**Supplementary material.** **Dietary antioxidants attenuate the endocrine stress response during long-duration flight of a migratory bird**

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Methods

Experimental diets

Starting in early-August 2016, birds were fed a semi-synthetic agar-based diet (Murphy and King, 1982) that was 42% carbohydrates, 23% protein, 20% fat (see Carbeck et al. 2018 for details). After ca. one month of acclimation to this diet and for the remainder of the experiment, birds were randomly assigned to either continue on the same diet (without anthocyanin supplement, n = 29) or the same diet with added anthocyanins (n = 25), hydrophilic dark purple pigments that are common in the diets of wild songbirds (McWilliams *et al.*, 2004; Schaefer *et al.*, 2008). Anthocyanin powder (Standardized Elderberry 6.5% Powder; Artemis International, Inc., Fort Wayne, IN, USA) was homogenously mixed into the semi-synthetic diet. A standard vitamin-mineral mix (AIN-76, MP Biomedicals, Solon, Ohio, USA) was included in both diets to ensure nutritional adequacy of other micronutrients and to provide the necessary Vitamin C for efficient use of the dietary anthocyanin supplement.

Housing conditions

From August 2016 until the start of the flight experiment and during winter season, groups of 15-20 birds were housed in separate outdoor aviaries (two aviaries per each experimental group) measuring 3.0 x 4.0 x 2.0 m each). During the flight sessions birds were housed in experimental indoor aviaries (1.5x2.5x2.5 m) located in wind-tunnel facility, in the section surrounding the wind-tunnel, where they were maintained at temperature ranging from 18°C to 22°C. During fall and spring, birds were maintained on a 13L:11D light cycle, while during winter season the light schedule was temporarily changed (for 3 weeks) to 10L:14D, to simulate natural light cycle of starlings wintering in the study area. Both outdoor and experimental indoor aviaries contained perches (7 and 2 perches in outdoor and experimental aviaries respectively) and were supplied with fresh food and water (*ad libitum*) and water-bath every day.

Experimental procedure and wind-tunnel flight training

During fall 2016 and spring 2017, we flew thirty-three each day for 15 days in a recirculating windtunnel at the MPIO under controlled conditions (15 °C, 70% humidity, 12 m\*s-1 wind speed). During the flight training period in fall (Sept-Dec) and spring (Feb-April), every 3-9 days, we randomly selected 4-6 individuals from each diet group to be transferred to the wind tunnel facility and start the 15-day flight training. Birds in each training cohort were housed in aviaries (3 x 4 x 2.5 m high) that were adjacent to the wind-tunnel and enclosed by nylon-mesh walls. This setup allowed us to release birds from their aviaries, and guide them to fly directly into the air stream without additional handling of birds. The section of the windtunnel where the birds flew was a 2 m long and 1.2 m wide octagon built of transparent plastic and glass. Flight-training for each cohort involved 15 consecutive days of specified amounts of flying in the windtunnel (day 1-3 flight time = 20 min, day 4 = 30 min, day 5 = 60 min, day 6 = 30 min, day 7 = 60 min, day 8 = 90 min, day 9 = no flight training, day 10 = 120 min, day 11 = 180 min, day 12 = no flight training, day 13 = 60 min, day 14 = 30 min, day 15 = up to 360 min). This resulted in a total flight-training time, excluding the longest flight on day 15, of 720 min. For each bird, we recorded actual flight time each day and summed the total time spent flying for the entire 15-day period.

Circulating levels of corticosterone

Plasma corticosterone concentrations at flight and rest timepoints were determined using an enzyme immunoassay kit (Cat. No. K014-H1; Corticosterone ELISA Kit, Arbor Assays) following a double diethyl ether extraction of a 10 µL plasma sample. Samples were re-dissolved in assay buffer and allowed to reconstitute over-night. A buffer blank and two stripped chicken plasma controls (with Cort added at concentrations of 10 and 5 ng mL-1, respectively) were taken through the entire procedure. The next day, 50 µL of each sample (in duplicate) was added to individual wells on an assay plate. Individuals were randomized across plates with repeated measures for the same individual run in the same plate. The inter-plate coefficient of variation (CV) was calculated as the average concentrations of the four controls (for both high and low concentrations) of the 6 plates and was 8.73±0.41%. The intra-plate CV was calculated as the average CV of the concentrations of all the unknown samples run on 6 plates and was 4.36±0.24%.

Results

Table S1. Means (± s.e.) of the considered variables.

|  |  |  |
| --- | --- | --- |
|  | Flight | Rest |
| Variable | Anthocyanins | Controls | Anthocyanins | Controls |
| Corticosterone (ng/mL) | 39.33 (±5.40) | 62.18 (±7.41) | 15.22 (±4.07) | 13.35 (±1.53) |
| Body mass (g) | 66.09 (±1.01) | 66.38 (±0.82) | 67.40 (±0.93) | 67.34 (±0.71) |
| Fat store (score)  | 1.75 (±0.12) | 1.76 (±0.15) | 2.18 (±0.13) | 2.12 (±0.11) |
| Muscle size (score) | 21.2 (±0.19) | 21.19 (±0.16) | 21.25 (±0.16) | 21.34 (±0.15) |