Supplementary Table I. Primer sequences of target genes of intermediary metabolism used to analyse hepatic expression.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Target Gene | Gene Abbreviation | EC Number | Sequence | Annealing Temp | Reference |
| **Glucose Metabolism** |  |  |  |  |  |
|  | glucokinase (hexokinase IV) | *gck* | EC 2.7.1.1 | CTGGTGTGATCAACCGAATG | 59.4°C | Wade *et al.* 201424 |
| ACCACTCCCCTCCTCTGACT | 64.2°C |
|  | glucose-6-phosphatase | *g6pca* | EC 3.1.3.9 | TTGCTGGTGTCATCACAGGT | 62.2°C | Wade *et al.* 201424 |
| CACTTCTGGGCTTTCTCCAG | 60.8°C |
|  | fructose-1,6-bisphosphatase | *fbp1* | EC 3.1.3.11 | GATATGTTGGTTCAATGGTGGCT | 61.7°C | Wade *et al.* 201424 |
| GGACTCGTTGGTGGATTGTG | 61.4°C |
|  | pyruvate kinase | *pk* | EC 2.7.1.40 | TCACGCCTCTCTCCTCTGAT | 62.4°C | Wade *et al.* 201424 |
| TTCTGGTGACCGCAATGATA | 59.6°C |
|  | phosphoenolpyruvate carboxykinase, cytosolic  | *pck1* | EC 4.1.1.32 | GCCTGACTCCCTACACATCT | 61.2°C | Wade *et al.* 201424 |
| CACGGTCTTGCTCTCCACA | 62.4°C |
|  | phosphoenolpyruvate carboxykinase, mitochondrial | *pck2* | EC 4.1.1.32 | TTGGTGCTGGGTTAGTGG | 59.6°C | Wade *et al.* 201424 |
| TGGTGAGTGGAGAGAGTTG | 58.7°C |
| **Fatty Acid Metabolism** |  |  |  |  |  |
|  | glucose-6-phosphate dehydrogenase | *g6pd* | EC 1.1.1.49 | CAAACTCAACACACACATCC | 57.7°C | Wade *et al.* 201424 |
| CTTCTCTACAATCACCCTGTTC | 58.6°C |
|  | fatty acid synthase | *fasn* | EC 2.3.1.85 | TGAATCTCACCACGCTTCAG | 60.5°C | Wade *et al.* 201424 |
| AGGCAGCAATAGAACCCTCA | 61.4°C |
|  | stearoyl CoA desaturase | *scd* | EC 1.14.19.1 | CCTGGTACTTCTGGGGTGAA | 61.3°C | Wade *et al.* 201424 |
| AAGGGGAATGTGTGGTGGTA | 61.3°C |
|  | carnitine palmitoyltransferase | *cpt1a* | EC 2.3.1.21 | TGATGGTTATGGGGTGTCCT | 60.8°C | Wade *et al.* 201424 |
| CGGCTCTCTTCAACTTTGCT | 61.1°C |
|  | ATP citrate lyase | *acyl* | EC 2.3.3.8 | CAACACCATTGTCTGTGCTC | 59.9°C | Wade *et al.* 201424 |
| GAAATGCTGCTTAACAAAGTCC | 58.9°C |
|  | sterol regulatory element binding protein 1c | *srebf1* | na | GTTCTGGGTGTGCATATTGC | 60.6°C | Wade *et al.* 201424 |
| GGATCAGAACTCCAGACACC | 59.9°C |
| **Control Genes** |  |  |  |  |  |
|  | luciferase | *luc* | na | GGTGTTGGGCGCGTTATTTA | 61.8°C | Wade *et al.* 201424 |
| CGGTAGGCTGCGAAATGC | 62.0°C |
|  | elongation factor 1 alpha | *eef1a1* | na | AAATTGGCGGTATTGGAAC | 56.6°C | Wade *et al.* 201424 |
| GGGAGCAAAGGTGACGAC | 60.8°C |
|  |  |  |  |  |  |  |

Supplementary Table II. Relative proportions of liver fatty acids in fish fed either a high proportion of protein or starch, and the increase of fish liver fatty acids compared with those contained in the diets.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Liver FA Proportion of Total (%) |  | Increase in FA Relative to Diet (%) |
|  | Protein | Starch |  | Protein | Starch |
| SFA |  |  |  |  |  |
|  | 14:0 | 4.3a | 3.0b |  | 4.3 | 3.0 |
|  | 16:0 | 25.8a | 28.7b |  | 5.8 | 3.8 |
|  | 18:0 | 8.3 | 9.2 |  | -0.1 | 3.7 |
|  | 20:0 | 0.2 | 0.2 |  | -2.0 | 0.2 |
|  | 22:0 | 0.2 | 0.1 |  | -2.0 | 0.1 |
|  | **sum** | **38.8a** | **41.3b** |  | **5.9** | **10.8** |
| MUFA |  |  |  |  |  |
|  | 16:1n-7 | 5.8 | 5.3 |  | -3.0 | -2.8 |
|  | 18:1n-9 cis | 19.8a | 25.3b |  | -5.1 | 10.9 |
|  | 18:1n-9 trans | 2.8 | 2.4 |  | 2.8 | -1.2 |
|  | 20:1n-9 | 0.6 | 0.5 |  | -1.7 | 0.5 |
|  | 20:1n-7 | 0.2 | 0.1 |  | 0.2 | 0.1 |
|  | 24:1n-9 | 0.3 | 0.3 |  | 0.3 | 0.3 |
|  | **sum** | **29.5a** | **33.9b** |  | **-6.6** | **7.9** |
| PUFA |  |  |  |  |  |
|  | 18:2n-6 trans | 0.9a | 2.0b |  | 0.9 | 1.0 |
|  | 18:2n-6 cis | 3.8 | 3.67 |  | -12.0 | -6.8 |
|  | 18:3n-6 | 0.4 | 0.5 |  | 0.4 | 0.5 |
|  | 18:3n-3 | 0.4 | 0.4 |  | -6.7 | 0.4 |
|  | 18:4n-3 | 0.7 | 0.5 |  | 0.7 | 0.5 |
|  | 20:2n-6 | 0.2 | 0.3 |  | 0.2 | 0.3 |
|  | 20:4n-6 (ARA) | 0.7a | 0.5b |  | 0.7 | 0.5 |
|  | 20:3n-3 | 0.3 | 0.3 |  | 0.3 | 0.3 |
|  | 20:5n-3 (EPA) | 7.8a | 4.2b |  | 2.6 | -14.7 |
|  | 22:2 | 0.4a | 0.2b |  | 0.4 | 0.2 |
|  | 22:4n-6 | 0.4 | 0.1 |  | 0.1 | 0.1 |
|  | 22:5n3 (DPA) | 2.7a | 1.8b |  | 2.7 | 1.8 |
|  | 22:6n-3 (DHA) | 6.3a | 4.5b |  | 3.2 | -9.6 |
|  | **sum** | **24.9a** | **18.8b** |  | **-6.4** | **-24.8** |
|  | **Total** | **93.0** | **94.0** |  |  |  |
|  |  |  |  |  |  |  |
|  | sum n-3 | 18.2a | 11.6b |  |  |  |
|  | sum n-6 | 6.2 | 7.1 |  |  |  |
|  | sum n-9 | 23.5a | 28.5b |  |  |  |
|  | (n-3)/(n-6) | 2.9a | 1.6b |  |  |  |
|  |  |  |  |  |  |  |

ARA – arachidonic acid; DHA – docosahexaenoic acid; DPA – docosapentaenoic acid; EPA – eicosapentaenoic acid; MUFA – monounsaturated fatty acid; PUFA – polyunsaturated fatty acid; SFA – saturated fatty acid. Superscripts denote significant (*P*<0.05) differences between the different diets, and no superscript indicates no significant difference.

Supplementary Table III. Percentage of lipid species and chemical structure as determined from 1H NMR spectra of hepatic TAG of barramundi (*Lates calcarifer*) fed with Protein or Starch diet.

|  |  |  |
| --- | --- | --- |
| *Lipid species (%)* | Protein | Starch |
| non n-3 | 85.8±0.67a | 89.6±0.44b |
| SFA | 28.81 ± 0.96a | 37.21 ± 1.04b |
| UFA | 71.19 ± 0.96a | 62.79 ± 1.04b |
|  PUFA | 29.81 ± 1.29a | 21.30 ± 0.91b |
|  DHA (22:6n-3) | 3.46 ± 0.22 a | 2.00 ± 0.18b |
|  Linoleic acid (18:2n-6) | 7.30 ± 0.44 | 6.95 ± 0.19 |
|  MUFA | 41.38 ± 0.85 | 42.03 ± 0.67 |
|  n-3 | 14.17 ± 0.67 a | 10.39 ± 0.44b |

Mean values ± S.E.M are presented (n=6). Superscripts denote significant (*P*<0.05) differences between the different diets, and no superscript indicates no significant difference.