**Supplementary table 1** – Element concentrations (mg/kg dry weight) in purified zebrafish diets. Data are mean ± SEM n=2-3.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| diet no. | Vanadium | | Manganese | | Iron | | Cobolt | | Copper | | Zinc | | Arsenic | | Selenium | | Strontium | |
| mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM |
| 1 | 0.426 | 0.023 | 32.9 | 1.8 | 130.7 | 12.0 | 1.224 | 0.085 | 3.897 | 0.279 | 103.4 | 6.3 | 0.0466 | 0.0044 | 0.085 | 0.001 | 4.58 | 0.33 |
| 2 | 0.409 | 0.022 | 31.1 | 1.6 | 122.3 | 1.3 | 1.145 | 0.036 | 3.925 | 0.108 | 95.6 | 4.5 | 0.0383 | 0.0010 | 0.184 | 0.029 | 4.41 | 0.14 |
| 3 | 0.418 | 0.004 | 31.3 | 0.3 | 122.3 | 7.4 | 1.247 | 0.014 | 3.802 | 0.054 | 95.6 | 0.3 | 0.0396 | 0.0011 | 0.305 | 0.006 | 4.43 | 0.09 |
| 4 | 0.432 | 0.008 | 32.5 | 0.7 | 123.5 | 4.5 | 1.210 | 0.001 | 3.999 | 0.002 | 97.3 | 1.8 | 0.0425 | 0.0014 | 0.496 | 0.004 | 4.52 | 0.04 |
| 5 | 0.443 | 0.014 | 32.9 | 0.9 | 128.6 | 5.7 | 1.215 | 0.033 | 3.953 | 0.116 | 99.0 | 3.2 | 0.0406 | 0.0020 | 0.650 | 0.099 | 4.59 | 0.13 |
| 6 | 0.452 | 0.008 | 32.9 | 0.7 | 126.3 | 3.8 | 1.184 | 0.003 | 4.713 | 0.004 | 99.9 | 1.3 | 0.0422 | 0.0004 | 1.014 | 0.024 | 4.62 | 0.03 |
| 7 | 0.470 | 0.010 | 32.9 | 0.7 | 129.2 | 3.4 | 1.092 | 0.017 | 4.976 | 0.099 | 104.4 | 1.7 | 0.0458 | 0.0026 | 29.533 | 0.628 | 4.77 | 0.10 |

cont’

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| diet no. | Silver | | Cadmium | | Tin | | Barium | | Mercury | | Lead | |
| mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM |
| 1 | 0.0041 | 0.0024 | 0.0204 | 0.0020 | 0.0245 | 0.0021 | 0.301 | 0.028 | 0.0028 | 0.0003 | 0.0458 | 0.0124 |
| 2 | 0.0008 | 0.0004 | 0.0189 | 0.0003 | 0.0240 | 0.0033 | 0.291 | 0.004 | 0.0023 | 0.0001 | 0.0227 | 0.0002 |
| 3 | 0.0007 | 0.0001 | 0.0180 | 0.0005 | 0.0204 | 0.0004 | 0.281 | 0.017 | 0.0023 | 0.0001 | 0.0221 | 0.0010 |
| 4 | 0.0006 | 0.0002 | 0.0177 | 0.0004 | 0.0219 | 0.0003 | 0.293 | 0.013 | 0.0021 | 0.0003 | 0.0232 | 0.0005 |
| 5 | 0.0006 | 0.0002 | 0.0190 | 0.0012 | 0.0233 | 0.0003 | 0.298 | 0.013 | 0.0020 | 0.0001 | 0.0237 | 0.0012 |
| 6 | 0.0004 | 0.0000 | 0.0191 | 0.0003 | 0.0240 | 0.0013 | 0.301 | 0.009 | 0.0023 | 0.0002 | 0.0257 | 0.0017 |
| 7 | 0.0007 | 0.0002 | 0.0189 | 0.0004 | 0.0228 | 0.0004 | 0.330 | 0.016 | 0.0022 | 0.0001 | 0.0246 | 0.0005 |

**\* Cu concentrations were statistically higher (p<0.05) in diet no. 7 than diets 1-5.**

**Supplementary table 2** – Growth, fultons condition factor, survival and rate of skeletal deformities in zebrafish fed diets with graded levels of Se from 27 dpf. Data are mean ± SEM n=3, except for 27 dpf which are analytical parallels.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | | | | | | | | | | | Deformities | | | | | | | | | | | | | |
| Age  (dpf) |  | Length | | Weight | | Fulton condition  factor | | Specific growth rate\* | | Survival | | Fish measured per tank | | Head | | neck | | Lordosis | | Scoliosis | | kyphosis | | Short body | | Total with deformity | |
| Unit → | mm | | g | | No unit | | %/day | | % | | no. | | % | | | | | | | | | | | | | |
| diet Se conc | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM |
| 27 | - | 8.9 | 0.002 | 0.010 | 0.0007 | 0.88 | 0.03 | ND | ND | ND | ND | 30 | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 62 | 0.09 | 16.0 | 0.1 | 0.083 | 0.003 | 1.15 | 0.01 | 5.7 | 0.1 | 96.3 | 1.5 | 17.7 | 0.3 | 3.8 | 1.9 | 3.8 | 1.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 7.6 | 3.8 |
| 62 | 0.18 | 16.8 | 0.2 | 0.093 | 0.002 | 1.10 | 0.02 | 6.0 | 0.2 | 95.6 | 1.3 | 17.0 | 1.0 | 2.1 | 2.1 | 10.4 | 5.5 | 2.1 | 2.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 14.6 | 7.5 |
| 62 | 0.30 | 17.4 | 0.2 | 0.108 | 0.004 | 1.15 | 0.03 | 6.4 | 0.2 | 94.1 | 2.0 | 17.0 | 1.2 | 8.4 | 6.0 | 6.2 | 3.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 12.9 | 10.3 |
| 62 | 0.50 | 17.0 | 0.2 | 0.094 | 0.004 | 1.11 | 0.04 | 6.1 | 0.2 | 96.3 | 0.7 | 17.0 | 0.6 | 4.2 | 4.2 | 0.0 | 0.0 | 3.9 | 2.0 | 0.0 | 0.0 | 1.9 | 1.9 | 0.0 | 0.0 | 8.1 | 5.6 |
| 62 | 0.65 | 17.1 | 0.2 | 0.097 | 0.005 | 1.10 | 0.03 | 6.1 | 0.2 | 97.0 | 2.0 | 16.7 | 0.7 | 6.0 | 3.6 | 4.2 | 4.2 | 1.9 | 1.9 | 2.1 | 2.1 | 0.0 | 0.0 | 0.0 | 0.0 | 12.0 | 0.5 |
| 62 | 1 | 15.8 | 0.3 | 0.081 | 0.004 | 1.10 | 0.03 | 5.3 | 0.2 | 96.3 | 0.7 | 16.7 | 0.7 | 9.7 | 5.0 | 7.6 | 4.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 17.4 | 8.2 |
| 62 | 30 | 16.1 | 0.4 | 0.085 | 0.008 | 1.13 | 0.02 | 5.7 | 0.3 | 93.3 | 0.0 | 16.7 | 1.2 | 2.1 | 2.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 5.6 | 3.1 | 7.7 | 3.9 |

\* 62 dpf values are for SGR between 27 and 62 dpf

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Age  (dpf) | diet Se conc | Vanadium | | Manganese | | Iron | | Cobolt | | Copper | | Zinc | | Arsenic | | Selenium | | Strontium | |
| mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM |
| 27 | - | 0.078 | 0.008 | 3.71 | 0.44 | 133 | 8 | 0.152 | 0.020 | 4.72 | 0.33 | 313 | 8 | 1.7700 | 0.0300 | 2.240 | 0.240 | 21.0 | 0.8 |
| 62 | 0.09 | 0.322 | 0.042 | 4.87 | 0.53 | 216 | 35 | 0.264 | 0.053 | 5.10 | 0.91 | 175 | 17 | 0.0522 | 0.0091 | 0.491 | 0.042 | 11.8 | 1.0 |
| 62 | 0.18 | 0.317 | 0.009 | 4.71 | 0.29 | 175 | 3 | 0.204 | 0.017 | 4.61 | 0.15 | 172 | 10 | 0.0452 | 0.0017 | 0.473 | 0.009 | 11.6 | 0.6 |
| 62 | 0.30 | 0.265 | 0.012 | 4.14 | 0.27 | 153 | 10 | 0.169 | 0.026 | 3.86 | 0.42 | 152 | 5 | 0.0438 | 0.0012 | 0.667 | 0.041 | 10.5 | 0.2 |
| 62 | 0.50 | 0.293 | 0.006 | 3.78 | 0.11 | 142 | 6 | 0.125 | 0.015 | 3.62 | 0.17 | 169 | 7 | 0.0451 | 0.0016 | 0.728 | 0.077 | 12.1 | 0.8 |
| 62 | 0.65 | 0.299 | 0.012 | 4.38 | 0.18 | 143 | 7 | 0.118 | 0.018 | 3.27 | 0.35 | 166 | 9 | 0.0450 | 0.0013 | 0.931 | 0.017 | 11.6 | 0.6 |
| 62 | 1 | 0.251 | 0.011 | 3.71 | 0.37 | 120 | 7 | 0.095 | 0.010 | 3.08 | 0.11 | 175 | 6 | 0.0433 | 0.0014 | 1.286 | 0.021 | 11.0 | 0.4 |
| 62 | 30 | 0.254 | 0.035 | 3.59 | 0.33 | 123 | 10 | 0.095 | 0.007 | 2.91 | 0.29 | 170 | 9 | 0.0410 | 0.0009 | 14.952 | 1.164 | 11.6 | 0.9 |

**Supplementary table 3** – Element concentrations (mg/kg DM) in whole zebrafish fed diets with graded levels of Se from 27 dpf. Data are mean ± SEM, n=3, except for 27 dpf which are analytical parallels.

Cont’

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Age  (dpf) | diet Se conc | Silver | | Cadmium | | Tin | | Barium | | Mercury | | Lead | |
| mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM |
| 27 | - | 0.028 | 0.004 | 0.040 | 0.006 | 0.149 | 0.008 | 0.040 | 0.006 | 0.149 | 0.008 | 0.044 | 0.015 |
| 62 | 0.09 | 0.006 | 0.000 | 0.021 | 0.001 | 0.045 | 0.005 | 0.021 | 0.001 | 0.045 | 0.005 | 0.051 | 0.002 |
| 62 | 0.18 | 0.005 | 0.001 | 0.018 | 0.001 | 0.036 | 0.009 | 0.018 | 0.001 | 0.036 | 0.009 | 0.040 | 0.003 |
| 62 | 0.30 | 0.003 | 0.000 | 0.018 | 0.001 | 0.026 | 0.004 | 0.018 | 0.001 | 0.026 | 0.004 | 0.041 | 0.005 |
| 62 | 0.50 | 0.003 | 0.000 | 0.017 | 0.001 | 0.025 | 0.001 | 0.017 | 0.001 | 0.025 | 0.001 | 0.044 | 0.006 |
| 62 | 0.65 | 0.003 | 0.000 | 0.017 | 0.001 | 0.025 | 0.003 | 0.017 | 0.001 | 0.025 | 0.003 | 0.047 | 0.005 |
| 62 | 1 | 0.004 | 0.000 | 0.017 | 0.000 | 0.028 | 0.000 | 0.017 | 0.000 | 0.028 | 0.000 | 0.054 | 0.004 |
| 62 | 30 | 0.003 | 0.001 | 0.017 | 0.003 | 0.030 | 0.005 | 0.017 | 0.003 | 0.030 | 0.005 | 0.044 | 0.003 |

**Supplementary table 4** – Glutathione, redox potential, TBARS and enzyme activity levels in zebrafish fed diets with graded levels of Se from 27 dpf. Data are mean ± SEM n=3, except for 27 dpf which are analytical parallels.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Age  (dpf) |  | Total Glutathione | | Oxidised glutathione | | Redox potential | | TBARS | | Total Sod activity | | Mn Sod activity | | Cu/Zn Sod activity | | Se-dependent Gpx activity | | Catalase activity | |
| Unit → | umol g-1 WW | | | | Volts | | nmol g-1 WW | | units min-1 mg protein-1 † | | | | | | | | | |
| diet Se conc | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM |
| 27 | - | 738 | 20 | 2.41 | 0.01 | -0.221 | 0.001 | ND | ND | 1.12 | 0.01 | 0.31 | 0.08 | 0.81 | 0.06 | 69.3 | 5.3 | ND | ND |
| 62 | 0.09 | 877 | 49 | 2.91 | 0.69 | -0.223 | 0.005 | 12.3 | 0.3 | 0.81 | 0.08 | 0.32 | 0.08 | 0.50 | 0.15 | 20.7 | 1.7 | 67.1 | 11.3 |
| 62 | 0.18 | 781 | 5 | 2.44 | 0.40 | -0.222 | 0.002 | 9.0 | 0.8 | 0.58 | 0.04 | 0.33 | 0.02 | 0.24 | 0.05 | 18.1 | 1.9 | 83.2 | 5.3 |
| 62 | 0.30 | 751 | 35 | 2.15 | 0.23 | -0.223 | 0.003 | 7.2 | 1.3 | 1.15 | 0.61 | 0.42 | 0.10 | 1.23 | 0.87 | 13.1 | 5.0 | 95.6 | 3.2 |
| 62 | 0.50 | 865 | 28 | 2.29 | 0.09 | -0.225 | 0.000 | 9.9 | 1.1 | 0.36 | 0.03 | 0.51 | 0.13 | <LOQ | <LOQ | 16.0 | 3.8 | 106.8 | 10.4 |
| 62 | 0.65 | 831 | 25 | 2.16 | 0.07 | -0.225 | 0.001 | 9.2 | 2.0 | 0.63 | 0.18 | 0.87 | 0.44 | <LOQ | <LOQ | 42.3 | 8.5 | 130.1 | 8.6 |
| 62 | 1 | 828 | 19 | 2.11 | 0.18 | -0.225 | 0.001 | 9.4 | 1.6 | 0.38 | 0.07 | 0.41 | 0.08 | <LOQ | <LOQ | 79.1 | 4.1 | 99.0 | 14.9 |
| 62 | 30 | 833 | 33 | 3.23 | 0.39 | -0.220 | 0.001 | 9.9 | 2.2 | 2.09 | 0.69 | 0.48 | 0.10 | 1.60 | 0.79 | 89.2 | 13.0 | 105.9 | 6.2 |

\*ND – No data as not analysed

†1 unit = amount of enzyme in the given assay to result in, Sod; exhibit 50% dismutation of the available superoxide radicals, Gpx; oxidation of 1 nmol NADPH to NADP+, Cat; 1 nmol formaldehyde catalysed from methanol

**Supplementary table 5** – Mean normalised mRNA expression of selected selenoproteins in zebrafish at 62 dpf that were fed diets with graded levels of Se from 27 dpf. Data are mean ± SEM n=3.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Age (dpf) |  | *gpx1a* | | *gpx1b* | | *sepp1a* | | *sepp1b* | |
| Unit → | MNE | |  | |  | |  | |
| diet Se conc | mean | SEM | mean | SEM | mean | SEM | mean | SEM |
| 62 | 0.09 | 0.27 | 0.02 | 0.31 | 0.03 | 0.32 | 0.05 | 0.36 | 0.09 |
| 62 | 0.18 | 0.26 | 0.06 | 0.30 | 0.02 | 0.40 | 0.13 | 0.44 | 0.16 |
| 62 | 0.30 | 0.23 | 0.03 | 0.23 | 0.03 | 0.10 | 0.02 | 0.25 | 0.09 |
| 62 | 0.50 | 0.25 | 0.04 | 0.28 | 0.04 | 0.14 | 0.09 | 0.17 | 0.04 |
| 62 | 0.65 | 0.45 | 0.02 | 0.47 | 0.01 | 0.26 | 0.01 | 0.43 | 0.10 |
| 62 | 1 | 0.41 | 0.07 | 0.40 | 0.04 | 0.30 | 0.04 | 0.40 | 0.09 |
| 62 | 30 | 0.70 | 0.10 | 0.49 | 0.13 | 0.42 | 0.02 | 0.19 | 0.07 |

**Supplementary table 6** – Organ actual and somatic index corrected (SIC) weights and spleen mineral contents in 254 dpf male zebrafish fed one of 3 different Se concentrations (mg/kg DM) from 27 dpf. Data are mean ± SEM, n=4-5.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| diet Se conc. | Spleen weight | | Spleen SIC | | heart weight | | heart SIC | | liver weight | | Liver SIC | | Testes | | Gonad SIC | |
| mg/fish | | mg organ/g fish | | mg/fish | | mg organ/g fish | | mg/fish | | mg organ/g fish | | mg/fish | | mg organ/g fish | |
| mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM |
| 0.09 | 1.2 | 0.2 | 3.0 | 0.3 | 1.8 | 0.2 | 4.5 | 0.3 | 10.4 | 1.4 | 25.9 | 1.9 | 3.4 | 0.5 | 8.3 | 0.7 |
| 0.65 | 1.0 | 0.1 | 2.3 | 0.2 | 2.3 | 0.4 | 5.4 | 0.7 | 9.4 | 1.8 | 20.9 | 3.1 | 4.7 | 0.7 | 10.5 | 1.2 |
| 30 | 0.7 | 0.1 | 2.3 | 0.3 | 1.5 | 0.4 | 4.3 | 0.8 | 9.0 | 0.7 | 30.7 | 6.4 | 2.4 | 0.8 | 6.0 | 1.2 |

cont’

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| diet Se conc. | Spleen vanadium | | | | Spleen iron | | | | Spleen arsenic | | | | Spleen selenium | | | |
| mg/kg WW | | ng/spleen WW | | mg/kg WW | | ng/spleen WW | | mg/kg WW | | ng/spleen WW | | mg/kg WW | | ng/spleen WW | |
| mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM |
| 0.09 | 1.4 | 0.1 | 1.5 | 0.1 | 963 | 79 | 695 | 50 | 1.2 | 0.1 | 1.4 | 0.1 | 0.7 | 0.1 | 0.9 | 0.1 |
| 0.65 | 2.1 | 0.2 | 1.6 | 0.1 | 971 | 144 | 496 | 83 | 2.0 | 0.2 | 1.6 | 0.1 | 1.5 | 0.1 | 1.4 | 0.1 |
| 30 | 3.9 | 0.4 | 2.2 | 0.1 | 1276 | 323 | 475 | 76 | 3.7 | 0.4 | 2.2 | 0.1 | 8.7 | 0.3 | 6.2 | 0.6 |

**\*** Below limit of quantification due to small sample size; Mn, Co, Cu, Zn, Sr, Ag, Cd, Sn, Ba, Hg, Pb

**Supplementary table 7** – Mating related parameters of zebrafish (154+ dpf) fed different levels of dietary Se (mg/kg DM) from 27 dpf. Data are mean ± SEM, n = various, based on reproductive outcomes (See table).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Age  (dpf) |  | Pairwise crossing no. per treatment | | Mating success† | | | | Egg number-1 successful crossing | | | | Embryo survival 24 hours post fertilisation | | | |
| Unit → | TM\*TF | TM\*NTF | TM\*TF | | TM\*NTF | | TM\*TF | | TM\*NTF | | TM\*TF | | TM\*NTF | |
| diet Se conc | no. | no. | % | normalised % | % | normalised % | mean | SEM | mean | SEM | mean | SEM | mean | SEM |
| 154+ | 0.09 | 13 | 24 | 38.5 | 58.2 | 12.5 | 52.5 | 242 | 64 | 337 | 65 | 52 | 16 | 63 | 4 |
| 154+ | 0.18 | 7 | 25 | 28.6 | 43.2 | 12.0 | 50.4 | 131 | 45 | 238 | 17 | 77 | 4 | 50 | 6 |
| 154+ | 0.30 | 12 | 25 | 33.3 | 50.4 | 12.0 | 50.4 | 335 | 115 | 337 | 103 | 74 | 8 | 56 | 4 |
| 154+ | 0.50 | 8 | 31 | 37.5 | 56.7 | 9.7 | 40.6 | 212 | 13 | 110 | 31 | 86 | 7 | 60 | 4 |
| 154+ | 0.65 | 12 | 21 | 58.3 | 88.2 | 23.8 | 100.0 | 217 | 54 | 116 | 25 | 65 | 7 | 65 | 3 |
| 154+ | 30 | 16 | 32 | 25.0 | 37.8 | 3.1 | 13.1 | 199 | 69 | 379 | - | 44 | 4 | 73 | - |

\* TF, treatment female; TM, treatment male; NTF, non treatment female used to test treatment diet outcomes specifically on male fish.

† Mating success is % of pairwise crossings that resulted in ≥20 fertilised eggs

‡ Some values have no variance as n=1, marked as -

**Supplementary table 8** – Element concentrations (mg/kg DM)\* and locomotor activity in the offspring of parents fed diets with graded levels of Se. Data are mean ± SEM, n=2-3.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Age  (dpf) | Parental diet Se conc. | Manganese | | Iron | | Copper | | Zinc | | Selenium | | Strontium | | Locomotor activity† | |
| mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM | mean | SEM |
| 6 | 0.09 | 1.39 | 0.35 | 115 | 19 | 3.14 | 0.79 | 135 | 32 | 0.34 | 0.04 | 4.75 | 0.06 | 2.3 | 0.1 |
| 6 | 0.18 | 1.44 | 0.23 | 127 | 14 | 4.18 | 0.62 | 147 | 10 | 0.60 | 0.06 | 3.88 | 0.99 | 2.4 | 0.1 |
| 6 | 0.30 | 1.21 | 0.05 | 109 | 2 | 3.94 | 0.77 | 147 | 8 | 0.61 | 0.10 | 4.38 | 0.65 | 2.5 | 0.1 |
| 6 | 0.50 | 1.49 | 0.18 | 121 | 11 | 3.79 | 0.95 | 159 | 19 | 0.97 | 0.37 | 6.21 | 1.46 | 1.8 | 0.2 |
| 6 | 0.65 | 1.35 | 0.11 | 103 | 8 | 3.53 | 0.37 | 136 | 8 | 1.13 | 0.13 | 6.62 | 1.15 | 1.4 | 0.1 |
| 6 | 30 | 1.22 | 0.10 | 100 | 5 | 4.61 | 0.22 | 135 | 3 | 12.46 | 1.63 | 5.75 | 0.13 | 2.1 | 0.1 |

\*Below limit of quantification due to small sample size; V, Co, As, Ag, Cd, Sn, Ba, Hg, Pb

†Locomotor activity is the normalised % of time larvae spent moving during a 24 h period starting at 5 dpf.

Notes on the effect of dietary Se level on organ morphology and spleen micro element concentrations in male zebrafish.

For the final sampling (254 dpf), male fish from groups representing deficient (0.09), replete (0.65), and excessive (30 mg Se/kg) dietary Se levels were fixed and prepared to determine the effects of dietary Se level on organ morphology and spleen element concentrations. Spleen, which is a central organ in Fe metabolism, was analysed for micro element concentration primarily to further investigate links between dietary Se level and Fe metabolism as suggested by ZF whole body Fe content and Cat activity, (Fig. 2B, 4B) which are both Fe dependent outcomes. No differences were found in the actual or somatic index corrected (SIC) weights of livers or hearts between groups (See supplementary table 6). The gonad SIC weight was 1.8 fold higher (p<0.05) in males fed 0.65 versus 30 mg Se/kg diet (8.3 ± 0.7ab, 10.5 ± 1.2a and 6.0 ± 1.2b mg testes/g fish n = 4-5 for 0.3, 0.65 and 30 mg Se/kg diet groups, respectively, Fig. 6B). A trend (p = 0.054) of lower spleen weights, but not spleen SIC weight (p = 0.16) occurred with increasing dietary Se (Spleen weight (dietary Se conc.); 1.24 ± 0.20 (0.09), 1.00 ± 0.11 (0.65), 0.71 ± 0.09 (30) mg/fish). Of the essential elements, only Se and Fe were within the LOQ of the method, due to the low weights of individual spleens. Spleen Se concentrations increased with increasing dietary Se (0.67 ± 0.12 (0.09), 1.53 ± 0.11 (0.65), 8.73 ± 0.25 (30) mg Se/kg WW), while there were no statistical differences between spleen Fe contents (average 1062 ± 113 mg Fe/kg WW). The 30 mg Se/kg group also had statistically higher levels of spleen V and arsenic than other groups. See supplement table six for raw data. Organs other than testes were also investigated to identify if dietary Se level affected their microscopic morphology. Organs were embedded in Technovit 7100 as per directions (Heraeus Kulzer, Wehrheim, Germany). Resin blocks were sectioned into 3 (head kidneys and heart) or 1 (testis and liver) μm thick slices, stained (toluidine blue), and examined for pathologies, specifically cell necrosis, vaculation, and nephrocalcinosis (head kidneys), in sections spaced approximately 50 µM apart. No obvious differences in the gross microscopic morphology of the heart, liver, testis or head kidneys between treatments.

Notes on the effect of dietary Se level on Fe status

Dietary Se had a dose response effect on ZF Fe-dependent Cat activity, that appeared unrelated to the changes observed in Gpx activity or whole body Fe status. In rats, Fe homeostasis can be disrupted by Se deficiency, increasing organ Fe content, particularly in the spleen([1](#_ENREF_1)), and increased spleen size can indicate Fe deficiency([2](#_ENREF_2)). No effect was found on ZF spleen Fe concentration, but a trend of larger spleens (p = 0.054) and statistically higher whole body levels of Fe in Se deficient ZF displayed similarities to Fe deficiency symptoms observed in rodents with suboptimal Se status. The ZF diet in the current study had 127 ± 2 mg Fe/kg which is adequate Fe for fish requirements in general (30-150 mg Fe/kg DM([3](#_ENREF_3))), but as shown for vitamin E deficiency([4](#_ENREF_4)), may be inadequate in combination with Se deficiency.

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