Supporting Information

Microplastic ingestion induces size-specific effects in Japanese quail

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1. **Plastic**

1.1 **Collection of plastic**

Several plastic items were collected from islets in Mausund, Norway (63°52’N, 8°39’E) in March 2020. Fig. S1 shows the environment where the plastic items were collected.

**Fig. S1** Collection of pictures from the field sampling of plastic items in Mausund islets (Trøndelag, Norway) in March 2020.

1.2 **Plastic cleaning and preparation**

The collected items (Fig. S2) were gently washed with ultrapure water and further analyzed for polymer type.

**Fig. S2** Example of plastic items collected, cleaned, and stored in the laboratory for further polymer identification and plastic selection for the experiment.

1.3 **Plastic selection for the experiment**

The polymer type was determined using a confocal Raman spectrometer (alpha300 R, WITec) and spectra were confirmed by comparison to the ST-Japan polymer database (L60002). Three items were selected according to unambiguous polymer identification, high abundance in marine environments, and a large volume available for performing the experiment. Fig. S3 – S5 show the three polymer types selected and their RAMAN spectrum.
Fig. S3 Plastic item 1 selected for the experiment and its RAMAN spectrum (red line) aligned with that of the polypropylene from the database (blue line). This item was identified as polypropylene (PP).

Fig. S4 Plastic item 2 selected for the experiment and its RAMAN spectrum (red line) aligned with that of the polyethylene from the database (blue line). This item was identified as polyethylene (PE).

Fig. S5 Plastic item 3 selected for the experiment and its RAMAN spectrum (red line) aligned with that of the polypropylene from the database (blue line). This item was identified as polypropylene (PP).

1.4 Plastic preparation for the experiment

Two different size classes were prepared: < 125 μm and 3 mm particles. First, the three plastic items were cut into smaller pieces with scissors. Then, half of the pieces were prepared by punching out 3 mm circular disks using a leather hole-puncher (Fig. S6). The other half of the plastic was cryo-milled to obtain particles < 125 μm (Fig. S7). The cryo-milling was performed through ca. 30 cycles of 2 min immersion in liquid nitrogen followed by 2 min of milling (Retsch Mixer Mill 400) at a frequency of 30 Hz until visual inspection indicated the desired size. The resulting powder was sieved using a vibratory sieve shaker (Retsch AS 200 basic) with a 125 μm sieve, and all MP < 125 μm were collected.
To evaluate the size distribution of the powder (< 125 µm) we used a Coulter counter (Beckam Coulter Miltisizer 4e). Item 1 PP had a mean distribution of 37.78 µm ± 19.0 µm (Fig. S8), Item 2 PE of 33.92 µm ± 11.1 µm (Fig. S9), and Item 3 PP of 43.86 µm ± 21.4 µm (Fig. S10). The overall mean was 38.52 µm ranging from 18.78 to 65.36 µm.

**Fig. S6** Plastic particles prepared in 3 mm size. From left to right; item 1 PP, item 2 PE, item 3 PP.

**Fig. S7** Plastic powder prepared < 125 µm size. From left to right; item 1 PP, item 2 PE, item 3 PP.

**Fig. S8.** Size distribution of MP item 1 PP.
**Fig. S9** Size distribution of MP item 2 PE.

**Fig. S10** Size distribution of MP item 3 PP.
2. Quail husbandry

In October 2020, 160 quail eggs were purchased from a local farm (Trøndelag, Norway) and kept in incubators (type 180, America A/S, 94 Thisted, Denmark and J. Hemel, Verl, Germany) for 16 days at 38 °C and 50–60 % humidity with automatic egg-turning (90° per hour). The last 2 days before hatching (day 16–18), the egg rotation was stopped, and humidity was increased to 70–75 %. Fifty-six chicks hatched successfully and, once dried (24h post-hatch), they were moved to cages lined with paper and equipped with a cardboard box shelter to increase animal welfare. Eight custom-built wooden cages (1.1 m x 0.9 m) with wire-mesh lids at a height of 30 cm were used. An infrared heat lamp set at 37 °C created a gradient of heat in each cage. The temperature of the heating lamp was decreased by 2–3 °C each week until quail reached 4 weeks of age when the lamps were removed. All cages were maintained in the same room at 22–25 °C with a light-dark cycle of 14–10 h. At week 2, cages were lined with wood chip bedding to encourage natural behaviors such as foraging and dust bathing. Quail were marked with flexible leg bands shortly after hatching for individual identification, which were replaced with larger sizes as the quail grew.

3. Experimental set up

![Experimental set up diagram](image)

Fig. S11 Experimental set up. Colors show the different treatments (in yellow control, in purple treatment 1 ingesting 3 mm MP, in red treatment 2 ingesting < 125 μm MP, in blue treatment 3 ingesting both 3 mm and < 125 μm MP). Wooden cages were custom-made with a dimension of 1.1 m x 0.9 m and a wire-mesh lid at a height of 30 cm following recommendations for animal welfare.)
4. Plastic exposure and recovery

To administer the plastic powder to the quail, the desired weight of the powder was placed in a ceramic dish where finely ground poultry food and water were added (amount determined visually) in order to form soft pellets of the desired consistency. Finally, to facilitate administration to the quail, the pellets were divided into smaller pellets (Fig. S12).

*Fig. S12* The different polymer types in a powder form (left). After grinded food and water were added to create the desired texture of the pellet (middle). The final small pellets created to facilitate oral administration to the quail (right).

Both 3 mm particles and powder pellets were administered orally to the quail. The 3 mm particles were carefully hand-fed using blunt forceps (Fig. S13), while the powder mixed with food and water was hand-fed using small spoons. To avoid bias for the oral administration, control quail were also captured and hand-fed food pellets following the same procedure.

*Fig. S13* Oral administration of a 3 mm particle to a male quail using tweezers
In total, T1 quail received 72 particles in total per individual (24 of each plastic item), those in T2 received 600 mg of small MP (200 mg of each plastic item), and those in T3 received a mixture of 36 particles (12 of each plastic item) together with 300 mg small MP (100 mg of each plastic item). The dose of MP per g of body mass is shown in Table S1.

**Table S1.** Dose of MP/g body mass (BM) for each period comprising four feeding events. The final MP/g BM is calculated based on the final BM and total mg MP exposure.

|                  | Period 1 | Period 2 | Period 3 | Final |
|------------------|----------|----------|----------|-------|
| MP feeding events| 4        | 4        | 4        | 12    |
| mg MP given total (feeding events) | 100 | 200 | 300 | 600 |
| Average body mass | 62/85 | 156 | 222 | 222 |
| Average mg MP/g BM | 1.61/1.17 | 1.28 | 1.35 | 2.70 |
| mg MP/g BM T1    | 1.67/1.21 | 1.32 | 1.39 | 2.76 |
| mg MP/g BM T2    | 1.66/1.19 | 1.25 | 1.30 | 2.61 |
| mg MP/g BM T3    | 1.53/1.12 | 1.23 | 1.32 | 2.64 |

After euthanasia, the stomach tract of the birds was opened to count the remaining 3 mm particles (Fig. S14).

![Gastrointestinal tract (gizzard and proventriculus) with remaining food and 3 mm particles. The crop cannot be observed in this picture, but it was always opened and evaluated. No particles were found in the crop in any individual.](image)

**5. Blood processing**

Blood collected in heparinized Eppendorf tubes was stored on ice until it could be centrifuged (max. 5 min) to separate the plasma and red blood cells (RBC) fractions (4,000 g for 5 min at 4 °C). Plasma was then collected and transferred into a new tube and stored at -80 °C, while RBC samples were washed with saline solution (NaCl 9 mg/mL) and centrifuged again (4,000 g for 5 min at 4 °C) before storing the supernatant at -80 °C.
6. Laboratory analyses

6.1 Oxidative stress parameters

The activities of CAT, GST and GPx were determined as described in Mennillo et al.². Briefly, CAT activity was determined based on the peroxidation of methanol in the presence of hydrogen peroxide (H₂O₂). The formaldehyde produced was quantified using a colorimetric assay with 4-amino-3-hydrazino-5-mercaptopo-1,2,4-triazole (Purpald) as the chromogen, and the absorbance was read at 540 nm. GST was determined using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate and absorbance was read at 340 nm. An extinction coefficient of 9.6 mM⁻¹ cm⁻¹ was used to calculate GST activity. The activity of GPx was determined by calculating the rate of NADPH oxidation coupled with glutathione reductase and tert-butyl hydroperoxide (12 mM) and the absorbance was read at 340 nm. The specific activity was determined using an extinction coefficient of 6.22 mM⁻¹ cm⁻¹. The activities of CAT, GST and GPx were then divided by the total protein concentration (mg mL⁻¹) determined by the Bradford assay. We used bovine serum albumin (BSA) to prepare the calibration curve and, after adding dye solution (Coomasie blue) to protein samples, the absorption was measured at 595 nm. SOD was quantified using a °Cayman SOD kit (Ann Arbor, MI, USA) following the instructions of the assay. The plate was read at 450 nm of absorbance. The activity of SOD is given in U L⁻¹. The TBARS assay used the measurement of malondialdehyde (MDA) to construct the standard curve and the absorption was read at 530 nm. All enzymes and protein determinations were done using a Synergy HT microplate reader from Bio-Tek Instruments Inc. (Winncski, Vermont, USA) for absorbance and fluorescence readings.

All samples were assayed in duplicate or triplicate. Therefore, we used two 96-well plates for each assay. The intra and inter-assay coefficient of variation (CV, mean % ± SE) are determined based on the final concentration for each replicate. The inter-assay CV is calculated based on 2 – 4 samples.

**Table S2.** Mean % ± SE intra- and inter-assay coefficient of variation (CV).

| Assay  | Intra-assay CV (%) | Inter-assay CV (%) |
|--------|--------------------|--------------------|
| Bradford | 9.98 ± 1.14        | 5.30 ± 2.58        |
| CAT    | 6.77 ± 0.81        | 8.52 ± 2.12        |
| GPx    | 10.44 ± 1.43       | 12.78 ± 2.78       |
| GST    | 9.53 ± 1.33        | 6.74 ± 2.54        |
| SOD    | 6.54 ± 1.53        | 12.16 ± 2.32       |
| TBARS  | 2.67 ± 0.75        | 25.74 ± 0.73*      |

*In the case of TBARS, a correction to homogenize values from both plates was carried out due to a great difference in the standard curves. Based on three samples that were measured in both plates, a correction of ±1.5 was applied for all samples from plate 2. After correction, the inter-assay CV was < 5%.*
6.2 Cytokines

All samples were assayed in duplicate in TNF-α and 20 samples in IL-1β. We performed the analyses in two 96-well plates for TNF-α and one plate for IL-1β. The intra and inter-assay CV (mean % ± SE) are determined based on the final concentration for each replicate. The inter-assay CV for TNF-α is calculated based on 4 samples.

Table S3. Mean % ± SE intra- and inter-assay coefficient of variation (CV).

| Assay     | Intra-assay CV (%) | Inter-assay CV (%) |
|-----------|--------------------|--------------------|
| TNF-α     | 7.13 ± 1.36        | 9.72 ± 3.09        |
| IL-1β     | 7.37 ± 1.37        | NA                 |

6.3 Reproductive hormones

After high-speed centrifugation to remove the lipid phase, plasma samples were extracted twice with diethyl-ether using 4 times the sample volume (1200 μL), frozen in liquid nitrogen, and the supernatant was subsequently collected. After evaporation of the organic phase overnight, samples were reconstituted with 300 μL of assay buffer and frozen at -80 °C for analyses.

All samples were assayed in duplicate. The sample size was 23 for testosterone and 32 for 17β-estradiol. We performed one assay for each reproductive hormone. The intra-assay CV (mean % ± SE) are determined based on the final concentration for each replicate.

Table S4. Mean % ± SE intra- and inter-assay coefficient of variation (CV).

| Assay            | Intra-assay CV (%) |
|------------------|--------------------|
| 17β-Estradiol (E₂) | 10.9 ± 2.92        |
| Testosterone (T)  | 8.5 ± 1.81         |
### 6.4 Plastic particles retention in the stomach

#### Table S5. Plastic particles recovered from stomach of quail T1 and T3.

| Treatment group | Quail | Total plastic particles | % Plastic found/ingested | Types of polymers |
|-----------------|-------|--------------------------|--------------------------|-------------------|
|                 |       |                          |                          | Grey   | White PP | White PE |
| T1              | 1     | 35                       | 49                       | 10     | 12       | 13       |
|                 | 2     | 38                       | 53                       | 15     | 12       | 11       |
|                 | 3     | 28                       | 39                       | 13     | 7        | 8        |
|                 | 4     | 18                       | 25                       | 5      | 7        | 6        |
|                 | 5     | 7                        | 10                       | 2      | 1        | 4        |
|                 | 6     | 46                       | 64                       | 19     | 15       | 12       |
|                 | 7     | 49                       | 68                       | 17     | 19       | 13       |
|                 | 8     | 19                       | 26                       | 5      | 7        | 7        |
|                 | 9     | 45                       | 63                       | 15     | 13       | 17       |
|                 | 10    | 44                       | 61                       | 12     | 15       | 17       |
|                 | 11    | 28                       | 39                       | 9      | 8        | 11       |
|                 | 12    | 32                       | 44                       | 7      | 14       | 11       |
|                 | 13    | 47                       | 65                       | 16     | 15       | 16       |
| **Mean ± SD**   |       | **33.5 ± 13.1**          | **46.6 ± 18.2**          |        |          |          |
| **Median**      |       | **35**                   | **48.6**                 |        |          |          |
| T3              | 1     | 26                       | 72                       | 8      | 8        | 10       |
|                 | 2     | 29                       | 81                       | 8      | 11       | 10       |
|                 | 3     | 36                       | 100                      | 13     | 10       | 15       |
|                 | 4     | 22                       | 61                       | 9      | 6        | 7        |
|                 | 5     | 32                       | 89                       | 10     | 10       | 12       |
|                 | 6     | 16                       | 44                       | 8      | 4        | 4        |
|                 | 7     | 29                       | 81                       | 11     | 8        | 9        |
|                 | 8     | 26                       | 72                       | 9      | 8        | 7        |
|                 | 9     | 0                        | 0                        | 0      | 0        | 0        |
|                 | 10    | 18                       | 50                       | 5      | 4        | 9        |
|                 | 11    | 21                       | 58                       | 8      | 7        | 6        |
|                 | 12    | 9                        | 25                       | 5      | 1        | 3        |
|                 | 13    | 27                       | 75                       | 10     | 8        | 9        |
|                 | 14    | 35                       | 97                       | 12     | 11       | 12       |
| **Mean ± SD**   |       | **23.3 ± 10.0**          | **64.7 ± 27.8**          |        |          |          |
| **Median**      |       | **26**                   | **72.2**                 |        |          |          |
7. Descriptive results for all investigated endpoints

Table S6. BCCP levels, oxidative stress parameters, cytokines, reproductive hormones in plasma of Japanese quail (Coturnix japonica). Sample size (n), mean ± SE, median and [range] are shown. Treatment (T) groups were as following: T1 (3 mm), T2 (< 125 μm), T3 (mix 3 mm and < 125 μm).

| BCCPs1 | n | Glu (mmol L⁻¹) | Amy (U L⁻¹) | TG (mmol L⁻¹) | AST (U L⁻¹) | Chol (mmol L⁻¹) | CK (μmol L⁻¹) | UA (μmol L⁻¹) |
|--------|---|----------------|-------------|--------------|-------------|----------------|--------------|-------------|
| All    | 56 | 19.2 ± 0.3     | 518 ± 53.7  | 3.9 ± 0.5    | 356 ± 11.9  | 4.4 ± 0.2      | 3526 ± 190  | 634 ± 26.2  |
| 18.9    | [14.8 – 24.4] | 411 [32.5 – 1320] | 2.6 [0.8 – 13.7] | 351 [190 – 571] | 4.2 [2.6 – 8.2] | 3255 [267 – 6650] | 601 [165 – 1120] |
| Control | 14 | 19.4 ± 0.7     | 588 ± 101   | 4.81 ± 1.19  | 307 ± 15   | 4.5 ± 0.4      | 3735 ± 413  | 605 ± 59.7  |
| 19.5    | [15.2 – 24.4] | 516 [87.9 – 1320] | 3 [0.8 – 13.7] | 320 [217 – 375] | 4.3 [2.6 – 8.2] | 3135 [1760 – 6650] | 543 [333 – 1120] |
| T1     | 13 | 19.7 ± 0.7     | 562 ± 157   | 4 ± 1        | 385 ± 30.1 | 4.3 ± 0.3      | 3349 ± 300  | 720 ± 51.6  |
| 19.7    | [17.3 – 22.8] | 420 [196 – 1240] | 2.3 [0.8 – 13.3] | 426 [200 – 517] | 4.1 [3.3 – 6.4] | 3245 [1885 – 5850] | 661 [479 – 1010] |
| T2     | 14 | 18.6 ± 0.6     | 502 ± 70.9  | 4.5 ± 0.9    | 352 ± 21.5 | 5.2 ± 0.3      | 3634 ± 522  | 625 ± 62.4  |
| 18.6    | [15.7 – 24.1] | 338 [52.5 – 954] | 3.5 [0.8 – 11.3] | 337 [234 – 489] | 5.4 [3.4 – 6.7] | 4147 [267 – 6300] | 610 [165 – 1060] |
| T3     | 14 | 19.2 ± 0.6     | 563 ± 144   | 2.2 ± 0.2    | 382 ± 24.5 | 3.8 ± 0.3      | 3372 ± 265  | 594 ± 31    |
| 19.2    | [14.8 – 23.3] | 396 [32.5 – 1250] | 2.3 [0.8 – 3.4] | 369 [190 – 571] | 3.3 [2.6 – 5.4] | 3270 [1240 – 4590] | 591 [336 – 795] |

| Oxidative stress2 | n | CAT (nmol min⁻¹ mg⁻¹) | SOD (U L⁻¹) | GST (nmol min⁻¹ mg⁻¹) | GPx (nmol min⁻¹ mg⁻¹) | TBARS (μM MDA) | Cytokines³ | TNF-α (pg mL⁻¹) | IL-1 β (pg mL⁻¹) |
|-------------------|---|-----------------------|-------------|-----------------------|-----------------------|---------------|------------|----------------|----------------|
| All               | 56 | 4.2 ± 0.1             | 15.9 ± 0.5  | 0.8 ± 0.03            | 1.2 ± 0.08            | 8.1 ± 0.5     | 69.4 ± 5.06 | 62.8 [12.5 – 158] | 29.3 [18.6 – 54] |
| Control           | 14 | 3.8 ± 0.2             | 14.4 ± 0.4  | 0.6 ± 0.05            | 0.8 ± 0.1             | 8.02 ± 0.9   | 78.9 ± 12.1 | 67.2 [18.6 – 152] | 30.5 [20.7 – 39] |
| T1                | 13 | 4.4 ± 0.3             | 16 ± 1.3    | 0.7 ± 0.05            | 1.3 ± 0.2             | 7.9 ± 0.9    | 70.9 ± 11.2 | 67.3 [12.5 – 157] | 30.6 [19.5 – 48.2] |
| T2                | 14 | 4.9 ± 0.3             | 16.9 ± 1    | 0.9 ± 0.07            | 1.3 ± 0.1             | 7.9 ± 0.8    | 63.3 ± 16.9 | 50.8 [13.4 – 148] | 25.7 [18.9 – 54] |
| T3                | 14 | 4.6 ± 0.2             | 16.1 ± 0.9  | 0.8 ± 0.07            | 1.4 ± 0.2             | 8.6 ± 1.3    | 64.7 ± 6.5  | 65.2 [31.4 – 122] | 29.2 [18.6 – 46.9] |

| Reproductive hormones⁴ | Males n | T (pg mL⁻¹) | Females n | E₂ (pg mL⁻¹) | Nutritional status⁵ | n | HSI | Ht (%) |
|------------------------|---------|-------------|-----------|-------------|---------------------|---|-----|--------|
| All                    | 23      | 1660 ± 218  | 32        | 365 ± 61.4   | 56                  | 2.9 ± 0.1 | 43.1 ± 0.6 |
| Control                | 9       | 1494 [166 – 4059] | 9       | 260 [41.4 – 1495] | 14                  | 2.8 [1.6 – 5.9] | 43 [31 – 53] |
| T1                     | 7       | 1748 ± 410  | 9         | 564 ± 132    | 12                  | 2.5 ± 0.1 | 41.7 ± 0.9 |
| T2                     | 5       | 1862 [407 – 2881] | 6       | 514 [50.6 – 1316] | 14                  | 2.5 [1.9 – 4.2] | 42.5 [36 – 48] |
| T3                     | 4       | 1641 ± 241  | 3         | 186 ± 25.1   | 12                  | 3.1 ± 0.3 | 45 ± 1.2 |
| 1494 [178 – 3150]      | 6       | 182 [118 – 260] | 9       | 449 ± 140    | 14                  | 3 [1.9 – 5.9] | 44 [40.4 – 53] |
| 1112 [501 – 4059]      | 8       | 352 [55.4 – 1495] | 8       | 181 ± 56.6   | 14                  | 2.9 [1.6 – 5] | 42 [31 – 52] |
| 1629 [166 – 2241]      | 9       | 161 [41.4 – 526] | 8       | 181 ± 56.6   | 14                  | 2.8 ± 0.3 | 43.6 ± 1 |

¹BCCPs: Glu (glucose); Amy (amylase); TG (triglycerides); AST (aspartate aminotransferase); Chol (cholesterol); CK (creatinine kinase); UA (uric acid); ²Oxidative stress parameters: CAT (catalase); SOD (superoxide dismutase); GST (glutathione-S-transferase); GPx (glutathione peroxidase); TBARS (thiobarbituric acid-reactive substances); ³Cytokines: TNF-α (tumor necrosis factor-alpha); IL-1 β (interleukin-1β) ⁴Reproductive hormones: T (testosterone); E₂ (17-β estradiol); ⁵Nutritional status parameters: Hepatosomatic index (HSI); haematocrit (Ht)

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8. Statistical Output

The following tables show AICc values for models explaining variation in the studied parameters. The best-supported models (ΔAICc<2), those in bold, were selected for further analysis. Dependent variables included treatment (control, treatment 1, treatment 2, treatment 3), sex, body mass, and cage (C1- C8) distribution. The sample size was 55 quail. As sex was an influential factor in some parameters (HSI, Ht, TG, CK, AST, Chol, Glu, TNF-α), the same models were run again with the sexes separated. Testosterone (T) was only performed for males (n = 23) while 17-β estradiol (E2) only for females (n = 32).

Table S7. AICc values for models explaining body mass (BM; mg) variation.

| Model                                      | df | AICc   | Delta | weight |
|--------------------------------------------|----|--------|-------|--------|
| BM ~ treatment * week + sex + cage + (1|ID) | 31 | 2501.5 | 0.00   | 0.73   |
| BM ~ treatment * week + cage + (1|ID) | 30 | 2503.5 | 1.97  | 0.73   |
| BM ~ treatment * week + (1|ID) | 26 | 2550.8 | 49.28 | <0.01  |
| BM ~ treatment + week + (1|ID) | 11 | 2634.6 | 133.1 | <0.01  |
| BM ~ week + (1|ID) | 8  | 2646.3 | 144.8 | <0.01  |
| BM ~ 1 + (1|ID) | 3  | 3745.6 | 1244.1 |<0.01   |

When evaluating differences in body mass, the best model included the interaction between treatment and week, sex, and cage (Table S7). Sex was found not-significant (Table S8), while cage was an influential factor, specially cage 2 (Table S8). To confirm that the interaction between week and treatment was not influenced by cage 2, we rerun the model excluding it and the results obtained were the same (Table S9).

Table S8. Output for models explaining body mass (BM; mg) variation

|            | Estimate | Std. Error | df | t-value | p-value |
|------------|----------|------------|----|---------|---------|
| Intercept  | 60.6     | 5.9        | 62.7 | 10.3    | <0.001  |
| T1         | 28.1     | 8.4        | 63.5 | 3.4     | <0.01   |
| T2         | 0.84     | 8.01       | 64.5 | 0.1     | 0.92    |
| T3         | 2.88     | 8.03       | 64.4 | 0.4     | 0.73    |
| Week 3     | 44.7     | 3.57       | 204 | 12.5    | <0.001  |
| Week 4     | 88.2     | 3.57       | 204 | 24.7    | <0.001  |
| Week 5     | 124.8    | 3.57       | 204 | 34.9    | <0.001  |
| Week 6     | 151.9    | 3.57       | 204 | 42.5    | <0.001  |
| Sex M      | -1.51    | 3.89       | 46  | -0.39   | 0.70    |
| Cage 2     | -51.7    | 7.70       | 46  | -6.71   | <0.001  |
| Cage 3     | -1.01    | 7.37       | 46  | -0.14   | 0.89    |
| Cage 4     | 5.25     | 7.43       | 46  | 0.71    | 0.48    |
| Cage 5     | -0.71    | 7.37       | 46  | -0.09   | 0.92    |
| T1 : week 3| 0.24     | 5.15       | 204 | 0.05    | 0.96    |
| T2 : week 3| 2.04     | 5.05       | 204 | 0.40    | 0.69    |
| T3 : week 3| 3.61     | 5.05       | 204 | 0.72    | 0.48    |
| T1 : week 4| 2.89     | 5.15       | 204 | 0.56    | 0.58    |
Since we found a significant interaction between week and treatment, resulting in different slopes in the growth of the quail, we also investigated potential statistical differences in the body mass of quail each week per separate (Table S10).

**Table S9.** Output for models explaining body mass (BM; mg) variation excluding cage 2

| Variable | Estimate | Std. Error | df  | t-value | p-value |
|----------|----------|------------|-----|---------|---------|
| Intercept| 60.3     | 6.23       | 51.6| 9.69    | <0.001  |
| T1       | 16.3     | 9.09       | 60.2| 1.79    | 0.08    |
| T2       | 0.84     | 8.38       | 53.1| 0.10    | 0.92    |
| T3       | 2.99     | 8.40       | 52.9| 0.36    | 0.72    |
| Week 3   | 44.7     | 3.43       | 176| 13.0    | <0.001  |
| Week 4   | 88.3     | 3.43       | 176| 25.8    | <0.001  |
| Week 5   | 124.8    | 3.43       | 176| 36.4    | <0.001  |
| Week 6   | 151.9    | 3.43       | 176| 44.3    | <0.001  |
| Sex M    | -0.78    | 4.45       | 40 | -0.17   | 0.86    |
| Cage 3   | -0.91    | 7.82       | 40 | -0.12   | 0.91    |
| Cage 4   | 5.04     | 7.89       | 40 | 0.64    | 0.53    |
| Cage 5   | -0.60    | 7.82       | 40 | -0.08   | 0.94    |
| T1 : week 3 | 8.26 | 6.26       | 176| 1.32    | 0.19    |
| T2 : week 3 | 2.04 | 4.85       | 176| 0.42    | 0.68    |
| T3 : week 3 | 3.61 | 4.85       | 176| 0.75    | 0.46    |
| T1 : week 4 | 16.5  | 6.26       | 176| 2.63    | <0.01   |
| T2 : week 4 | 11.9  | 4.85       | 176| 2.47    | 0.01    |
| T3 : week 4 | 8.56  | 4.85       | 176| 1.77    | 0.07    |
| T1 : week 5 | 19.9  | 6.26       | 176| 3.19    | <0.01   |
| T2 : week 5 | 12.6  | 4.85       | 176| 2.60    | 0.01    |
| T3 : week 5 | -10.6 | 4.84       | 176| -2.18   | 0.03    |
| T1 : week 6 | 22.9  | 6.25       | 176| 3.67    | <0.001  |
| T2 : week 6 | 17.9  | 4.85       | 176| 3.71    | <0.001  |
| T3 : week 6 | 10.1  | 4.85       | 176| 2.08    | 0.03    |

Since we found a significant interaction between week and treatment, resulting in different slopes in the growth of the quail, we also investigated potential statistical differences in the body mass of quail each week per separate (Table S10).

**Table S10.** Output for models explaining body mass (BM, mg) variation. Models were selected based on AICc contrast.

| Selected model      | ANOVA output                                      | Dunnett test |
|---------------------|--------------------------------------------------|--------------|
| w1                  | BM ~ treatment + cage                            |              |
|                     | Treatment: $F_3 = 1.19; P = 0.32$                 |              |
|                     | Cage: $F_{17} = 7.59; P < 0.01$                   | C2 – C1 P = 0.02 |
| Page | BM ~ treatment + cage | Treatment: $F_3 = 1.59; P = 0.20$ | Cage: $F_4 = 11.10; P < 0.01$ |
|------|-----------------------|---------------------------------|-------------------------------|
| w2   | BM ~ treatment + cage | Treatment: $F_3 = 1.69; P = 0.18$ | Cage: $F_4 = 10.88; P < 0.01$ |
| w3   | BM ~ treatment + cage | Treatment: $F_3 = 2.63; P = 0.06$ |                                |
| w4   | BM ~ treatment + cage | Treatment: $F_3 = 2.88; P = 0.05$ |                                |
| w5   | BM ~ treatment + cage | Treatment: $F_3 = 2.94; P = 0.04$ |                                |
| w6   | BM ~ treatment + cage + sex | Treatment: $F_3 = 1.31; P = 0.26$ |                                |

**BM ~ treatment + cage**

Treatment: $F_3 = 1.69; P = 0.18$

Cage: $F_4 = 10.88; P < 0.01$

| C2 – C1 | P = 0.03 |
| C3 – C1 | P > 0.05 |
| C4 – C1 | P > 0.05 |
| C5 – C1 | P > 0.05 |
| C6 – C1 | P > 0.05 |
| C7 – C1 | P > 0.05 |
| C8 – C1 | P < 0.01 |

**BM ~ treatment + cage**

Treatment: $F_3 = 2.63; P = 0.06$

Cage: $F_4 = 9.67; P < 0.01$

| C2 – C1 | P = 0.06 |
| C3 – C1 | P > 0.05 |
| C4 – C1 | P > 0.05 |
| C5 – C1 | P > 0.05 |
| C6 – C1 | P > 0.05 |
| C7 – C1 | P > 0.05 |
| C8 – C1 | P < 0.01 |

**BM ~ treatment + cage**

Treatment: $F_3 = 2.88; P = 0.05$

Cage: $F_4 = 15.28; P < 0.01$

| C2 – C1 | P = 0.01 |
| C3 – C1 | P > 0.05 |
| C4 – C1 | P > 0.05 |
| C5 – C1 | P > 0.05 |
| C6 – C1 | P > 0.05 |
| C7 – C1 | P > 0.05 |
| C8 – C1 | P < 0.01 |

**BM ~ treatment + cage + sex**

Treatment: $F_3 = 2.94; P = 0.04$

Sex: $F_3 = 1.31; P = 0.26$

Cage: $F_4 = 9.66; P < 0.01$

| C2 – C1 | P > 0.05 |
| C3 – C1 | P > 0.05 |
| C4 – C1 | P > 0.05 |
| C5 – C1 | P > 0.05 |
| C6 – C1 | P > 0.05 |
| C7 – C1 | P > 0.05 |
| C8 – C1 | P < 0.01 |
Table S11. AICc values for models explaining variation in HSI (Hepatosomatic Index).

| Model                      | df | AICc  | Delta | weight |
|----------------------------|----|-------|-------|--------|
| HSI all                    |    |       |       |        |
| HSI ~ treatment + sex      | 6  | -363.8| 0.00  | 0.941  |
| HSI ~ treatment * sex      | 9  | -358.2| 5.61  | 0.057  |
| HSI ~ treatment * sex + cage| 13 | -351.3| 12.50 | <0.01  |
| HSI ~ 1                    | 2  | -340.8| 22.99 | <0.01  |
| HSI ~ treatment            | 5  | -336.4| 27.42 | <0.01  |
| HSI males                  |    |       |       |        |
| HSI ~ 1                    | 2  | -166.9| 0.00  | 0.637  |
| HSI ~ treatment            | 5  | -165.8| 1.13  | 0.363  |
| HSI ~ treatment + cage     | 9  | -148.2| 18.68 | <0.01  |
| HSI females                |    |       |       |        |
| HSI ~ 1                    | 2  | -201.6| 0.00  | 0.857  |
| HSI ~ treatment            | 5  | -197.7| 3.97  | 0.118  |
| HSI ~ treatment + cage     | 9  | -194.5| 7.09  | 0.025  |

Table S12. Output for models explaining HSI variation.

| Selected model | ANOVA output                      |
|----------------|-----------------------------------|
| HSI all        | Sex: $F_{1,50} = 34.16; P < 0.001$|
| HSI male       | Treatment: $F_3 = 1.37; P = 0.26$|

Table S13. AICc values for models explaining variation in Ht (hematocrit %).

| Model                      | df | AICc  | Delta | weight |
|----------------------------|----|-------|-------|--------|
| Ht all                     |    |       |       |        |
| Ht ~ sex                   | 3  | 301.4 | 0.00  | 0.574  |
| Ht ~ sex + treatment + weight| 7  | 303.1 | 1.74  | 0.240  |
| Ht ~ sex + treatment       | 6  | 303.7 | 2.32  | 0.180  |
| Ht ~ sex + treatment + weight + cage | 11 | 310.7 | 9.32  | <0.01  |
| Ht ~ sex * treatment + weight + cage | 14 | 318.2 | 16.84 | <0.01  |
| Ht ~ 1                     | 2  | 327.5 | 26.08 | <0.01  |
| Ht males                   |    |       |       |        |
| Ht ~ 1                     | 2  | 130.8 | 0.00  | 0.934  |
| Ht ~ treatment             | 5  | 136.9 | 6.12  | 0.044  |
| Ht ~ treatment + weight    | 6  | 138.3 | 7.50  | 0.02   |
| Ht ~ treatment + weight + cage | 10 | 157.3 | 26.47 | <0.01  |
| Ht females                 |    |       |       |        |
| Ht ~ 1                     | 2  | 172.8 | 0.00  | 0.769  |
| Ht ~ treatment             | 5  | 175.8 | 3.01  | 0.170  |
| Ht ~ treatment + weight    | 6  | 177.9 | 5.14  | 0.06   |
| Ht ~ treatment + weight + cage | 10 | 184.9 | 12.11 | <0.01  |

Table S14. Output for models explaining Ht variation.

| Selected model | ANOVA output                      |
|----------------|-----------------------------------|
| Ht all         | Sex: $F_1 = 32.35, P < 0.01$      |
|                | Body weight: $F_1 = 2.94, P = 0.09$|
|                | Treatment: $F_3 = 3.07, P = 0.03$  |
We found that T1 quail tended to show higher Ht than control quail (post-hoc $t = 2.12, P = 0.09$), but this could instead be due to arbitrary slight dehydration than an effect of MP. When analyzed separately by sex, no further differences were found (Table S13).

Table S15. AICc values for models explaining variation in BCCPs. Note: BM = body mass, TG = triglycerides, CK = creatine kinase, AST = aspartate aminotransferase, UA = uric acid, Chol = cholesterol, Amy = amylase, Glu = glucose. Residuals for TG, CK, AST and UA were normally distributed, while residuals for Chol, Amy and Glu were not normally distributed.

| Model                               | df | AICc | Delta | weight |
|-------------------------------------|----|------|-------|--------|
| **TG**                              |    |      |       |        |
| TG ~ treatment + sex + BM           | 7  | 281.6| 0.00  | 0.859  |
| TG ~ treatment + sex                | 6  | 285.5| 3.84  | 0.126  |
| TG ~ treatment + sex + BM + cage    | 11 | 289.9| 8.27  | <0.01  |
| TG ~ 1                              | 2  | 194.8| 13.14 | <0.01  |
| TG ~ treatment                       | 5  | 296.8| 15.13 | <0.01  |
| TG males                            |    |      |       |        |
| TG ~ BM                             | 3  | 58.0 | 0.00  | 0.53   |
| TG ~ 1                              | 2  | 58.3 | 0.28  | 0.46   |
| TG ~ treatment + BM                 | 6  | 65.8 | 7.82  | 0.01   |
| TG ~ treatment + BM + cage          | 10 | 81.3 | 23.26 | <0.01  |
| TG females                          |    |      |       |        |
| TG ~ BM                             | 3  | 173.7| 0.00  | 0.86   |
| TG ~ BM + treatment                 | 6  | 177.5| 3.86  | 0.13   |
| TG ~ 1                              | 2  | 182.3| 8.59  | 0.01   |
| TG ~ BM + treatment + cage          | 10 | 188.5| 14.77 | <0.01  |
| **CK**                              |    |      |       |        |
| CK ~ sex                            | 3  | 952.5| 0.00  | 0.868  |
| CK ~ 1                              | 2  | 957.8| 5.34  | 0.06   |
| CK ~ treatment + sex                | 6  | 958.0| 5.49  | 0.06   |
| CK ~ treatment + sex + BM           | 7  | 960.5| 8.00  | 0.02   |
| CK ~ treatment + sex + BM + cage    | 11 | 968.0| 15.55 | <0.01  |
| CK males                            |    |      |       |        |
| CK ~ treatment                       | 5  | 395.6| 0.00  | 0.66   |
| CK ~ 1                              | 2  | 397.9| 2.29  | 0.21   |
| CK ~ treatment + BM                 | 6  | 398.9| 3.35  | 0.12   |
| CK ~ treatment + BM + cage          | 10 | 415.1| 19.54 | <0.01  |
| CK females                          |    |      |       |        |
| CK ~ 1                              | 2  | 557.0| 0.00  | 0.95   |
| CK ~ treatment                       | 5  | 563.8| 6.85  | 0.03   |
| CK ~ treatment + BM                 | 6  | 565.2| 8.21  | 0.01   |
| CK ~ treatment + BM + cage          | 10 | 576.9| 19.92 | <0.01  |
| **AST**                             |    |      |       |        |
| AST ~ treatment + sex               | 6  | 607.1| 0.00  | 0.577  |
| AST ~ treatment + sex + BM          | 7  | 697.9| 0.79  | 0.389  |
| AST ~ treatment                     | 5  | 614.6| 7.45  | 0.01   |
| AST ~ 1                             | 2  | 614.8| 7.66  | 0.01   |
| AST ~ treatment + sex + BM + cage   | 10 | 615.9| 8.79  | <0.01  |
| AST males                           |    |      |       |        |
| AST ~ 1                             | 2  | 233.8| 0.00  | 0.57   |
| AST ~ treatment                     | 5  | 234.6| 0.85  | 0.38   |
| AST ~ treatment + BM                | 6  | 238.6| 4.81  | 0.05   |
| AST ~ treatment + BM + cage         | 10 | 256.1| 22.37 | <0.01  |
| AST females                         |    |      |       |        |
| AST ~ 1                             | 2  | 375.3| 0.00  | 0.58   |
| AST ~ treatment                     | 5  | 376.9| 1.66  | 0.26   |
| AST ~ treatment + BM                | 6  | 377.9| 2.69  | 0.15   |
Similar to AST (detailed in the main Manuscript), CK levels were also higher in males than females ($F_1 = 7.83, P < 0.01; t = 2.79, P < 0.01; \text{Fig S18}$), and when analyzed separately by sex, only males showed a difference between treatment groups ($F_3 = 3.84, P = 0.03$) with T2 and T3 males showing lower CK levels than control males (both $P < 0.05$). Because the pattern of CK was not the same as AST, we could rule out a possible muscular damage.

|            | $\text{AST} \sim \text{treatment} + \text{BM} + \text{cage}$ | $t$ | $P$   |
|------------|---------------------------------------------------------------|-----|-------|
| UA         | $\text{UA} \sim 1$                                           | 2   | 740   |
|            |                                                               |     | 0.00  |
|            | $\text{UA} \sim \text{treatment}$                            | 5   | 743.4 |
|            |                                                               |     | 3.40  |
|            |                                                               |     | 0.140 |
|            | $\text{UA} \sim \text{treatment} + \text{sex}$              | 6   | 744.7 |
|            |                                                               |     | 4.69  |
|            |                                                               |     | 0.07  |
|            | $\text{UA} \sim \text{treatment} + \text{sex} + \text{BM}$  | 7   | 747.3 |
|            |                                                               |     | 7.32  |
|            |                                                               |     | 0.02  |
|            | $\text{UA} \sim \text{treatment} + \text{sex} + \text{BM} + \text{cage}$ | 11  | 755.9 |
|            |                                                               |     | 15.93 |
|            |                                                               |     | <0.01 |

|            | $\text{Chol} \sim \text{treatment} + \text{BM}$             | 6   | 176.9 |
|            |                                                               |     | 0.00  |
|            | $\text{Chol} \sim \text{treatment} + \text{BM} + \text{sex}$| 7   | 178.0 |
|            |                                                               |     | 1.09  |
|            |                                                               |     | 0.317 |
|            | $\text{Chol} \sim \text{treatment}$                         | 5   | 180.1 |
|            |                                                               |     | 3.20  |
|            | $\text{Chol} \sim 1$                                         | 2   | 183.5 |
|            |                                                               |     | 6.60  |
|            |                                                               |     | 0.02  |
|            | $\text{Chol} \sim \text{treatment} + \text{BM} + \text{sex} + \text{cage}$ | 11  | 186.1 |
|            |                                                               |     | 9.22  |
|            |                                                               |     | <0.01 |

|            | $\text{Amy} \sim \text{weight}$                              | 3   | 628.7 |
|            |                                                               |     | 0.00  |
|            | $\text{Amy} \sim 1$                                          | 2   | 630.5 |
|            |                                                               |     | 1.81  |
|            |                                                               |     | 0.275 |
|            | $\text{Amy} \sim \text{treatment} + \text{BM}$              | 6   | 634.9 |
|            |                                                               |     | 6.20  |
|            |                                                               |     | 0.03  |
|            | $\text{Amy} \sim \text{treatment} + \text{BM} + \text{sex}$ | 7   | 636.4 |
|            |                                                               |     | 7.67  |
|            |                                                               |     | 0.02  |
|            | $\text{Amy} \sim \text{treatment} + \text{BM} + \text{sex} + \text{cage}$ | 10  | 644.8 |
|            |                                                               |     | 16.11 |
|            |                                                               |     | <0.01 |

|            | $\text{Glu} \sim \text{sex}$                                 | 3   | 236.1 |
|            |                                                               |     | 0.00  |
|            | $\text{Glu} \sim \text{sex} + \text{treatment}$             | 6   | 242.1 |
|            |                                                               |     | 6.03  |
|            |                                                               |     | 0.05  |
|            | $\text{Glu} \sim \text{sex} + \text{treatment} + \text{cage}$ | 10  | 243.7 |
|            |                                                               |     | 7.60  |
|            |                                                               |     | 0.02  |
|            | $\text{Glu} \sim \text{sex} + \text{treatment} + \text{cage} + \text{BM}$ | 11  | 246.8 |
|            |                                                               |     | 10.73 |
|            |                                                               |     | <0.01 |
|            | $\text{Glu} \sim 1$                                          | 2   | 249.7 |
|            |                                                               |     | 13.63 |
|            |                                                               |     | <0.01 |

|            | $\text{Glu} \sim 1$                                          | 2   | 90.1  |
|            |                                                               |     | 0.00  |
|            | $\text{Glu} \sim \text{treatment}$                          | 5   | 95.0  |
|            |                                                               |     | 4.88  |
|            |                                                               |     | 0.08  |
|            | $\text{Glu} \sim \text{treatment} + \text{cage}$           | 9   | 108.7 |
|            |                                                               |     | 18.59 |
|            |                                                               |     | <0.01 |
|            | $\text{Glu} \sim \text{treatment} + \text{cage} + \text{BM}$ | 10  | 109.2 |
|            |                                                               |     | 19.06 |
|            |                                                               |     | <0.01 |

|            | $\text{Glu} \sim 1$                                          | 2   | 145.5 |
|            |                                                               |     | 0.00  |
|            | $\text{Glu} \sim \text{treatment}$                          | 5   | 151.9 |
|            |                                                               |     | 6.41  |
|            |                                                               |     | 0.04  |
|            | $\text{Glu} \sim \text{treatment} + \text{BM}$             | 9   | 154.2 |
|            |                                                               |     | 8.69  |
|            |                                                               |     | 0.01  |
|            | $\text{Glu} \sim \text{treatment} + \text{BM} + \text{cage}$ | 10  | 158.2 |
|            |                                                               |     | 12.74 |
|            |                                                               |     | <0.01 |
**Table S16.** AICc values for models explaining variation in oxidative stress parameters. Note: CAT = catalase, GST = glutathione-S-transferase, TBARS = thiobarbituric acid-reactive substances, SOD = superoxide dismutase, GPx = glutathione peroxidase. Residuals for CAT, GST and TBARS were normally distributed, while residuals for SOD and GPx were not normally distributed.

| Model                      | df | AICc  | Delta | weight |
|----------------------------|----|-------|-------|--------|
| **CAT**                    |    |       |       |        |
| CAT ~ treatment            | 5  | 152.9 | 0.00  | 0.792  |
| CAT ~ treatment + cage      | 9  | 157.1 | 4.19  | 0.098  |
| CAT ~ 1                    | 2  | 157.3 | 4.45  | 0.086  |
| CAT ~ treatment + cage + sex| 10 | 159.8 | 6.92  | 0.025  |
| **GST**                    |    |       |       |        |
| GST ~ treatment            | 5  | -1.4  | 0.00  | 0.692  |
| GST ~ 1                    | 2  | 1.4   | 2.84  | 0.167  |
| GST ~ treatment + cage      | 9  | 2.2   | 3.65  | 0.112  |
| GST ~ treatment + cage + sex| 10 | 5.0   | 6.37  | 0.029  |
| **TBARS**                  |    |       |       |        |
| TBARS ~ 1                  | 2  | 287.0 | 0.00  | 0.942  |
| TBARS ~ treatment          | 5  | 293.7 | 6.72  | 0.033  |
| TBARS ~ treatment + cage    | 9  | 294.9 | 7.98  | 0.017  |
| TBARS ~ treatment + cage + sex| 10 | 296.5 | 9.55  | <0.01  |
| **SOD**                    |    |       |       |        |
| SOD ~ 1                    | 2  | 295.2 | 0.00  | 0.712  |
| SOD ~ treatment            | 5  | 298.3 | 3.04  | 0.156  |
| SOD ~ treatment + cage      | 9  | 299.9 | 4.64  | 0.070  |
| SOD ~ treatment + cage + sex| 10 | 300.1 | 4.86  | 0.063  |
| **GPx**                    |    |       |       |        |
| GPx ~ 1                    | 2  | 107.6 | 0.00  | 0.512  |
| GPx ~ treatment            | 4  | 108.0 | 0.40  | 1.419  |
| GPx ~ treatment + cage      | 9  | 112.3 | 4.72  | 0.048  |
| GPx ~ treatment + cage + sex| 10 | 113.9 | 6.38  | 0.021  |

**Table S17.** Output for models explaining variation for CAT, GST and GPx.

| Selected model | ANOVA / Kruskal-Wallis output | Dunnett / Dunn’s test |
|----------------|--------------------------------|-----------------------|
| **CAT**        | **CAT ~ treatment**            | Treatment: $F_{3,51} = 3.93, P = 0.01$ |
|                |                                | C – T1: $P > 0.05$    |
|                |                                | C – T2: $t = 3.25, P < 0.01$ |
|                |                                | C – T3: $t = 2.39, P = 0.05$ |
| **GST**        | **GST ~ treatment**            | Treatment: $F_{3,51} = 3.33, P = 0.03$ |
|                |                                | C – T1: $P > 0.05$    |
|                |                                | C – T2: $t = 2.76, P = 0.02$ |
|                |                                | C – T3: $t = 2.26, P = 0.07$ |
| **GPx**        | **GPx ~ treatment**            | Treatment: $\chi^2 = 12.86, P < 0.01$ |
|                |                                | C – T1: $P > 0.05$    |
|                |                                | C – T2: $z = -3.11, P = 0.01$ |
|                |                                | C – T3: $z = -3.09, P < 0.01$ |

**Table S18.** AICc values for models explaining variation in cytokines.

| Model                      | df | AICc  | Delta | weight |
|----------------------------|----|-------|-------|--------|
| **TNF-α**                  |    |       |       |        |
| TNF-α ~ treatment + sex    | 6  | 530.3 | 0.00  | 0.99   |
| TNF-α ~ treatment + sex + cage | 10 | 539.4 | 9.15  | 0.01   |
| TNF-α ~ treatment + sex + cage + weight | 13 | 546.1 | 15.8  | <0.01  |
| TNF-α ~ 1                  | 2  | 559.0 | 28.8  | <0.01  |
### Table S19. AICc values for models explaining variation in E₂ (17-β Estradiol) concentrations.

| Model | df  | AICc  | Delta | weight |
|-------|-----|-------|-------|--------|
| E₂    |     |       |       |        |
| E₂ ~ treatment                     5  | 467.9 | 0.00  | 0.59  |
| E₂ ~ 1                             2  | 468.6 | 0.71  | 0.41  |
| E₂ ~ treatment + weight            9  | 478.5 | 10.56 | <0.01 |
| E₂ ~ treatment + weight + cage     10 | 482.1 | 14.23 | <0.01 |
| T    |     |       |       |        |
| T ~ 1                             2  | 388.7 | 0.00  | 0.98  |
| T ~ treatment                      5  | 397.1 | 8.41  | 0.02  |
| T ~ treatment + weight             6  | 399.7 | 11.02 | <0.01 |
| T ~ treatment + weight + cage       10 | 419.1 | 30.31 | <0.01 |

### Table S20. Output for the Dunn’s Test for E₂ variation in females.

| Comparison     | Z-value | P-adj (Holm method) |
|----------------|---------|---------------------|
| Control – T1   | 2.44    | 0.07                |
| Control – T2   | 0.65    | 0.76                |
| T1 – T2        | -1.85   | 0.18                |
| Control – T3   | 2.91    | 0.02                |
| T1 – T3        | 0.24    | 0.81                |
| T2 – T3        | 2.28    | 0.08                |
9. Additional Results

**Fig. S15.** Body mass (g) of Japanese quail (Coturnix japonica) measured once per week in A) females (n = 32) and B) males (n = 23). The figure compares four treatment groups, Control (blue, n = 14), T1 (treatment 1: 3 mm particles, purple, n = 13), T2 (treatment 2: powder < 125 μm, pink, n = 14) and T3 (treatment 3: mixture of 3 mm and < 125 μm particles, green, n = 14). Error bars show standard error.

**Fig. S16** Boxplot showing Hepatosomatic Index (HSI) in Japanese quail (Coturnix japonica) for each treatment group (C n= 14, T1: 3 mm MP n=14, T2: < 125 μm MP n=13, T3: mixture 3 mm and < 125 μm MP n=14). As sex was an influential factor, the treatment groups are separated by sex.
Fig. S17 Boxplot showing Hematocrit (Ht, %) in Japanese quail (Coturnix japonica) for each treatment group (C n= 14, T1: 3 mm MP n=14, T2: < 125 μm MP n=13, T3: mixture 3 mm and < 125 μm MP n=14). As sex was an influential factor, the treatment groups are separated by sex.
Fig. S18 Boxplots showing BCCPs in blood of Japanese quail (Coturnix japonica) for each treatment group (C n= 14, T1: 3 mm MP n=14, T2: < 125 μm MP n=13, T3: mixture 3 mm and < 125 μm MP n=14). When sex was found to be an influential factor, the treatment groups are separated by sex. Statistical differences are shown (* P < 0.05).
**Fig. S19** Boxplot showing testosterone levels in blood samples of male Japanese quail (Coturnix japonica) for each treatment group (C n= 14, T1: 3 mm MP n=14, T2: < 125 μm MP n=13, T3: mixture 3 mm and < 125 μm MP n=14).

**Fig. S20** Boxplots showing oxidative stress parameters in blood of Japanese quail (Coturnix japonica) for each treatment group (C n= 14, T1: 3 mm MP n=14, T2: < 125 μm MP n=13, T3: mixture 3 mm and < 125 μm MP n=14).
Fig. S21 Boxplots showing cytokine levels in blood of Japanese quail (Coturnix japonica) for each treatment group (C n= 14, T1: 3 mm MP n=14, T2: < 125 μm MP n=13, T3: mixture 3 mm and < 125 μm MP n=14). When sex was found to be an influential factor, the treatment groups are separated by sex.
References

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