Effect of early peptide diets on European sea bass (*Dicentrarchus labrax*) skeletal development

A. Printzia, b\*, S. Jodetb, V. Fournierc, S. Colletb, L. Madecb, V. Simonb, J-L. Zambonino-Infanteb, G. Koumoundourosa, D. Mazuraisb

a Biology Department, University of Crete, Crete, 70013, Greece.

b IFREMER, University of Brest, CNRS, IRD, LEMAR, F-29280, Plouzané, France.

c Symrise Aqua Feed, Symrise Group, Elven, 56250, France.

\*, to whom correspondence should be addressed: aprintzi@ifremer.fr

Table S1. Abiotic measurements throughout the experiment. Values are expressed as means ± standard deviation. Common conditions were applied up to 42dph (4 replicates/diet). From 42dph onward, the measurements refer to each experimental diet (C, P6, P12) separately. dph, days-post-hatching. T, Temperature. O2, Oxygen saturation.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Condition** | **dph** | **T (C°)** | **O2 (%)** | **ph** | **Light (lux)** |
| 4 repl/diet | 1-10 | 19.7±0.2 | 96±0.1 | 8.0±0.1 | 0-1 |
| 11-20 | 19.5±0.5 | 95.3±0.4 | 8.0±0.1 | 2-7 |
| 21-30 | 18.0±0.3 | 94±0.1 | 8.0±0.1 | 7-10 |
| 31-41 | 18.1±0.5 | 86.3±3.9 | 8.0±0.1 | 10-59 |
|  |
| C | 42-116 | 17.9±0.2 | 99.1±1.5 | 8.0±0.1 | 66 |
| P6 | 17.8±0.4 | 99.1±1.3 | 8.0±0.1 |
| P12 | 17.8±0.3 | 98.9±1.4 | 8.0±0.1 |

Table S2. Feeding regime throughout the experiment (6-116 dph). Artemia nau., Artemia nauplii. df, dry feeds (C, P6, P12).

|  |  |  |
| --- | --- | --- |
| **Dph** | **Feed**  | **nauplii·larvae-1** **g·meal-1** |
| 6-12 | Artemia nau. | 15-33 |
| df  | - |
| 13-20 | Artemia nau. | 10-28 |
| df  | 0.2-0.5 |
| 21-41 | Artemia nau. | - |
| df  | 0.6 |
| 42-59 | Artemia nau. | - |
| df  | 2.4-3.5 |
| 60-90 | Artemia nau. | - |
| df  | 4-5 |
| 91-116 | Artemia nau. | - |
| df  | 1.5-4 |

Table S3. Abiotic measurements during the swimming challenge tests (SCT). T, Temperature. O2, Oxygen saturation.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Condition** | **T (C°)** | **O2 (%)** | **ph** | **Ammonia (mg/L)** | **Light (lux)** |
| C | 19.7±0.5 | 92.0±7.6 | 7.8±0.1 | 0-0.25 | 66 |
| P6 | 19.7±0.2 | 89.2±5.0 | 7.9±0.1 | 0-0.25 | 66 |
| P12 | 19.6±0.3 | 92.4±2.7 | 7.9±0.1 | 0-0.25 | 66 |

Table S4. Accession numbers and nucleotide sequences of the primers used for the RT-PCR.

|  |  |  |
| --- | --- | --- |
| Gene(Accession no.) | Forward primer(5’ – 3’) | Reverse primer(5’ – 3’) |
| ***Amy***(DLAgn\_00008180) | TCCAGATCTCCCCTCCAAATGA | CACTGAACCAGCTTCCACAT |
| ***Bglap***(DLAgn\_00186150) | TGACTTCAGATGCCTCCGCT | GATTCCCTGCGTGTCCATCA |
| ***Myog***(DLAgn\_00091480) | CCAACCCAGAGTGTCGTCGT | GCAGAGATGCTGTCCACGAT |
| ***Myod***(DLAgn\_00164520) | CGACGGGATGGACTTTAACGG | GTTGTCGGTGGAGATTCGCT |
| ***Myf5***(DLAgn\_00209310) | CTCTGATGGCATGGTTGACAG | CTCCGACTGTCTTATCGCAC |
| ***Cls15a1***(DLAgn\_00138210) | AGTCAAAAAGCGCTACTGTCTG | TTGAACTTGCCGAGCCATGA |
| ***Cls15a2***(DLAgn\_00053970) | CATGAAAGCGGTGCTGACACT | ACATGGCCGATCACATAGACG |
| ***Trypsin***(DLAgn\_00254490) | ATGAGCTCCACTGTTGACAGGA | ACATAGTCGAGAGGCCGAGA |
| ***Slc6a19a***(DLAgn\_00199200) | TTCTTCAGTCAGGGCGTGG | AGCCACAGAGTGCTCTGACTT |
| ***Gcn2***(DLAgn\_00021030) | TTGGAAGGTTAAAAGGCCTCC | AAGTCCACAGTCACGTAGCA |
| ***Sparc***(DLAgn\_00112870) | TGCTGAGAATCCCTGCCTGA | AGGGTCCGATGTAGTCCAGG |
| ***Tnni2***(DLAgn\_00164600) | ACTCTGAAAAGCTGTATGCTGG | CAGCTCCAGAGGAGGACATTT |
| ***Tgfb1***(DLAgn\_00035970) | CTGGCTCAAAGGGACTGATGA | TGGTCAGGATGTAGGGTGGT |
| ***Foxo1***(DLAgn\_00035360) | GCAGGCTGGAAGAACTCCATTA | TGCTCTTGGTGAGCTTGCTA |
| ***Pparg***(DLAgn\_00094900) | AGGACACGCACAACTCAATC | TGTGCGTCTGAAGAAACCCT |
| ***Col1a1***(DLAgn\_00196080) | CGGCCTTGGTGGAAACTTCT | TGAATCCCTGAGGTCCGCTA |
| ***Ef1***(DLAgn\_00204500) | AACCCAGAAACACCGAAACT | TGTAGATCAGGTGGCCGGTA |
| ***Rpl13a***(DLAgn\_00023060) | CGCAACAAGCTGAAGTACCTGG | GCCTGGCCTCTCTTGGTTTT |
| ***Gapdh***(DLAgn\_00059000) | CAAACGAGGACTAGAACAACCC | TCCAGGTCGATGAAAGGGTC |

Table S5. Summary of statistical analysis on the effect of replicate within each diet (C, P6, P12) on gene expression data of RNA samplings 1,2 (rS1-rS2). Statistical significant differences (p<0.05) are indicated with the asterisks (\*\*). df, degrees of freedom. MS, Mean Square.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sampling** | **Gene** | **Diet** | **df** | **H** | **p** |
| rS1 | *amy* | C | 3 | 2.72 | 0.44 |
| P6 | 3 | 6.75 | 0.08 |
| P12 | 3 | 2.73 | 0.44 |
| rS2 | C | 3 | 2.59 | 0.46 |
| P6 | 3 | 6.90 | 0.08 |
| P12 | 3 | 6.59 | 0.09 |
| rS1 | *prss1* | C | 3 | 5.00 | 0.17 |
| P6 | 3 | 5.15 | 0.16 |
| P12 | 3 | 1.11 | 0.78 |
| rS2 | C | 3 | 4.54 | 0.21 |
| P6 | 3 | 1.77 | 0.62 |
| P12 | 3 | 5.21 | 0.16 |
| rS1 | *csl6a19a* | C | 3 | 2.72 | 0.44 |
| P6 | 3 | 1.95 | 0.58 |
| P12 | 3 | 3.85 | 0.28 |
| rS2 | C | 3 | 0.44 | 0.93 |
| P6 | 3 | 6.90 | 0.08 |
| P12 | 3 | 9.36 | \*\* |
| rS1 | *gcn2* | C | 3 | 5.25 | 0.15 |
| P6 | 3 | 2.24 | 0.52 |
| P12 | 3 | 4.56 | 0.21 |
| rS2 | C | 3 | 3.72 | 0.29 |
| P6 | 3 | 4.13 | 0.25 |
| P12 | 3 | 4.44 | 0.22 |
| rS1 | *pept1* | C | 3 | 0.47 | 0.69 |
| P6 | 3 | 4.27 | 0.23 |
| P12 | 3 | 4.92 | 0.18 |
| rS2 | C | 3 | 4.54 | 0.21 |
| P6 | 3 | 1.67 | 0.64 |
| P12 | 3 | 0.49 | 0.92 |
| rS1 | *pept2* | C | 3 | 4.50 | 0.21 |
| P6 | 3 | 3.84 | 0.28 |
| P12 | 3 | 0.92 | 0.82 |
| rS2 | C | 3 | 8.74 | \*\* |
| P6 | 3 | 1.67 | 0.64 |
| P12 | 3 | 0.49 | 0.92 |
| rS1 | *myf5* | C | 3 | 0.72 | 0.87 |
| P6 | 3 | 1.95 | 0.58 |
| P12 | 3 | 1.16 | 0.68 |
| rS2 | C | 3 | 4.23 | 0.24 |
| P6 | 3 | 4.13 | 0.25 |
| P12 | 3 | 3.11 | 0.38 |
| rS1 | *myod* | C | 3 | 1.47 | 0.69 |
| P6 | 3 | 1.22 | 0.75 |
| P12 | 3 | 4.97 | 0.17 |
| rS2 | C | 3 | 4.74 | 0.19 |
| P6 | 3 | 2.59 | 0.25 |
| P12 | 3 | 3.11 | 0.38 |
| rS1 | *myog* | C | 3 | 4.25 | 0.24 |
| P6 | 3 | 1.80 | 0.61 |
| P12 | 3 | 4.68 | 0.20 |
| rS2 | C | 3 | 2.28 | 0.52 |
| P6 | 3 | 1.97 | 0.58 |
| P12 | 3 | 2.59 | 0.46 |
| rS1 | *sparc* | C | 3 | 0.72 | 0.87 |
| P6 | 3 | 4.09 | 0.25 |
| P12 | 3 | 0.68 | 0.88 |
| rS2 | C | 3 | 5.97 | 0.11 |
| P6 | 3 | 1.05 | 0.79 |
| P12 | 3 | 7.67 | 0.05 |
| rS1 | *bglap* | C | 3 | 2.50 | 0.48 |
| P6 | 3 | 1.95 | 0.58 |
| P12 | 3 | 7.59 | 0.06 |
| rS2 | C | 3 | 6.92 | 0.07 |
| P6 | 3 | 0.74 | 0.86 |
| P12 | 3 | 5.67 | 0.13 |
|  |  |  |  |  |  |

Table S6. Summary of statistical analysis on gene expression data between the diets (C, P6, P12). The phenotype predictor within each diet refers to the external categorization in lordotic (L) or normal (N). Statistical significant differences (p<0.05) are indicated with the asterisks (\*\*). df, degrees of freedom.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene** | **Predictor** | **df** | **F** | **p** |
| *bglap* | Diet | 2 | 8.00 | \*\* |
| Phenotype | 3 | 0.98 | 0.41 |
| Replicate | 3 | 0.18 | 0.91 |
| col1 | Diet | 2 | 0.76 | 0.47 |
| Phenotype | 3 | 2.31 | 0.09 |
| Replicate | 3 | 2.12 | 0.11 |
| foxo | Diet | 2 | 19.43 | \*\* |
| Phenotype | 3 | 1.30 | 0.29 |
| Replicate | 3 | 19.22 | \*\* |
| myf | Diet | 2 | 42.23 | 0.00 |
| Phenotype | 3 | 1.81 | 0.16 |
| Replicate | 3 | 2.34 | 0.09 |
| *myod* | Diet | 2 | 3.70 | \*\* |
| Phenotype | 3 | 0.24 | 0.87 |
| Replicate | 3 | 0.93 | 0.44 |
| *myog* | Diet | 2 | 1.16 | 0.32 |
| Phenotype | 3 | 1.01 | 0.40 |
| Replicate | 3 | 1.33 | 0.28 |
| *pparg* | Diet | 2 | 0.93 | 0.40 |
| Phenotype | 3 | 0.01 | 0.99 |
| Replicate | 3 | 1.29 | 0.29 |
| *sparc* | Diet | 2 | 9.16 | \*\* |
| Phenotype | 3 | 0.34 | 0.80 |
| Replicate | 3 | 0.06 | 0.98 |
| *tgfb* | Diet | 2 | 2.51 | 0.09 |
| Phenotype | 3 | 0.45 | 0.72 |
| Replicate | 3 | 6.95 | \*\* |
| *tnni2* | Diet | 2 | 1.34 | 0.27 |
| Phenotype | 3 | 0.63 | 0.60 |
| Replicate | 3 | 2.62 | 0.06 |
|  |  |  |  |  |