlings and 3DPH larvae were affected significantly (P<0.05) with dietary protein and energy levels.

Spawning performance - Results indicated that
spawning performance criteria of *A. latus* broodstock, except fecundity and survival rate in 3DPH larvae (%), were not significantly affected (P<0.05) by dietary protein and energy levels (Table 3).

Biochemical composition- With the exception of body crude protein and lipid, body composition of broodstock was not significantly affected (P<0.05) by dietary protein and energy levels (Table 4). Biochemical composition of eggs, hatchlings and 3DPH larvae are summarized in Table 5.

### Table 2- Growth and biometry of eggs, hatchlings and 3DPH 1 larvae of *Acanthopagrus latus* fed different dietary protein and energy levels

<table>
<thead>
<tr>
<th>Diets</th>
<th>P40E22.5</th>
<th>P40E23.5</th>
<th>P40E24.5</th>
<th>P50E22.5</th>
<th>P50E23.5</th>
<th>P50E24.5</th>
<th>P60E22.5</th>
<th>P60E23.5</th>
<th>P60E24.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>342.7±37.7</td>
<td>331.3±29.6</td>
<td>372.1±34.1</td>
<td>352.6±</td>
<td>326.1±</td>
<td>333.2±</td>
<td>378.7±</td>
<td>362.8±30.9</td>
<td>316.1±</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>439.0±33.0</td>
<td>412.6±26.0</td>
<td>458.8±29.3</td>
<td>432.0±</td>
<td>408.2±</td>
<td>405.8±</td>
<td>470.8±</td>
<td>445.1±22.7</td>
<td>428.6±</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>96.3±70⁵</td>
<td>81.3±55.6⁵</td>
<td>86.7±63.4⁶</td>
<td>79.4±</td>
<td>55.1⁶</td>
<td>45.3⁷</td>
<td>53.8⁸</td>
<td>56.9⁹</td>
<td>53.6⁸</td>
</tr>
<tr>
<td>Egg diameter (μm)</td>
<td>0.75±0.002⁶</td>
<td>0.757±</td>
<td>0.729±</td>
<td>0.747±</td>
<td>0.761±</td>
<td>0.749±</td>
<td>0.760±</td>
<td>0.747±</td>
<td>0.753±</td>
</tr>
<tr>
<td>oil globule droplet diameter (μm)</td>
<td>181.5±1.1⁶</td>
<td>184.0±1.0³</td>
<td>185.5±0.6¹</td>
<td>185.8±</td>
<td>191.5±</td>
<td>187.6±</td>
<td>188.2±</td>
<td>185.3±</td>
<td>186.3±</td>
</tr>
<tr>
<td>Hatchling length (mm)</td>
<td>1.55±0.02²</td>
<td>1.46±0.01³</td>
<td>1.43±0.02⁷</td>
<td>1.42±</td>
<td>1.48±</td>
<td>1.47³</td>
<td>1.47±</td>
<td>1.41±</td>
<td>1.44±</td>
</tr>
<tr>
<td>yolk sac length (μm)</td>
<td>581.7±12.7⁴</td>
<td>605.2±5.6⁵</td>
<td>566.1±7.2⁹</td>
<td>610.5±</td>
<td>572.8±</td>
<td>589.5±</td>
<td>598.6±</td>
<td>585.9±</td>
<td>594.7±</td>
</tr>
<tr>
<td>oil globule droplet diameter (μm)</td>
<td>181.9±2.7⁴</td>
<td>175.6±1.6³</td>
<td>171.1±1.7⁴</td>
<td>178.1±3.9</td>
<td>170.7²</td>
<td>168.5±</td>
<td>165.7±1.4³</td>
<td>170.7²</td>
<td>1.1³</td>
</tr>
<tr>
<td>3DPH 1 larvae length (mm)</td>
<td>2.36±0.05⁴</td>
<td>2.38±0.02⁴</td>
<td>2.18±0.06³</td>
<td>2.39±</td>
<td>2.56±</td>
<td>2.19³</td>
<td>2.00±</td>
<td>2.30±</td>
<td>2.4±0.03³</td>
</tr>
<tr>
<td>oil globule droplet diameter (μm)</td>
<td>70.5±5.9⁶</td>
<td>67.5±5.7⁹</td>
<td>87.0±4.8⁶</td>
<td>67.5±</td>
<td>66.0±</td>
<td>64.5²</td>
<td>5.0⁵</td>
<td>70.5±5.0</td>
<td>73.5±5.1³</td>
</tr>
</tbody>
</table>

Mean ± SE values with different superscript in each row are significantly (P<0.05) different.

1. Days post-hatching

### Table 3- Spawning performance of *Acanthopagrus latus* broodstock fed different dietary protein and energy levels

<table>
<thead>
<tr>
<th>Diets</th>
<th>P40E22.5</th>
<th>P40E23.5</th>
<th>P40E24.5</th>
<th>P50E22.5</th>
<th>P50E23.5</th>
<th>P50E24.5</th>
<th>P60E22.5</th>
<th>P60E23.5</th>
<th>P60E24.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative fecundity (10⁶ eggs Kg⁻¹ female weight)</td>
<td>833±2⁴</td>
<td>1349±2³</td>
<td>1469±1¹</td>
<td>1311±2²</td>
<td>1737±5⁴</td>
<td>1478±2²</td>
<td>1374±2²</td>
<td>1952±2⁴</td>
<td>1367±2⁴</td>
</tr>
<tr>
<td>Floating eggs (%)</td>
<td>59.6±3.5⁶</td>
<td>54.1±3.6⁶</td>
<td>60.5±3.3⁶</td>
<td>59.8±³</td>
<td>58.2±3.3⁶</td>
<td>54.2±3.0⁸</td>
<td>57.2±³</td>
<td>66.3±³</td>
<td>59.3±³</td>
</tr>
<tr>
<td>Fertilizability (%)</td>
<td>83.8±3.7</td>
<td>81.4±2.9</td>
<td>81.9±2.9</td>
<td>78.4±3.2</td>
<td>77.6±3.1</td>
<td>74.7±3.6</td>
<td>74.3±4.4</td>
<td>76.0±3.6</td>
<td>81.3±3.1</td>
</tr>
<tr>
<td>Hatchability (%)</td>
<td>45.7±3.1⁶</td>
<td>60.3±³</td>
<td>51.2±³</td>
<td>36.7±³</td>
<td>29.1±³</td>
<td>42.0±³</td>
<td>38.8±³</td>
<td>53.9±³</td>
<td>41.2±6.8⁶</td>
</tr>
<tr>
<td>Survival rate at 3DPH1 (%)</td>
<td>87.3±6.3⁶</td>
<td>76.2±5.7⁶</td>
<td>94.9±4.0⁸</td>
<td>93.9±4.4⁹</td>
<td>60.0±6.3³</td>
<td>21.1±6.4⁹</td>
<td>91.6±5.2⁹</td>
<td>50.1±6.4⁹</td>
<td>58.1±6.3³</td>
</tr>
</tbody>
</table>

Mean ± SE values with different superscript in each row are significantly (P<0.05) different.

1. Days post-hatching

### Table 4- Biochemical composition of whole body of *Acanthopagrus latus* broodstock fed different dietary protein and energy levels

<table>
<thead>
<tr>
<th>Diets</th>
<th>P40E22.5</th>
<th>P40E23.5</th>
<th>P40E24.5</th>
<th>P50E22.5</th>
<th>P50E23.5</th>
<th>P50E24.5</th>
<th>P60E22.5</th>
<th>P60E23.5</th>
<th>P60E24.5</th>
<th>Initial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>65.3±1.9</td>
<td>67.0±0.6</td>
<td>63.6±2.1</td>
<td>66.0±1.0</td>
<td>66.6±0.4</td>
<td>64.9±1.1</td>
<td>64.1±2.7</td>
<td>63.9±1.8</td>
<td>65.7±0.6</td>
<td>63.7±0.8</td>
</tr>
<tr>
<td>Crude protein 1</td>
<td>59.9±0.6⁶</td>
<td>59.5±0.8⁶</td>
<td>58.1±0.9³</td>
<td>62.7±0.8³</td>
<td>58.6±1.0⁸</td>
<td>57.1±1.4³</td>
<td>60.1±1.2⁶</td>
<td>58.9±1.0³</td>
<td>57.4±1.2³</td>
<td>55.8±0.8³</td>
</tr>
<tr>
<td>Crude lipid 1</td>
<td>19.6±0.9³</td>
<td>20.6±0.9³</td>
<td>27.0±1.3³</td>
<td>18.2±0.7³</td>
<td>21.0±1.0³</td>
<td>21.2±1.1³</td>
<td>18.5±1.0³</td>
<td>23.9±0.9³</td>
<td>22.9±0.7³</td>
<td>21.4±1.1³</td>
</tr>
<tr>
<td>Fibre</td>
<td>1.9±0.2</td>
<td>1.0±0.1</td>
<td>1.0±0.1</td>
<td>1.6±0.1</td>
<td>2.0±0.1</td>
<td>2.0±0.1</td>
<td>2.0±0.2</td>
<td>1.3±0.1</td>
<td>1.8±0.2</td>
<td>1.9±0.2</td>
</tr>
<tr>
<td>Ash</td>
<td>13.2±1.1</td>
<td>15.1±1.0</td>
<td>13.4±2.1</td>
<td>13.0±0.7</td>
<td>13.3±1.1</td>
<td>14.0±1.2</td>
<td>13.3±1.0</td>
<td>12.3±0.8</td>
<td>12.5±0.6</td>
<td>10.5±0.8</td>
</tr>
</tbody>
</table>

1. Dry-weight basis; mean ± SE values with different superscript in each row are significantly (P<0.05) different.
Table 5- Biochemical composition of eggs, hatchlings and 3DPH1 larvae of *Acanthopagrus latus* fed different dietary protein and energy levels (Dry-weight basis)

<table>
<thead>
<tr>
<th>Diets</th>
<th>P40E22.5</th>
<th>P40E23.5</th>
<th>P40E24.5</th>
<th>P50E22.5</th>
<th>P50E23.5</th>
<th>P50E24.5</th>
<th>P60E22.5</th>
<th>P60E23.5</th>
<th>P60E24.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>56.8±1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.1±0.9&lt;sup&gt;e&lt;/sup&gt;</td>
<td>57.8±1.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.4±1.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>54.7±2.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>57.9±0.7&lt;sup&gt;e&lt;/sup&gt;</td>
<td>56.6±0.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>52.0±1.8&lt;sup&gt;e&lt;/sup&gt;</td>
<td>54.1±1.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Egg</td>
<td>54.0±0.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>45.5±0.4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>51.1±0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>43.4±2.1&lt;sup&gt;f&lt;/sup&gt;</td>
<td>51.6±1.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>49.5±2.1&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>42.5±0.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>43.4±2.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>49.4±2.1&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hatchling</td>
<td>50.3±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.3±0.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>53.0±0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>41.8±0.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>45.3±0.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>46.2±0.1&lt;sup&gt;ce&lt;/sup&gt;</td>
<td>53.0±0.1&lt;sup&gt;de&lt;/sup&gt;</td>
<td>48.6±0.8&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>47.9±0.2&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>15.9±1.8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16.7±1.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>11.0±0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.3±0.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>16.5±0.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>15.6±1.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>17.7±0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.7±0.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16.3±1.0&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>3DPH1 larvae</td>
<td>7.5±0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.8±0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.9±0.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>11.4±0.2&lt;sup&gt;de&lt;/sup&gt;</td>
<td>12.7±0.2&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>12.9±0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.0±0.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>9.9±0.3&lt;sup&gt;e&lt;/sup&gt;</td>
<td>11.2±0.2&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean ± SE values with different superscript in each row are significantly (P<0.05) different.

1 Days post-hatching

4. Discussion

Growth and anabolism of fish are supported by energy obtained from the catabolism of dietary protein and energy sources. Similarly, dietary lipids also influence the dietary protein requirement of fish profoundly. Many studies have been conducted to study the potential sparing of dietary protein by increasing the concentration of non-protein energy. These studies have indicated that at inadequate energy level, dietary protein may be used as an energy source, whereas at high protein level, a proportion of this protein will be deaminated and carbon skeleton used as an energy source (El-Sayed et al., 2008). At adequate energy level, dietary protein can be spared for anabolic functions (Morais et al., 2001). Afzal Khan et al., (2005) found that dietary protein level did not affect the diameter of spawned eggs of *L. rohita* and Spin and Lochmann (2008). These differences could be attributed to the species-specific growth rates. *A. latus* has been reported to have a low growth rate (Hesp et al., 2004) and this probably might be another reason for the low growth performance observed in this study.

Egg diameter is considered an important criterion for the assessment of spawning performance in fish. The effect of dietary protein and energy levels on egg diameter of *A. latus* was significant. Similar finding have also been reported in *Cyprinus carpio* (Manissery et al., 2001) and *O. niloticus* (El-Sayed et al., 2003). Afzal Khan et al., (2005) found that dietary protein level did not affect the diameter of spawned eggs of *L. rohita* and Spin and Lochmann (2008) reported that egg diameters were not different for eggs from *I. punctatus* broodfish fed different lipid diets.

The present results indicated that dietary protein significantly affected length of hatchlings and 3DPH larvae of *A. latus*. Hatchling length was increased with decreasing dietary protein from 60 to 40% regardless of energy levels. Also, the length of 3DPH larvae was significantly affected by energy levels, but effects of dietary protein and energy showed irregular trends. Similar results have been reported by El-Sayed et al., (2003, 2008) where growth of
Nile tilapia fry was found to increase with increasing dietary protein content of broodstock diets from 30 to 40%. Also Duray et al., (1994) showed that increasing lipid levels from 12% to 18% in broodstock rabbit fish produced large newly hatched larvae. However, Sink and Lochmann (2008) reported that there was no difference in weight or length of I. punctatus fry at 14 day from broodstock fed with different lipid diets. Results of OGD of eggs and hatchlings were noticeable and the effect of dietary protein at 50 and 40% levels were highly significant.

Fecundity, another important parameter to assess spawning performance of fish, is known to be affected by nutritional deficiencies in broodstock diet (Izquierdo et al., 2001). Diet containing high (60%) dietary protein level and intermediate (23.5 MJ/Kg) energy level produced higher relative fecundity in A. latus. Also, relative fecundity was significantly affected by energy levels. However, the increase in relative fecundity was comparable among increasing dietary protein levels, presumably dietary protein levels used were above the minimum requirement, and any increase in dietary protein did not further affect the relative fecundity. These results appear to be in agreement with the results of Sink and Lochmann (2008) who reported spawning success in I. punctatus increased as dietary energy concentration increased. Fernández-Palacios et al., (1995) stated that in gilthead sea bream, the total number of eggs produced / Kg of female was more sensitive to the dietary essential fatty acid (EFA) levels than the other parameters. Similar observation was made in Paralichthys olivaceus (Furuita et al., 2000). However, El-Sayed et al. (2008) described at low (30%) and intermediate (35%) dietary protein levels, increasing dietary energy levels resulted in a significant decrease in spawning performance of O. niloticus, probably because the P/E ratio of these diets was not appropriate for optimum performance. In C. carpio maximum fecundity was at high dietary protein levels (350 g Kg\(^{-1}\)) (Manissery et al., 2001).

Although, Afzal Khan et al. (2005) expressed that low dietary protein levels (250 and 300 g Kg\(^{-1}\)) produced higher relative fecundity in L. rohita.

The percentage of buoyant eggs on the water surface has been broadly used to evaluate egg quality in marine fish (Izquierdo et al., 2001). However, in A. latus buoyant eggs (%), despite being at their maximum number in fish fed with high (60%) dietary protein level and intermediate (23.5 MJ/Kg) energy level diet, were not affected by dietary protein and energy levels. Ceredà et al. (1994) reported that eggs of D. labrax fish fed with low (34%) protein diet had significantly lower buoyancy than those fed with high (51%) protein diet.

Nutrition influences the fertilizability and hatchability (%) of fish eggs; Results showed these criteria, however, were not significantly affected by dietary protein and energy levels. Although, hatchability (%) was at its maximum in fish fed with P40E23.5 diet with noticeable values. Similar findings have also been reported by Afzal Khan et al. (2005) in L. rohita. Irregardless, El-Sayed et al. (2008) reported that fertilized eggs produced from O. niloticus broodstock fed with 30 and 35% protein diet displayed lower hatchability than the eggs of fish fed with a 40% protein diet. However, increasing energy level at each dietary protein level did not significantly affect these parameters. Conversely, Sink and Lochmann (2008) described that eggs from I. punctatus broodfish fed with the 10% lipid diet showed greater hatching success than eggs from broodfish fed with 4% lipid diet. Ceredà et al. (1994) reported that the eggs of D. labrax fish fed with 51% protein diet had significantly higher hatchability than those fed with 34% protein diet fish. In addition, increased egg quality, fecundity, fertilization success and egg hatching success in fish have been linked to diets enriched with highly unsaturated fatty acids (Izquierdo et al., 2001).

Survival rate (%) at 3DPH larvae was significantly affected by dietary protein and energy levels. Furuita et al. (2000) stated that survival at
3PDH was positively correlated with dietary lipids in *P. olivaceus*, and Fernandez-Palacios et al. (1995) found a significant decrease in the survival of gilthead sea bream larvae at 3DPH when broodstock were fed with 21% lipid diet. Similar trends have been reported by Duray et al., 1994 for rabbit fish and Sink and Lochmann (2008) for *I. punctatus*.

Body crude lipid was significantly affected by energy and protein levels. Body lipid increased with increasing energy. Interestingly body crude protein was found to be significantly affected by energy levels. This might be because all diets contained efficient dietary protein levels. Similarly, increasing protein in high dietary protein levels (300, 350 and 400 g Kg⁻¹) in *L. rohita* did not significantly affect muscle protein of females (Afzal Khan et al., 2005). On the contrary, El-Sayed et al. (2008) stated that body protein of *O. niloticus* was significantly increased with increasing dietary protein levels. There are however, relatively few studies on the influence of dietary protein and lipid on body composition of broodfish.

The chemical composition of eggs is often examined to evaluate egg quality, as the egg must satisfy nutritional needs for embryonic and larval development (Furuita et al., 2002). This study showed that protein in eggs was significantly increased with decreasing dietary protein levels. However, increasing energy levels at each dietary protein level did not significantly affect this composition. Also lipid in eggs was not significantly affected by dietary protein and energy levels. Similarly, El-Sayed et al. (2008) reported that only egg crude protein was significantly affected by protein and energy content of *O. niloticus* broodstock diets and indicated at all energy levels, eggs crude protein significantly increased with increasing dietary protein levels. These differences might be related to the difference in species, diet composition, dietary protein and energy levels, P/GE ratio and experimental design.

Afzal Khan et al. (2005) stated that the fat content in eggs of *L. rohita* did not fluctuate with variations in dietary protein. However, egg protein was increased with increasing dietary protein levels. Ceredà et al. (1994) showed that the egg proximate composition of *D. labrax* was the same regardless of dietary treatment. Similar observations were made in *O. niloticus* (El-Sayed et al., 2003), Plectorhynchus cinctus (Li et al., 2005) and *I. punctatus* (Sink and Lochmann, 2008).

In the present study, at 40 and 60% dietary protein levels, increasing energy levels resulted in significantly increased crude protein of hatchlings and 3DPH larvae. Also, crude lipid of hatchlings and 3DPH larvae significantly increased by increasing dietary protein levels at 23.5 MJ/Kg energy level. However, Li et al. (2005) showed the levels of total lipid in larvae of *P. cinctus* was independent of dietary treatment and El-Sayed et al. (2003) reported that the chemical composition in fry of *O. niloticus* was not significantly affected by dietary protein levels.

It is concluded the best reproductive performance of *A. latus* broodstock was achieved at 40% dietary protein and 23.5MJ/Kg energy. This finding is of practical implication and in light of scarce knowledge on *Acanthopagrus* nutrition, signifying the necessity for more research under different nutritional culturing conditions.

5. Acknowledgment

Authors are thankful to Director, South Iranian Aquaculture Research Center, Ahwaz, Iran for financial grant No. 4-74-12-87042, and Director and Staff at Mariculture Research Station, Mahshahr, Iran for providing necessary facilities for the experiment and the University of Marine Science and Technology, Khoramshahr, Iran, for its support during the tenure of this project. We would like to express our thanks to Mr. Udaya Sankar Sethi for assistance during the trials.
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gilthead seabream (Sparus aurata) growth, nutrient utilization efficiency, rates of gastric evacuation and digestive enzyme activities.

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