Supplementary Material

## Supplementary Tables

**Table S1.** Dissolved oxygen (DO) (mg L-1), pH, temperature (ºC), salinity, ammonia (NH4) and nitrites (NO2) monitored weekly on different treatments testing the combined effects of temperature (T – 20 and 25ºC) and salinity (S – 15, 20 and 25) in the fatty acid profile of *Hediste diversicolor* fed a commercial aquafeed during 10, 20 and 40 days (D10, D20 and D40). Average values (± SD) (n = 5). The values between brackets represented the maximum and minimum values.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Samples | Treatment | DO (mg L-1) | pH | Temp. (ºC) | Salinity | NH4 (mg L-1) | NO2 (mg L-1) |
| D10 | T20S15 | 7.71 ± 0.39 (8.10 - 6.70) | 8.37 ± 0.09 (8.45 - 8.08) | 20.57 ± 0.76 (21.70 - 19.40) | 14.87 ± 0.59 (15.60 - 13.80) | 0.94 ± 0.54 (1.50 – 0.15) | 0.52 ± 0.40 (1.00 – 0.10) |
| T20S20 | 7.74 ± 0.31 (8.20 - 7.20) | 8.40 ± 0.08 (8.53 - 8.26) | 20.50 ± 0.83 (21.90 - 19.30) | 19.92 ± 0.98 (21.00 - 18.20) | 0.90 ± 0.59 (1.50 – 0.15) | 0.81 ± 0.69 (2.00 – 0.10) |
| T20S25 | 7.76 ± 0.25 (8.10 - 7.30) | 8.34 ± 0.09 (8.46 - 8.12) | 20.55 ± 0.75 (21.80 - 19.40) | 24.89 ± 0.93 (26.00 - 23.40) | 0.55 ± 0.38 (1.00 – 0.15) | 1.02 ± 0.81 (2.00 – 0.10) |
|  |  |  |  |  |  |  |
| T25S15 | 7.01 ± 0.18 (7.50 - 6.80) | 8.32 ± 0.09 (8.46 - 8.16) | 25.27 ± 0.78 (26.70 - 23.40) | 15.59 ± 0.48 (16.40 - 14.80) | 1.21 ± 0.51 (1.50 - 0.25) | 0.87 ± 0.71 (2.00 – 0.10) |
| T25S20 | 6.93 ± 0.20 (7.20 - 6.60) | 8.39 ± 0.06 (8.49 - 8.31) | 25.45 ± 0.82 (27.00 - 23.46) | 20.55 ± 0.99 (21.60 - 18.00) | 0.78 ± 0.80 (1.50 – 0.00) | 0.68 ± 0.38 (1.00 – 0.10) |
| T25S25 | 6.95 ± 0.24 (7.40 - 6.50) | 8.34 ± 0.10 (8.50 - 8.12) | 25.35 ± 0.78 (26.80 - 23.50) | 26.01 ± 0.74 (27.20 - 24.90) | 0.86 ± 0.70 (1.50 – 0.15) | 0.85 ± 0.66 (2.00 – 0.10) |
|  |  |  |  |  |  |  |  |
| D20 | T20S15 | 7.83 ± 0.29 (8.40 - 7.20) | 8.36 ± 0.11 (8.59 - 8.14) | 20.10 ± 0.79 (21.80 - 19.00) | 15.05 ± 0.44 (15.60 - 13.60) | 0.91 ± 0.56 (1.50 – 0.15) | 1.21 ± 0.72 (2.00 – 0.50) |
| T20S20 | 7.98 ± 0.30 (8.50 - 7.40) | 8.39 ± 0.08 (8.52 - 8.16) | 20.11 ± 0.80 (21.70 - 19.10) | 19.87 ± 0.88 (21.40 - 17.60) | 0.87 ± 0.52 (1.50 – 0.15) | 1.23 ± 0.75 (2.00 – 0.10) |
| T20S25 | 7.88 ± 0.28 (8.20 - 7.10) | 8.40 ± 0.05 (8.50 - 8.28) | 20.09 ± 0.81 (21.80 - 19.00) | 24.86 ± 0.71 (25.90 - 23.40) | 0.63 ± 0.54 (1.50 – 0.15) | 0.81 ± 0.66 (2.00 – 0.10) |
|  |  |  |  |  |  |  |
| T25S15 | 6.96 ± 0.28 (7.50 - 6.50) | 8.28 ± 0.11 (8.46 - 7.99) | 25.17 ± 0.80 (26.70 - 23.30) | 15.48 ± 0.50 (16.30 - 14.10) | 0.74 ± 0.65 (1.50 – 0.00) | 1.05 ± 0.64 (2.00 – 0.10) |
| T25S20 | 7.00 ± 0.31 (7.70 - 6.50) | 8.27 ± 0.15 (8.49 - 7.91) | 25.23 ± 0.81 (26.90 - 23.20) | 20.48 ± 0.72 (21.60 - 18.80) | 0.76 ± 0.67 (1.50 – 0.00) | 1.13 ± 0.70 (2.00 – 0.10) |
| T25S25 | 6.97 ± 0.33 (7.50 - 6.30) | 8.28 ± 0.12 (8.46 - 8.05) | 25.27 ± 0.80 (27.10 - 23.40) | 25.64 ± 0.92 (27.70 - 23.90) | 0.58 ± 0.53 (1.50 – 0.15) | 1.09 ± 0.62 (2.00 – 0.10) |
|  |  |  |  |  |  |  |  |
| D40 | T20S15 | 7.57 ± 0.48 (8.50 - 6.30) | 8.23 ± 0.14 (8.49 - 7.95) | 20.52 ± 0.91 (22.30 - 19.00) | 15.16 ± 0.47 (15.60 - 13.30) | 0.65 ± 0.57 (1.50 – 0.15) | 1.11 ± 0.68 (2.00 – 0.25) |
| T20S20 | 7.61 ± 0.44 (8.30 - 6.50) | 8.35 ± 0.12 (8.52 - 7.97) | 20.47 ± 0.92 (22.30 - 18.90) | 20.19 ± 0.70 (21.20 - 18.20) | 0.60 ± 0.53 (1.50 – 0.15) | 1.40 ± 0.97 (2.00 – 0.10) |
| T20S25 | 7.58 ± 0.45 (8.20 - 5.90) | 8.27 ± 0.20 (8.52 - 7.73) | 20.48 ± 0.89 (22.30 - 19.00) | 24.90 ± 0.61 (26.20 - 23.10) | 0.48 ± 0.50 (1.50 – 0.00) | 1.19 ± 0.69 (2.00 – 0.25) |
|  |  |  |  |  |  |  |
| T25S15 | 6.81 ± 0.41 (7.60 - 5.60) | 8.22 ± 0.17 (8.50 - 7.81) | 25.38 ± 0.70 (26.90 - 23.30) | 15.65 ± 0.45 (16.60 - 14.50) | 0.43 ± 0.55 (1.50 – 0.00) | 0.96 ± 0.64 (2.00 – 0.10) |
| T25S20 | 6.78 ± 0.36 (7.50 - 5.40) | 8.25 ± 0.18 (8.50 - 7.71) | 25.54 ± 0.71 (27.00 - 23.40) | 20.43 ± 0.67 (21.60 - 18.00) | 0.49 ± 0.54 (1.50 – 0.00) | 1.07 ± 0.66 (2.00 – 0.00) |
| T25S25 | 6.73 ± 0.39 (7.30 - 5.30) | 8.17 ± 0.17 (8.48 - 7.79) | 25.44 ± 0.71 (27.00 - 23.30) | 25.58 ± 0.69 (27.00 - 24.30) | 0.45 ± 0.55 (1.50 – 0.00) | 1.15 ± 0.90 (2.00 – 0.25) |

**Table S2.** Polychaete biomass and aquafeed supplied to different treatments testing the combined effects of temperature (T – 20 and 25ºC) and salinity (S – 15, 20 and 25) in the fatty acid profile of *Hediste diversicolor* fed a commercial aquafeed during 10, 20 and 40 days (D10, D20 and D40). Average values (± SD) (n = 5).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|   |   |   | Biomass Start |   | Biomass End |  | Feed supplied |   | Survival (%) |
|  | Treatment |   | *Nº* | *Biomass (g)* | *Avg weight (g)* |   | *Nº* | *Biomass (g)* | *Avg weight (g)* |   | *Total (g)* |  |  |
| D10 | T20S15 |   | 10 | 1.86 ± 0.16 | 0.19 ± 0.02 |   | 7 ± 2 | 1.76 ± 0.56 | 0.26 ± 0.03 |   | 0.28 ± 0.02 |   | 66.00 ± 16.73 |
| T20S20 |   | 10 | 1.83 ± 0.22 | 0.18 ± 0.02 |   | 7 ± 2 | 1.84 ± 0.56 | 0.28 ± 0.02 |   | 0.28 ± 0.03 |   | 66.00 ± 21.91 |
| T20S25 |   | 10 | 1.73 ± 0.25 | 0.17 ± 0.03 |   | 7 ± 1 | 1.80 ± 0.45 | 0.25 ± 0.03 |   | 0.26 ± 0.04 |   | 72.00 ± 10.95 |
| T25S15 |   | 10 | 2.16 ± 0.21 | 0.22 ± 0.02 |   | 8 ± 1 | 2.36 ± 0.40 | 0.30 ± 0.03 |   | 0.32 ± 0.04 |   | 78.00 ± 13.04 |
| T25S20 |   | 10 | 2.14 ± 0.17 | 0.21 ± 0.02 |   | 7 ± 1  | 2.09 ± 0.31 | 0.30 ± 0.01 |   | 0.31 ± 0.01 |   | 70.00 ± 10.00 |
| T25S25 |   | 10 | 2.20 ± 0.24 | 0.22 ± 0.02 |   | 7 ± 1 | 2.12 ± 0.33 | 0.31 ± 0.04 |   | 0.32 ± 0.02 |   | 70.00 ± 12.25 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D20 | T20S15 |   | 10 | 1.76 ± 0.17 | 0.18 ± 0.02 |   | 6 ± 1 | 2.60 ± 0.27 | 0.45 ± 0.09 |   | 0.65 ± 0.08 |   | 60.00 ± 14.14 |
| T20S20 |   | 10 | 1.82 ± 0.19 | 0.18 ± 0.02 |   | 5 ± 1 | 1.80 ± 0.57 | 0.38 ± 0.11 |   | 0.68 ± 0.07 |   | 48.00 ± 8.37 |
| T20S25 |   | 10 | 1.69 ± 0.21 | 0.17 ± 0.02 |   | 6 ± 1 | 2.04 ± 0.48 | 0.34 ± 0.05 |   | 0.61 ± 0.08 |   | 60.00 ± 14.14 |
| T25S15 |   | 10 | 2.18 ± 0.20 | 0.22 ± 0.02 |   | 7 ±1 | 3.10 ± 0.40 | 0.44 ± 0.04 |   | 0.80 ± 0.08 |   | 70.00 ± 10.00 |
| T25S20 |   | 10 | 2.26 ± 0.23 | 0.23 ± 0.02 |   | 7 ± 1 | 2.64 ± 0.46 | 0.40 ± 0.05 |   | 0.90 ± 0.30 |   | 68.00 ± 8.37 |
| T25S25 |   | 10 | 2.06 ± 0.17 | 0.21 ± 0.02 |   | 7 ± 2 | 2.67 ± 0.32 | 0.40 ± 0.08 |   | 0.85 ± 0.29 |   | 70.00 ± 18.71 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D40 | T20S15 |   | 10 | 1.84 ± 0.34 | 0.18 ± 0.03 |   | 5 ± 1 | 3.21 ± 0.61 | 0.64 ± 0.08 |   | 1.76 ± 0.28 |   | 50.00 ± 7.07 |
| T20S20 |   | 10 | 1.76 ± 0.24 | 0.18 ± 0.02 |   | 5 ± 2 | 2.94 ± 1.04 | 0.67 ± 0.08 |   | 1.67 ± 0.22 |   | 46.00 ± 21.91 |
| T20S25 |   | 10 | 1.69 ± 0.26 | 0.17 ± 0.03 |   | 5 ± 1 | 2.87 ± 0.42 | 0.55 ± 0.08 |   | 1.63 ± 0.26 |   | 52.00 ± 4.47 |
| T25S15 |   | 10 | 2.14 ± 0.15 | 0.21 ± 0.02 |   | 5 ± 1 | 3.42 ± 1.02 | 0.62 ± 0.13 |   | 1.99 ± 0.15 |   | 54.00 ± 5.48 |
| T25S20 |   | 10 | 2.09 ± 0.18 | 0.21 ± 0.02 |   | 5 ± 1 | 2.86 ± 1.38 | 0.58 ± 0.19 |   | 2.05 ± 0.42 |   | 48.00 ± 8.37 |
| T25S25 |   | 10 | 2.17 ± 0.23 | 0.22 ± 0.02 |   | 4 ± 2 | 2.54 ± 0.91 | 0.71 ± 0.17 |   | 2.06 ± 0.17 |   | 38.00 ± 17.89 |

**Table S3.** Results of the two-way nested ANOVA or Kruskal-Wallis test to evaluate the existence of significant differences in feeding rate (FR), specific growth rate (SGR) and daily growth rate of *Hediste diversicolor* fed a commercial aquafeed during 10, 20 and 40 days (D10, D20 and D40) in different treatments of combined temperature (T – 20 and 25ºC) and salinity (S – 15, 20 and 25). S(T) - Salinity groups nested within temperature groups; The values with asterisk (\*) resulted from Kruskal-Wallis test; Significant differences when *p* < 0.05.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | *Test between S(T) levels* | *F or H\* - value* | *p – value* | *R2 (%)* |
| FR | D10 | 0.24 | 0.914 | 10.80 |
| D20 | 0.93 | 0.463 | 21.49 |
| D40 | 0.35 | 0.843 | 13.46 |
|  |  |  |  |  |
| SGR | D10 | 0.44 | 0.776 | 11.29 |
| D20 | 1.70 | 0.183 | 31.34 |
| D40 | 0.30\* | 0.860\* | - |
|  |  |  |  |  |
| DGR | D10 | 0.51 | 0.730 | 12.80 |
| D20 | 1.69 | 0.186 | 33.13 |
| D40 | 0.85 | 0.510 | 24.44 |

**Table S4.** Fatty acid (FA) profile (µg mg-1 DW) of *Hediste diversicolor* fed a commercial aquafeed for 10 days and maintained under different combinations of water temperature (T – 20 and 25 ºC) and salinity (S – 15, 20 and 25), along with conspecifics initially stocked (Initial) and collected from the wild at the same time point (Wild10). Average values (± SD) (n = 5). SFA - saturated FA; MUFA - monounsaturated FA; PUFA - polyunsaturated FA; HUFA - highly unsaturated FA. PUFA are defined as all FA with 2 or 3 double bonds and HUFA as all FA with ≥ 4 double bonds.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| FA | Initial | T20S15 | T20S20 | T20S25 | T25S15 | T25S20 | T25S25 | Wild10 |
| 14:0 | 0.41 ± 0.01 | 0.90 ± 0.10 | 0.79 ± 0.19 | 0.88 ± 0.27 | 1.02 ± 0.34 | 1.02 ± 0.21 | 1.14 ± 0.18 | 0.40 ± 0.02 |
| 16:0 | 4.14 ± 0.31 | 6.62 ± 1.29 | 5.81 ± 1.96 | 6.92 ± 2.39 | 7.97 ± 2.67 | 9.18 ± 1.77 | 9.36 ± 1.31 | 4.25 ± 0.34 |
| 18:0 | 2.44 ± 0.09 | 2.34 ± 0.24 | 2.33 ± 0.38 | 2.80 ± 0.31 | 2.60 ± 0.52 | 3.44 ± 1.18 | 2.72 ± 0.29 | 2.40 ± 0.16 |
| **∑SFA** | **6.99 ± 0.27** | **9.87 ± 1.61** | **8.93 ± 2.52** | **10.59 ± 2.84** | **11.59 ± 3.37** | **13.63 ± 3.08** | **13.22 ± 1.72** | **7.06 ± 0.47** |
|  |  |  |  |  |  |  |  |  |
| 16:1 *n*-7 | 1.05 ± 0.18 | 1.11 ± 0.09 | 1.03 ± 0.26 | 1.26 ± 0.42 | 1.07 ± 0.14 | 1.32 ± 0.24 | 1.32 ± 0.32 | 0.93 ± 0.14 |
| 18:1 *n*-14 | 2.18 ± 0.07 | 2.33 ± 0.15 | 2.20 ± 0.35 | 2.09 ± 0.27 | 1.96 ± 0.19 | 2.17 ± 0.15 | 2.19 ± 0.08 | 2.05 ± 0.10 |
| 18:1 *n*-9 | 1.48 ± 0.06 | 3.11 ± 0.57 | 3.17 ± 0.85 | 3.47 ± 0.77 | 3.04 ± 0.62 | 3.96 ± 0.54 | 4.35 ± 0.68 | 1.47 ± 0.02 |
| 18:1 *n*-7 | 1.97 ± 0.05 | 2.39 ± 0.23 | 2.44 ± 0.47 | 2.44 ± 0.41 | 2.20 ± 0.31 | 2.68 ± 0.25 | 2.88 ± 0.31 | 1.94 ± 0.09 |
| 20:1 *n*-13 | 1.31 ± 0.11 | 1.30 ± 0.30 | 1.42 ± 0.27 | 1.28 ± 0.12 | 1.29 ± 0.15 | 1.45 ± 0.13 | 1.68 ± 0.11 | 1.28 ± 0.09 |
| 20:1 *n*-11  | 0.91 ± 0.01 | 1.25 ± 0.17 | 1.30 ± 0.26 | 1.36 ± 0.19 | 1.26 ± 0.18 | 1.41 ± 0.11 | 1.57 ± 0.23 | 0.89 ± 0.03 |
| 20:1 *n*-9 | 0.75 ± 0.02 | 0.75 ± 0.02 | 0.77 ± 0.04 | 0.76 ± 0.02 | 0.74 ± 0.02 | 0.77 ± 0.05 | 0.87 ± 0.06 | 0.74 ± 0.02 |
| 22:1 *n*-11 | ND | 0.95 ± 0.11 | 1.03 ± 0.09 | 0.97 ± 0.07 | 0.95 ± 0.05 | 0.93 ± 0.07 | 1.86 ± 0.24 | ND |
| **∑MUFA** | **9.66 ± 0.25** | **13.19 ± 1.17** | **13.35 ± 2.22** | **13.62 ± 2.14** | **12.51 ± 1.44** | **14.69 ± 0.79** | **16.73 ± 1.91** | **9.29 ± 0.45** |
|  |  |  |  |  |  |  |  |  |
| 18:2*n*-6 (LA) | 0.70 ± 0.03 | 2.28 ± 0.51 | 2.18 ± 0.99 | 2.30 ± 0.86 | 2.16 ± 0.61 | 2.65 ± 0.77 | 2.70 ± 0.82 | 0.74 ± 0.03 |
| 18:3 *n*-3 (ALA) | 1.04± 0.06 | 1.11 ± 0.06 | 1.11 ± 0.23 | 1.06 ± 0.12 | 1.08 ± 0.06 | 1.14 ± 0.11 | 1.10 ± 0.07 | 0.99 ± 0.05 |
| Δ5,11 20:2  | ND | 1.10 ± 0.10 | 1.00 ± 0.12 | 1.10 ± 0.07 | 1.10 ± 0.05 | 1.20 ± 0.11 | 1.29 ± 0.08 | ND |
| 20:2 *n*-6 | 1.04 ± 0.05 | 1.36 ± 0.20 | 1.34 ± 0.27 | 1.38 ± 0.27 | 1.34 ± 0.18 | 1.45 ± 0.24 | 1.53 ± 0.27 | 1.04 ± 0.05 |
| 20:3 *n*-6 (DGLA) | ND | ND | ND | ND | ND | ND | ND | ND |
| 20:3 *n*-3 (ETE) | ND | ND | ND | ND | ND | ND | ND | ND |
| Δ7,13 22:2  | 1.12 ± 0.05 | 1.31 ± 0.09 | 1.25 ± 0.15 | 1.25 ± 0.06 | 1.21 ± 0.10 | 1.23 ± 0.09 | 1.29 ± 0.08 | 1.23 ± 0.05 |
| Δ7,13,16 22:3  | ND | ND | ND | ND | ND | ND | ND | ND |
| **∑PUFA** | **3.89 ± 0.09** | **7.15 ± 0.87** | **6.88 ± 1.70** | **7.09 ± 1.34** | **6.89 ± 0.97** | **7.67 ± 1.01** | **7.91 ± 1.20** | **4.00 ± 0.14** |
|  |  |  |  |  |  |  |  |  |
| 20:4 *n*-6 (ARA) | 1.77 ± 0.07 | 1.62 ± 0.13 | 1.36 ± 0.34 | 1.31 ± 0.29 | 1.33 ± 0.21 | 1.37 ± 0.22 | 1.39 ± 0.19 | 2.09 ± 0.19 |
| 20:4 *n*-3 (ETA) | ND | ND | ND | ND | ND | ND | ND | ND |
| 20:5 *n*-3 (EPA) | 3.31 ± 0.13 | 3.65 ± 0.40 | 3.09 ± 1.48 | 2.73 ± 1.22 | 2.92 ± 0.77 | 3.40 ± 0.95 | 3.15 ± 0.92 | 4.06 ± 0.39 |
| 22:4 *n*-6 (AdA) | 1.39 ± 0.07 | 1.42 ± 0.22 | 1.16 ± 0.27 | 1.13 ± 0.19 | 1.12 ± 0.13 | 1.21 ± 0.15 | 1.29 ± 0.14 | 1.85 ± 0.18 |
| 22:5 *n*-3 (DPA) | 1.34 ± 0.01 | 1.33 ± 0.13 | 1.33 ± 0.27 | 1.22 ± 0.16 | 1.20 ± 0.16 | 1.31 ± 0.13 | 1.28 ± 0.16 | 1.69 ± 0.13 |
| 22:6 *n*-3 (DHA) | ND | 1.18 ± 0.20 | 1.08 ± 0.24 | 1.02 ± 0.22 | 1.11 ± 0.17 | 1.77 ± 0.70 | 1.83 ± 0.48 | ND |
| **∑HUFA** | **7.81 ± 0.16** | **9.20 ± 1.03** | **8.03 ± 2.58** | **7.40 ± 2.04** | **7.68 ± 1.41** | **9.05 ± 1.54** | **8.93 ± 1.84** | **9.69 ± 0.80** |
|  |  |  |  |  |  |  |  |  |
| **Total FA** | **28.35 ± 0.50** | **39.41 ± 4.03** | **37.19 ± 8.56** | **38.70 ± 8.19** | **38.67 ± 5.71** | **45.04 ± 4.70** | **46.79 ± 5.37** | **30.03 ± 1.67** |

**Table S5.** Fatty acid (FA) profile (µg mg-1 DW) of *Hediste diversicolor* fed a commercial aquafeed for 20 days and maintained under different combinations of water temperature (T – 20 and 25 ºC) and salinity (S – 15, 20 and 25), along with conspecifics initially stocked (Initial) and collected from the wild at the same time point (Wild20). Average values (± SD) (n = 5). SFA - saturated FA; MUFA - monounsaturated FA; PUFA - polyunsaturated FA; HUFA - highly unsaturated FA. PUFA are defined as all FA with 2 or 3 double bonds and HUFA as all FA with ≥ 4 double bonds.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| FA | Initial | T20S15 | T20S20 | T20S25 | T25S15 | T25S20 | T25S25 | Wild20 |
| 14:0 | 0.41 ± 0.01 | 1.71 ± 0.21 | 1.88 ± 0.34 | 1.88 ± 0.18 | 2.43 ± 1.06 | 1.49 ± 0.51 | 1.58 ± 0.14 | 0.45 ± 0.02 |
| 16:0 | 4.14 ± 0.31 | 12.41 ± 1.54 | 13.68 ± 1.89 | 14.08 ± 2.19 | 18.38 ± 5.77 | 13.13 ± 3.63 | 13.48 ± 0.83 | 4.98 ± 0.47 |
| 18:0 | 2.44 ± 0.09 | 3.22 ± 0.28 | 3.64 ± 0.51 | 3.68 ± 0.52 | 4.17 ± 0.64 | 3.73 ± 0.89 | 3.48 ± 0.23 | 2.11 ± 0.08 |
| **∑SFA** | **6.99 ± 0.27** | **17.35 ± 1.99** | **19.20 ± 2.67** | **19.64 ± 2.88** | **24.98 ± 7.37** | **18.35 ± 4.86** | **18.54 ± 1.08** | **7.54 ± 0.56** |
|  |  |  |  |  |  |  |  |  |
| 16:1 *n*-7 | 1.05 ± 0.18 | 1.90 ± 0.30 | 2.26 ± 0.51 | 2.20 ± 0.25 | 2.22 ± 0.67 | 1.69 ± 0.50 | 1.83 ± 0.18 | 0.88 ± 0.17 |
| 18:1 *n*-14 | 2.18 ± 0.07 | 2.38 ± 0.23 | 2.36 ± 0.17 | 2.43 ± 0.32 | 2.23 ± 0.35 | 2.14 ± 0.32 | 2.11 ± 0.14 | 2.12 ± 0.10 |
| 18:1 *n*-9 | 1.48 ± 0.06 | 6.29 ± 0.81 | 7.41 ± 1.65 | 7.34 ± 0.74 | 7.07 ± 1.88 | 5.96 ± 1.76 | 6.62 ± 0.83 | 1.50 ± 0.05 |
| 18:1 *n*-7 | 1.97 ± 0.05 | 3.54 ± 0.34 | 3.96 ± 0.56 | 4.10 ± 0.57 | 3.65 ± 0.79 | 3.36 ± 0.85 | 3.48 ± 0.37 | 2.17 ± 0.11 |
| 20:1 *n*-13  | 1.31 ± 0.11 | 1.18 ± 0.28 | 1.48 ± 0.13 | 1.66 ± 0.23 | 1.20 ± 0.14 | 1.10 ± 0.21 | 1.37 ± 0.22 | 1.28 ± 0.05 |
| 20:1 *n*-11  | 0.91 ± 0.01 | 1.89 ± 0.50 | 2.32 ± 0.37 | 2.41 ± 0.26 | 2.25 ± 0.55 | 2.21 ± 0.76 | 2.22 ± 0.22 | 0.86 ± 0.02 |
| 20:1 *n*-9 | 0.75 ± 0.02 | 0.75 ± 0.04 | 0.81 ± 0.02 | 0.89 ± 0.07 | 0.75 ± 0.02 | 0.75 ± 0.02 | 0.76 ± 0.04 | 0.76 ± 0.02 |
| 22:1 *n*-11 | ND | 1.74 ± 0.24 | 1.40 ± 0.18 | 1.59 ± 0.45 | 1.03 ± 0.08 | 1.02 ± 0.17 | 1.11 ± 0.21 | ND |
| **∑MUFA** | **9.66 ± 0.25** | **19.66 ± 2.32** | **21.99 ± 3.32** | **22.63 ± 1.89** | **20.41 ± 4.31** | **18.23 ± 4.06** | **19.52 ± 1.77** | **9.57 ± 0.15** |
|  |  |  |  |  |  |  |  |  |
| 18:2*n*-6 (LA) | 0.70 ± 0.03 | 4.49 ± 1.72 | 5.55 ± 2.12 | 5.84 ± 0.86 | 5.71 ± 1.45 | 5.06 ± 1.61 | 5.40 ± 0.36 | 0.72 ± 0.07 |
| 18:3 *n*-3 (ALA) | 1.04± 0.06 | 1.27 ± 0.22 | 1.31 ± 0.27 | 1.45 ± 0.19 | 1.33 ± 0.14 | 1.32 ± 0.23 | 1.32 ± 0.05 | 1.07 ± 0.03 |
| Δ5,11 20:2  | ND | 1.23 ± 0.04 | 1.27 ± 0.11 | 1.28 ± 0.06 | 1.25 ± 0.12 | 1.21 ± 0.31 | 1.23 ± 0.09 | ND |
| 20:2 *n*-6 | 1.04 ± 0.05 | 2.29 ± 0.71 | 2.56 ± 0.76 | 2.72 ± 0.53 | 2.46 ± 0.64 | 2.73 ± 1.20 | 2.65 ± 0.43 | 1.03 ± 0.09 |
| 20:3 *n*-6 (DGLA) | ND | ND | ND | ND | ND | ND | ND | ND |
| 20:3 *n*-3 (ETE) | ND | ND | ND | ND | ND | ND | ND | ND |
| Δ7,13 22:2  | 1.12 ± 0.05 | 1.33 ± 0.11 | 1.38 ± 0.12 | 1.47 ± 0.15 | 1.18 ± 0.07 | 1.26 ± 0.22 | 1.27 ± 0.14 | 1.19 ± 0.05 |
| Δ7,13,16 22:3  | ND | 1.08 ± 0.08 | 1.03 ± 0.04 | 1.09 ± 0.06 | 0.95 ± 0.04 | 1.02 ± 0.09 | 1.00 ± 0.06 | ND |
| **∑PUFA** | **3.89 ± 0.09** | **11.69 ± 2.75** | **13.10 ± 3.32** | **13.86 ± 1.72** | **12.87 ± 2.35** | **12.60 ± 3.58** | **12.87 ± 0.69** | **4.01 ± 0.17** |
|  |  |  |  |  |  |  |  |  |
| 20:4 *n*-6 (ARA) | 1.77 ± 0.07 | 1.45 ± 0.36 | 1.33 ± 0.34 | 1.44 ± 0.19 | 1.35 ± 0.10 | 1.40 ± 0.33 | 1.44 ± 0.21 | 1.96 ± 0.22 |
| 20:4 *n*-3 (ETA) | ND | ND | ND | ND | ND | ND | ND | ND |
| 20:5 *n*-3 (EPA) | 3.31 ± 0.13 | 3.77 ± 1.59 | 3.25 ± 1.47 | 4.26 ± 1.14 | 4.03 ± 0.78 | 4.83 ± 1.46 | 4.40 ± 0.80 | 4.03 ± 0.66 |
| 22:4 *n*-6 (AdA) | 1.39 ± 0.07 | 1.28 ± 0.24 | 1.14 ± 0.26 | 1.25 ± 0.18 | 1.02 ± 0.04 | 1.14 ± 0.27 | 1.17 ± 0.16 | 1.62 ± 0.17 |
| 22:5 *n*-3 (DPA) | 1.34 ± 0.01 | 1.23 ± 0.20 | 1.19 ± 0.17 | 1.34 ± 0.17 | 1.14 ± 0.06 | 1.25 ± 0.21 | 1.28 ± 0.14 | 1.67 ± 0.16 |
| 22:6 *n*-3 (DHA) | ND | 1.87 ± 0.60 | 1.28 ± 0.50 | 1.94 ± 0.31 | 1.31 ± 0.16 | 1.58 ± 0.73 | 1.38 ± 0.19 | ND |
| **∑HUFA** | **7.81 ± 0.16** | **9.59 ± 2.91** | **8.19 ± 2.70** | **10.24 ± 1.77** | **8.85 ± 1.07** | **10.21 ± 2.98** | **9.67 ± 1.35** | **9.28 ± 1.20** |
|  |  |  |  |  |  |  |  |  |
| **Total FA** | **28.35 ± 0.50** | **58.30 ± 8.58** | **62.48 ± 11.22** | **66.37 ± 7.15** | **67.11 ± 14.17** | **59.39 ± 14.89** | **60.60 ± 3.70** | **30.41 ± 1.82** |

**Table S6.** Two-way nested analysis of similarities (ANOSIM) evaluating differences in the fatty acid profile of *Hediste diversicolor* fed a commercial aquafeed during 10, 20 and 40 days (D10, D20 and D40) in different treatments of combined temperature (T – 20 and 25ºC) and salinity (S – 15, 20 and 25). S(T) - Salinity groups nested within temperature groups; Significant differences when *p* < 0.05.

|  |  |  |  |
| --- | --- | --- | --- |
| Samples | Tests between T and S(T) levels | *R-value* | *p-value* |
| D10 | T | 0.222 | 0.300 |
| S(T) | 0.124 | 0.068 |
|  |  |
| D20 | T | 0.481 | 0.100 |
| S(T) | 0.101 | 0.089 |
|  |  |
| D40 | T | -0.034 | 0.500 |
| S(T) | 0.051 | 0.241 |

**Table S7.** One-way analysis of similarities (ANOSIM) between the fatty acid profile of *Hediste diversicolor* fed a commercial aquafeed during 10, 20 and 40 days (D10, D20 and D40) in different treatments of combined temperature (T – 20 and 25ºC) and salinity (S – 15, 20 and 25) and wild conspecifics collected on the same date (Wild10, Wild20 and Wild40). Significant differences when *p* < 0.05.

|  |  |  |
| --- | --- | --- |
| Pairwise tests | *R* | *p* |
| *D10 samples* |
| T20S15 – Wild10 | 1.000 | 0.008 |
| T20S20 – Wild10 | 0.872 | 0.008 |
| T20S25 – Wild10 | 1.000 | 0.008 |
|  |   |   |
| T25S15 – Wild10 | 1.000 | 0.008 |
| T25S20 – Wild10 | 1.000 | 0.008 |
| T25S25 – Wild10 | 1.000 | 0.008 |
| *D20 samples* |
| T20S15 – Wild20 | 1.000 | 0.008 |
| T20S20 – Wild20 | 1.000 | 0.008 |
| T20S25 – Wild20 | 1.000 | 0.008 |
|  |   |   |
| T25S15 – Wild20 | 1.000 | 0.008 |
| T25S20 – Wild20 | 1.000 | 0.008 |
| T25S25 – Wild20 | 1.000 | 0.008 |
| *D40 samples* |
| T20S15 – Wild40 | 1.000 | 0.008 |
| T20S20 – Wild40 | 1.000 | 0.008 |
| T20S25 – Wild40 | 1.000 | 0.008 |
|  |   |   |
| T25S15 – Wild40 | 1.000 | 0.008 |
| T25S20 – Wild40 | 1.000 | 0.008 |
| T25S25 – Wild40 | 1.000 | 0.008 |

**Table S8.** Similarity percentages (SIMPER) analysis (cut-off 50%) between the fatty acid (FA) profile of *Hediste diversicolor* fed a commercial aquafeed for 10 days in different treatments of combined temperature (T – 20 and 25ºC) and salinity (S – 15, 20 and 25) and wild conspecifics collected on the same date (Wild10).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| T20S15 & Wild10 |  | T20S20 & Wild10 |   | T20S25 & Wild10 |
| Avg. Dissimilarity: 14.76% |  | Avg. Dissimilarity: 16.12% |   | Avg. Dissimilarity: 16.74% |
| FA | Contrib.% | Cum.% |   | FA | Contrib.% | Cum.% |   | FA | Contrib.% | Cum.% |
| 22:6 *n*-3 (DHA) | 14.14 | 14.14 |   | 22:6 *n*-3 (DHA) | 12.34 | 12.34 |   | Δ5,1120:2 | 12.08 | 12.08 |
| Δ5,1120:2 | 13.51 | 27.65 |   | 22:1 | 12.08 | 24.42 |   | 22:6 *n*-3 (DHA) | 11.32 | 23.39 |
| 22:1 | 12.17 | 39.82 |   | Δ5,1120:2 | 11.78 | 36.20 |   | 22:1 | 11.09 | 34.48 |
| 18:2 *n*-6 (LA) | 11.30 | 51.12 |   | 18:2 *n*-6 (LA) | 9.30 | 45.50 |   | 18:2 *n*-6 (LA) | 9.80 | 44.28 |
|   |   |   |   | Δ918:1 | 8.44 | 53.94 |   | Δ918:1 | 9.38 | 53.67 |
|   |   |   |   |   |   |   |   |   |   |   |
| T25S15 & Wild10 |   | T25S20 & Wild10 |   | T25S25 & Wild10 |
| Avg. Dissimilarity: 16.37% |   | Avg. Dissimilarity: 18.50% |   | Avg. Dissimilarity: 20.02% |
| FA | Contrib.% | Cum.% |   | FA | Contrib.% | Cum.% |   | FA | Contrib.% | Cum.% |
| 22:6 *n*-3 (DHA) | 12.45 | 12.45 |   | 22:6 *n*-3 (DHA) | 14.02 | 14.02 |   | 22:1 | 13.38 | 13.38 |
| Δ5,1120:2 | 12.43 | 24.89 |   | Δ5,1120:2 | 11.13 | 25.15 |   | 22:6 *n*-3 (DHA) | 13.09 | 26.47 |
| 22:1 | 11.14 | 36.02 |   | 18:2 *n*-6 (LA) | 10.11 | 35.26 |   | Δ5,1120:2 | 10.58 | 37.05 |
| 18:2 *n*-6 (LA) | 9.62 | 45.64 |   | Δ918:1 | 9.77 | 45.03 |   | Δ918:1 | 9.78 | 46.83 |
| 16:0 | 8.31 | 53.96 |   | 22:1 | 9.27 | 54.36 |   | 18:2 *n*-6 (LA) | 9.32 | 56.15 |

**Table S9.** Similarity percentages (SIMPER) analysis (cut-off 50%) between the fatty acid (FA) profile of *Hediste diversicolor* fed a commercial aquafeed for 20 days in different treatments of combined temperature (T – 20 and 25ºC) and salinity (S – 15, 20 and 25), and wild conspecifics collected on the same date (Wild20).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| T20S15 & Wild20 |  | T20S20 & Wild20 |   | T20S25 & Wild20 |
| Avg. Dissimilarity: 24.83% |  | Avg. Dissimilarity: 26.23% |   | Avg. Dissimilarity: 26.72% |
| FA | Contrib.% | Cum.% |   | FA | Contrib.% | Cum.% |   | FA | Contrib.% | Cum.% |
| 18:2 *n*-6 (LA) | 10.57 | 10.57 |   | 18:2 *n*-6 (LA) | 11.56 | 11.56 |   | 18:2 *n*-6 (LA) | 11.80 | 11.80 |
| Δ918:1 | 10.25 | 20.82 |   | Δ918:1 | 10.72 | 22.28 |   | Δ918:1 | 10.30 | 22.10 |
| 22:6 *n*-3 (DHA) | 9.96 | 30.78 |   | 16:0 | 8.03 | 30.30 |   | 22:6 *n*-3 (DHA) | 9.23 | 31.32 |
| 22:1 | 9.70 | 40.47 |   | 22:1 | 7.85 | 38.16 |   | 22:1 | 8.11 | 39.43 |
| Δ5,1120:2 | 7.76 | 48.23 |   | Δ5,1120:2 | 7.38 | 45.53 |   | 16:0 | 7.87 | 47.31 |
| 16:0 | 7.74 | 55.97 |   | 22:6 *n*-3 (DHA) | 7.22 | 52.75 |   | Δ5,1120:2 | 7.10 | 54.41 |
|   |   |   |   |   |   |   |   |   |   |   |
| T25S15 & Wild20 |   | T25S20 & Wild20 |   | T25S25 & Wild20 |
| Avg. Dissimilarity: 26.03% |   | Avg. Dissimilarity: 23.99% |   | Avg. Dissimilarity: 24.38% |
| FA | Contrib.% | Cum.% |   | FA | Contrib.% | Cum.% |   | FA | Contrib.% | Cum.% |
| 18:2 *n*-6 (LA) | 12.13 | 12.13 |   | 18:2 *n*-6 (LA) | 12.36 | 12.36 |   | 18:2 *n*-6 (LA) | 12.86 | 12.86 |
| Δ918:1 | 10.37 | 22.50 |   | Δ918:1 | 9.98 | 22.34 |   | Δ918:1 | 10.83 | 23.69 |
| 16:0 | 10.22 | 32.72 |   | 22:6 *n*-3 (DHA) | 9.16 | 31.50 |   | 16:0 | 8.66 | 32.35 |
| 22:6 *n*-3 (DHA) | 7.59 | 40.31 |   | 16:0 | 8.34 | 39.84 |   | 22:6 *n*-3 (DHA) | 8.44 | 40.79 |
| 14:0 | 7.36 | 47.68 |   | Δ5,1120:2 | 7.84 | 47.68 |   | Δ5,1120:2 | 7.83 | 48.61 |
| Δ5,1120:2 | 7.32 | 55.00 |   | 22:3 *n*-6 | 7.06 | 54.74 |   | 22:1 | 7.24 | 55.86 |

**Table S10.** Similarity percentages (SIMPER) analysis (cut-off 50%) between the fatty acid (FA) profile of *Hediste diversicolor* fed a commercial aquafeed for 40 days in different treatments of combined temperature (T – 20 and 25ºC) and salinity (S – 15, 20 and 25), and wild conspecifics collected on the same date (Wild40).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| T20S15 & Wild40 |  | T20S20 & Wild40 |   | T20S25 & Wild40 |
| Avg. Dissimilarity: 30.04% |  | Avg. Dissimilarity: 31.47% |   | Avg. Dissimilarity: 31.34% |
| FA | Contrib.% | Cum.% |   | FA | Contrib.% | Cum.% |   | FA | Contrib.% | Cum. % |
| 22:6 *n*-3 (DHA) | 10.84 | 10.84 |   | 22:6 *n*-3 (DHA) | 10.40 | 10.40 |   | 22:6 *n*-3 (DHA) | 10.34 | 10.34 |
| 18:2 *n*-6 (LA) | 9.49 | 20.34 |   | 18:2 *n*-6 (LA) | 9.28 | 19.68 |   | 18:2 *n*-6 (LA) | 9.01 | 19.35 |
| 22:1 | 7.24 | 27.57 |   | 20:2 *n*-6 | 7.23 | 26.91 |   | 20:2 *n*-6 | 7.64 | 26.99 |
| Δ918:1 | 7.18 | 34.76 |   | Δ918:1 | 7.17 | 34.08 |   | Δ918:1 | 6.68 | 33.67 |
| 20:2 *n*-6 | 6.68 | 41.44 |   | Δ5,1120:2 | 6.39 | 40.47 |   | 22:1 | 6.23 | 39.90 |
| Δ5,1120:2 | 6.27 | 47.79 |   | 22:1 | 5.97 | 46.44 |   | Δ5,1120:2 | 6.07 | 45.96 |
| 20:3 *n*-6  |  6.17 | 53.88  |   | 22:3 *n*-6 | 5.67 | 52.12 |   | 22:3 *n*-6 | 6.04 | 52.0 |
|   |   |   |   |   |   |   |   |   |   |   |
| T25S15 & Wild40 |   | T25S20 & Wild40 |   | T25S25 & Wild40 |
| Avg. Dissimilarity: 30.40% |   | Avg. Dissimilarity: 28.91% |   | Avg. Dissimilarity: 32.41% |
| FA | Contrib.% | Cum.% |   | FA | Contrib.% | Cum.% |   | FA | Contrib.% | Cum.% |
| 22:6 *n*-3 (DHA) | 9.90 | 9.90 |   | 22:6 *n*-3 (DHA) | 10.74 | 10.74 |   | 22:6 *n*-3 (DHA) | 9.81 | 9.81 |
| 18:2 *n*-6 (LA) | 8.57 | 18.47 |   | 18:2 *n*-6 (LA) | 8.16 | 18.90 |   | 18:2 *n*-6 (LA) | 8.83 | 18.64 |
| 20:2 *n*-6 | 7.20 | 25.68 |   | 22:1 | 7.68 | 26.58 |   | 20:2 *n*-6 | 7.55 | 26.18 |
| Δ918:1 | 6.84 | 32.52 |   | 20:2 *n*-6 | 7.34 | 33.93 |   | Δ918:1 | 7.31 | 33.50 |
| Δ5,1120:2 | 6.83 | 39.35 |   | Δ5,1120:2 | 6.78 | 40.70 |   | Δ5,1120:2 | 6.47 | 39.97 |
| 22:1 | 6.27 | 45.62 |   | Δ918:1 | 6.43 | 47.14 |   | 22:3 *n*-6 | 5.87 | 45.84 |
| 22:3 *n*-6 | 6.03 | 51.65 |   |  22:3 *n*-6 |  6.38 | 53.52  |   | 22:1 | 5.57 | 51.41 |

## Supplementary Figures

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**Figure S1.** Evolution of fatty acid classes (expressed as % of total fatty acid methyl esters - FAME) of *Hediste diversicolor* fed a commercial aquafeed over time (10, 20 and 40 days) under different treatments of combined temperature (T – 20 and 25 ºC) and salinity (S – 15, 20 and 25) contrasted with that of initially stocked conspecifics (Initial). Average values (± SD) (n = 5). Abbreviations: SFA - saturated fatty acids; MUFA - monounsaturated FA; PUFA - polyunsaturated FA; HUFA - highly unsaturated FA. PUFA are defined as all FA with 2 or 3 double bonds and HUFA as all FA with ≥ 4 double bonds.