**The compositional and metabolic responses of gilthead seabream (*Sparus aurata*) to a gradient of dietary fish oil and associated n-3 long-chain polyunsaturated fatty acid content**

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**Supplementary file to a manuscript by Houston et al (2017)**

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**Supplementary table S1**. Proximate composition of body compartments in *S. aurata* presented as wet weight (g.Kg-1), means and standard deviations (n=3). Superscripts indicate Tukey’s HSD after ANOVA (P < 0.05).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Whole Bodies** | **Initial** | **D1** | **SD** |  | **D2** | **SD** |  | **D3** | **SD** |  | **D4** | **SD** |  | **D5** | **SD** |  | | **D6** | **SD** |  | **Pr(>F)** |
| Protein wet | 148.7 | 154.8 | ± 9.7 |  | 159.1 | ± 5.3 |  | 157.0 | ± 7.4 |  | 157.1 | ± 4.0 |  | 155.3 | ± 2.6 | |  | 164.1 | ± 9.4 |  | 0.782 |
| Lipid | 103.5 | 181.2 | ± 11.7 |  | 172.7 | ± 9.3 |  | 188.4 | ± 15.1 |  | 176.1 | ± 6.5 |  | 164.6 | ± 6.4 | |  | 166.9 | ± 16.4 |  | 0.371 |
| Ash wet | 27.4 | 14.3 | ± 1.6 |  | 16.3 | ± 0.7 |  | 16.6 | ± 1.8 |  | 15.5 | ± 0.7 |  | 16.3 | ± 0.5 | |  | 17.3 | ± 1.3 |  | 0.264 |
| Moisture | 692.8 | 627.3 | ± 9.2 |  | 628.7 | ± 2.3 |  | 623.2 | ± 0.9 |  | 631.4 | ± 15.2 |  | 633.9 | ± 2.2 | |  | 637.4 | ± 7.1 |  | 0.562 |
| **Carcass** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | |  |  |  |  |  |
| Protein wet |  | 179.20 | ± 11.7 |  | 173.70 | ± 2.2 |  | 171.20 | ± 1.7 |  | 182.60 | ± 10.7 |  | 172.70 | ± 3.1 | |  | 160.7 | ± 6.5 |  | 0.123 |
| Lipid |  | 162.7 | ± 7.3 | a | 149.7 | ± 9.1 | ab | 151.8 | ± 9.6 | ab | 129.5 | ± 8.8 | b | 154.6 | ± 10.0 | | ab | 139.9 | ± 10.1 | ab | 0.044 |
| Ash wet |  | 32.1 | ± 2.9 |  | 27.3 | ± 4.7 |  | 26.2 | ± 4.3 |  | 31.2 | ± 4.4 |  | 29.5 | ± 4.5 | |  | 36.1 | ± 6.1 |  | 0.361 |
| Moisture |  | 612.9 | ± 15.2 |  | 639.4 | ± 2.5 |  | 625.3 | ± 10.6 |  | 630.7 | ± 2.6 |  | 623.8 | ± 21.8 | |  | 622.3 | ± 7.1 |  | 0.425 |
| **Viscera** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | |  |  |  |  |  |
| Protein wet |  | 69.2 | ± 8.9 |  | 62.9 | ± 3.3 |  | 64.4 | ± 6.2 |  | 51.4 | ± 10.3 |  | 62.8 | ± 11.1 | |  | 63.1 | ± 4.9 |  | 0.419 |
| Lipid |  | 448.8 | ± 23.2 |  | 450.9 | ± 14.4 |  | 452.6 | ± 36 |  | 502.2 | ± 59.9 |  | 514.5 | ± 45.6 | |  | 525.1 | ± 47.8 |  | 0.261 |
| Ash wet |  | 6.7 | ± 0.5 |  | 7.3 | ± 1.1 |  | 8.9 | ± 0.1 |  | 6.6 | ± 0.7 |  | 8 | ± 1.7 | |  | 6.4 | ± 0.4 |  | 0.114 |
| Moisture |  | 475.3 | ± 15.9 |  | 478.9 | ± 16.3 |  | 474.1 | ± 32.6 |  | 439.8 | ± 50.4 |  | 414.6 | ± 33.6 | |  | 405.5 | ± 43.4 |  | 0.186 |
| **Liver** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | |  |  |  |  |  |
| Protein wet |  | 101.4 | ± 13.4 |  | 95.3 | ± 2.7 |  | 103.3 | ± 5.6 |  | 107.0 | ± 11.1 |  | 99.3 | ± 1.6 | |  | 101.9 | ± 5.9 |  | 0.776 |
| Lipid |  | 323.7 | ± 6.3 | a | 317.8 | ± 31.8 | ab | 311.0 | ± 22.8 | ab | 233.1 | ± 30.7 | bc | 220.1 | ± 22.8 | | c | 186.2 | ± 31.8 | c | < 0.001 |
| Ash wet |  | 6.4 | ± 1.0 |  | 6.1 | ± 1.9 |  | 6.9 | ± 0.8 |  | 7.6 | ± 0.9 |  | 7.8 | ± 0.8 | |  | 8.2 | ± 1.7 |  | 0.5172 |
| Moisture |  | 473.2 | ± 7.2 | d | 486.5 | ± 23.1 | bcd | 498.7 | ± 19.8 | bd | 550.9 | ± 16.6 | abc | 554.6 | ± 24.3 | | ab | 568.8 | ± 24.1 | a | 0.0014 |

**Supplementary table S2**. Lipid and fatty acids in the liver of S. aurata as mg.g-1 fatty acid per tissue. Data are summarised as mean and standard deviation (n=3\*3). Superscripts indicate Tukey’s HSD after ANOVA (P < 0.05).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **D1** | **SD** |  | **D2** | **SD** |  | **D3** | **SD** |  | **D4** | **SD** |  | **D5** | **SD** |  | **D6** | **SD** |  | **Pr(>F)** |
| **Total lipid** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Lipid (%) | 29.9 | ± 6 | a | 26.8 | ± 4.5 | a | 25.4 | ± 4.7 | a | 29.9 | ± 5.1 | a | 24.4 | ± 6.5 | a | 13.1 | ± 4.4 | b | < 0.001 |
| **Fatty acid** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 14:0 | 2.8 | ± 0.7 | b | 2.6 | ± 0.6 | b | 2.8 | ± 0.7 | b | 3.8 | ± 0.8 | ab | 4.5 | ± 1.3 | b | 3.8 | ± 1.5 | ab | 0.001 |
| 16:0 | 38.6 | ± 9.1 | a | 34.5 | ± 6.5 | a | 34.2 | ± 7.4 | a | 40.1 | ± 5.7 | a | 33.6 | ± 9.6 | a | 18.4 | ± 6.4 | b | < 0.001 |
| 18:0 | 12.7 | ± 2.8 | a | 11.5 | ± 2.3 | a | 10.7 | ± 1.9 | a | 12.5 | ± 1.6 | a | 10.5 | ± 3.6 | ab | 6.8 | ± 2.6 | b | < 0.001 |
| 20:0 | 0.4 | ± 0.1 | a | 0.4 | ± 0.1 | a | 0.4 | ± 0.1 | a | 0.5 | ± 0.1 | a | 0.4 | ± 0.1 | a | 0.2 | ± 0.1 | b | < 0.001 |
| 22:0 | 0.2 | ± 0.1 | ab | 0.3 | ± 0.1 | a | 0.3 | ± 0.0 | a | 0.3 | ± 0.0 | a | 0.2 | ± 0.1 | a | 0.1 | ± 0.0 | b | < 0.001 |
| 16:1n-9 | 2 | ± 0.6 | a | 1.4 | ± 0.3 | bc | 1.4 | ± 0.3 | bc | 1.5 | ± 0.3 | ab | 1 | ± 0.3 | cd | 0.5 | ± 0.2 | d | < 0.001 |
| 16:1n-7 | 4.3 | ± 1.2 | bc | 4.2 | ± 0.9 | c | 4.6 | ± 1.2 | bc | 6.5 | ± 1.2 | ab | 7 | ± 2.0 | a | 5.7 | ± 2.2 | abc | < 0.001 |
| 18:1n-9 | 109 | ± 25.6 | a | 90.9 | ± 18.6 | ab | 87.6 | ± 19.7 | ab | 96.7 | ± 15.5 | a | 63.4 | ± 19.1 | b | 20.9 | ± 9.2 | a | < 0.001 |
| 18:1n-7 | 8.1 | ± 1.9 | a | 7.3 | ± 1.5 | a | 7.1 | ± 1.7 | ab | 8.9 | ± 1.2 | a | 7.4 | ± 2.3 | a | 4.5 | ± 1.8 | b | < 0.001 |
| 20:1n | 3.4 | ± 1.0 | a | 3.6 | ± 0.9 | a | 3.5 | ± 1.0 | a | 4.6 | ± 1.3 | a | 3.1 | ± 1.2 | a | 1.5 | ± 0.7 | b | < 0.001 |
| 22:1n | 1 | ± 0.4 | ab | 1 | ± 0.3 | ab | 1 | ± 0.2 | ab | 1.4 | ± 0.4 | a | 1.1 | ± 0.4 | ab | 0.7 | ± 0.2 | b | 0.003 |
| 24:1n-9 | 1 | ± 0.2 |  | 0.9 | ± 0.2 |  | 0.9 | ± 0.2 |  | 1 | ± 0.4 |  | 0.9 | ± 0.3 |  | 0.6 | ± 0.2 |  | 0.061 |
| 18:2n-9 | 2.7 | ± 1.0 | a | 1.5 | ± 0.6 | b | 1.2 | ± 0.4 | b | 0.9 | ± 0.3 | bc | 0.4 | ± 0.2 | cd | 0.2 | ± 0.1 | c | < 0.001 |
| 18:2n-6 | 33.4 | ± 8.4 | a | 29.9 | ± 7.6 | a | 29.9 | ± 7.5 | a | 33.7 | ± 8.1 | a | 23.9 | ± 6.6 | a | 9.7 | ± 3.7 | b | < 0.001 |
| 18:3n-6 | 2.4 | ± 1.0 | a | 1.3 | ± 0.7 | b | 1.2 | ± 0.5 | bc | 0.8 | ± 0.3 | bcd | 0.4 | ± 0.2 | cd | 0.2 | ± 0.1 | d | < 0.001 |
| 20:2n-9 | 2.2 | ± 0.7 | a | 1.6 | ± 0.4 | b | 1.4 | ± 0.4 | b | 1.3 | ± 0.4 | b | 0.7 | ± 0.2 | cd | 0.2 | ± 0.1 | c | < 0.001 |
| 20:2n-6 | 1.3 | ± 0.4 | ab | 1.3 | ± 0.4 | ab | 1.3 | ± 0.4 | ab | 1.6 | ± 0.5 | a | 0.9 | ± 0.3 | bc | 0.4 | ± 0.2 | c | < 0.001 |
| 20:3n-6 | 1.2 | ± 0.4 | a | 0.9 | ± 0.3 | a | 0.9 | ± 0.3 | a | 0.8 | ± 0.2 | a | 0.5 | ± 0.2 | b | 0.2 | ± 0.1 | b | < 0.001 |
| 20:4n-6 | 0.1 | ± 0.1 | d | 0.3 | ± 0.0 | c | 0.4 | ± 0.0 | c | 0.6 | ± 0.1 | b | 0.7 | ± 0.2 | ab | 0.8 | ± 0.2 | a | < 0.001 |
| 18:3n-3 | 6.7 | ± 1.9 | ab | 6.3 | ± 1.8 | ab | 6.2 | ± 1.7 | ab | 7.3 | ± 2.1 | a | 4.8 | ± 1.3 | b | 1.3 | ± 0.5 | c | < 0.001 |
| 18:4n-3 | 0.7 | ± 0.2 | ab | 0.6 | ± 0.2 | b | 0.6 | ± 0.2 | b | 0.8 | ± 0.3 | ab | 1.1 | ± 0.3 | a | 1.1 | ± 0.5 | a | 0.0019 |
| 20:3n-3 | 0.4 | ± 0.1 | ab | 0.4 | ± 0.1 | ab | 0.4 | ± 0.1 | ab | 0.6 | ± 0.2 | a | 0.3 | ± 0.1 | b | 0.1 | ± 0.1 | c | < 0.001 |
| 20:4n-3 | 0.5 | ± 0.2 | c | 0.5 | ± 0.1 | c | 0.6 | ± 0.2 | bc | 1 | ± 0.2 | a | 1 | ± 0.3 | a | 0.9 | ± 0.4 | ab | < 0.001 |
| 20:5n-3 | 1.2 | ± 0.5 | c | 2.2 | ± 0.4 | c | 2.8 | ± 0.5 | bc | 4.9 | ± 1.1 | b | 7.1 | ± 2.0 | a | 7.5 | ± 2.7 | a | < 0.001 |
| 22:5n-3 | 0.7 | ± 0.3 | b | 1.3 | ± 0.4 | b | 1.7 | ± 0.4 | b | 3.5 | ± 1.0 | a | 4.5 | ± 1.4 | a | 4.7 | ± 2.0 | a | < 0.001 |
| 22:6n-3 | 1.6 | ± 0.5 | c | 2.7 | ± 0.6 | c | 3.2 | ± 0.6 | c | 6.1 | ± 1.3 | b | 8.4 | ± 2.3 | ab | 9.6 | ± 3.1 | a | < 0.001 |

**Supplementary table S3**. Lipid content and fatty acids in the mid intestine of *S. aurata* presented as mg.g-1 fatty acid per tissue. Data are summarised as mean and standard deviation (n=3\*3). Superscripts indicate Tukey’s HSD after ANOVA (P < 0.05).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Diet** | **D1** | **SD** |  | **D2** | **SD** |  | **D3** | **SD** |  | **D4** | **SD** |  | **D5** | **SD** |  | **D6** | **SD** |  | **Pr(>F)** |
| **Total lipid** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Lipid (%) | 16.4 | ± 3.6 | a | 13.8 | ± 2.2 | ab | 11.1 | ± 3.5 | bc | 10.5 | ± 1.9 | bc | 11.1 | ± 2.4 | bc | 9.5 | ± 1.5 | c | < 0.001 |
| **Fatty acid** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 14:0 | 0.9 | ± 0.2 | b | 1.3 | ± 0.3 | b | 1.2 | ± 0.5 | b | 1.5 | ± 0.3 | b | 2.6 | ± 0.8 | a | 3.3 | ± 0.8 | a | < 0.001 |
| 16:0 | 19.4 | ± 3.9 | a | 17.8 | ± 3.4 | ab | 15.7 | ± 4.5 | ab | 14.1 | ± 2 | b | 15.5 | ± 3.6 | ab | 13.6 | ± 2.5 | b | 0.01 |
| 18:0 | 5.7 | ± 1.2 | a | 5.4 | ± 0.8 | ab | 4.3 | ± 1.1 | bc | 4.1 | ± 0.6 | c | 4.1 | ± 0.8 | bc | 3.9 | ± 0.5 | c | < 0.001 |
| 20:0 | 0.4 | ± 0.1 | a | 0.4 | ± 0.1 | ab | 0.3 | ± 0.1 | abc | 0.3 | ± 0 | cd | 0.3 | ± 0.1 | bcd | 0.2 | ± 0 | d | < 0.001 |
| 22:0 | 0.2 | ± 0.1 | a | 0.2 | ± 0.1 | ab | 0.2 | ± 0.1 | abc | 0.1 | ± 0 | bc | 0.1 | ± 0 | bc | 0.1 | ± 0 | c | < 0.001 |
| 16:1n-9 | 0.5 | ± 0.1 | a | 0.4 | ± 0.1 | ab | 0.3 | ± 0.1 | bc | 0.3 | ± 0.1 | bc | 0.3 | ± 0.1 | bc | 0.2 | ± 0.1 | c | < 0.001 |
| 16:1n-7 | 1.2 | ± 0.3 | b | 1.6 | ± 0.4 | b | 1.5 | ± 0.6 | b | 1.9 | ± 0.3 | b | 3.1 | ± 1 | a | 3.5 | ± 0.9 | a | < 0.001 |
| 18:1n-9 | 63.4 | ± 17.3 | a | 45.3 | ± 10.8 | b | 32.3 | ± 14.7 | bc | 28.3 | ± 7.2 | c | 23.5 | ± 6.5 | c | 9.1 | ± 2 | d | < 0.001 |
| 18:1n-7 | 4.3 | ± 1.1 | a | 3.6 | ± 0.8 | ab | 2.7 | ± 1.1 | bc | 2.5 | ± 0.5 | bc | 2.8 | ± 0.7 | bc | 2.1 | ± 0.4 | c | < 0.001 |
| 20:1n | 1.3 | ± 0.4 | a | 1.1 | ± 0.2 | ab | 0.9 | ± 0.4 | ab | 0.8 | ± 0.1 | b | 1 | ± 0.3 | ab | 0.9 | ± 0.2 | b | 0.007 |
| 22:1n | 0.4 | ± 0.1 | ab | 0.4 | ± 0.2 | ab | 0.3 | ± 0.2 | b | 0.3 | ± 0.1 | b | 0.5 | ± 0.2 | ab | 0.6 | ± 0.1 | a | 0.004 |
| 24:1n-9 | 0.6 | ± 0.2 | a | 0.5 | ± 0.1 | ab | 0.4 | ± 0.2 | ab | 0.4 | ± 0.1 | b | 0.5 | ± 0.2 | ab | 0.5 | ± 0.1 | ab | 0.022 |
| 18:2n-6 | 24.3 | ± 6.4 | a | 18.5 | ± 3.8 | ab | 14.3 | ± 5.6 | bc | 12.7 | ± 2.7 | bc | 11.9 | ± 3.2 | cd | 6.4 | ± 1.2 | d | < 0.001 |
| 20:2n-6 | 0.4 | ± 0.1 | a | 0.3 | ± 0.1 | ab | 0.3 | ± 0.1 | bc | 0.2 | ± 0 | c | 0.2 | ± 0.1 | cd | 0.1 | ± 0 | d | < 0.001 |
| 20:4n-6 | 0.1 | ± 0.1 | d | 0.2 | ± 0 | c | 0.2 | ± 0 | bc | 0.3 | ± 0 | b | 0.5 | ± 0.1 | a | 0.5 | ± 0.1 | a | < 0.001 |
| 18:3n-3 | 5.4 | ± 1.7 | a | 3.9 | ± 0.9 | b | 2.9 | ± 1.4 | bc | 2.6 | ± 0.7 | bc | 2.3 | ± 0.7 | cd | 0.8 | ± 0.2 | d | < 0.001 |
| 18:4n-3 | 0 | ± 0 | c | 0.1 | ± 0.1 | bc | 0.2 | ± 0.1 | bc | 0.2 | ± 0.1 | b | 0.5 | ± 0.2 | a | 0.6 | ± 0.2 | a | < 0.001 |
| 20:4n-3 | 0 | ± 0 | c | 0 | ± 0.1 | c | 0.1 | ± 0.1 | bc | 0.2 | ± 0 | b | 0.3 | ± 0.1 | a | 0.3 | ± 0.1 | a | < 0.001 |
| 20:5n-3 | 0.6 | ± 0.1 | d | 1.2 | ± 0.1 | cd | 1.4 | ± 0.4 | cd | 1.9 | ± 0.2 | c | 3.5 | ± 0.8 | b | 4.6 | ± 1 | a | < 0.001 |
| 22:5n-3 | 0.3 | ± 0.1 | c | 0.5 | ± 0.1 | bc | 0.5 | ± 0.1 | bc | 0.7 | ± 0.1 | b | 1 | ± 0.3 | a | 1.3 | ± 0.3 | a | < 0.001 |
| 22:6n-3 | 1.1 | ± 0.2 | e | 2 | ± 0.2 | d | 2.4 | ± 0.5 | cd | 3.1 | ± 0.2 | c | 4.7 | ± 0.7 | b | 6.7 | ± 1.1 | a | < 0.001 |