Appendix A

METHODS FOR NUTRITIONAL BODY COMPOSITION ASSAYS

Each fly was individually homogenised in 180 µL of phosphate-buffered saline (PBS) (1.35 mM potassium chloride, 68.5 mM sodium chloride, pH 7.4) (P4417, Sigma Aldrich, Saint-Louis, MO, USA) using a microtube homogeniser (BeadBug™ 3 Position Bead Homogeniser, Benchmark Scientific, Sayreville, NJ, USA) and a 3 mm Ø zirconium bead. Flies were homogenised at 200 RCF for one minute. The samples were then centrifuged at 200 RCF at 4 °C for 15 minutes.

To determine estimates of soluble proteins, 1.5 µL supernatant was transferred to a 96-well microplate in duplicate. The Bradford assay (Bradford, 1976) was used to determine total protein content using Bradford reagent (Sigma-Aldrich, St Louis, MO, USA). Serial dilutions of bovine albumin standard (Sigma-Aldrich) were used to create a standard curve (0, 25, 50, 100, 200 µg/mL). Optical density was read at 595 nm.

For carbohydrate and lipid determinations, 20 µL 20% Na2SO4 (w/v), 4.5 µL PBS buffer, and 1500 µL chloroform:methanol (1:2 v/v) was added to the remaining homogenate. Samples were then vortexed for one minute and centrifuged at 200 RCF at 4 °C for 15 minutes. One hundred microlitres of the supernatant was set aside, in duplicate, in 2 mL Eppendorf tubes for lipid analysis. The remainder of the supernatant was evaporated overnight in a fume hood and then reconstituted using 250 µL chloroform:methanol (1:2 v/v). The anthrone method was used to determine the water-soluble carbohydrate content of these samples, with a glucose dilution range (0, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 mg mL-1) used as a calibration standard. Two hundred microlitres of standard or sample were transferred into a 15 mL tube with 4.8 mL of anthrone reagent (1.42g L-1in 70% sulphuric acid) (Anthrone, ACS reagent 97% obtained from Sigma-Aldrich). The samples and standards were incubated at 90 °C for 15 minutes in a water bath and then cooled on ice for five minutes. Two hundred microlitres of each sample and standard was transferred into a 96-well microplate, in duplicate, and the absorbance was read at 625 nm.

Total lipid determinations were made using the vanillin colorimetric assay. Calibration standards were made by dissolving glyceryl trioleate (Sigma-Aldrich) in chloroform:methanol (1:2 v/v) (dilution range 0, 0.1, 0.2, 0.5, 1.0 µg µL-1). Samples were completely evaporated under a fume hood overnight. After which samples were incubated with 10 µL 98% sulphuric acid at 90 °C for two minutes and then cooled on ice for 5 minutes. Then, 210 µL of vanillin reagent (1.2 g L-1 vanillin dissolved in 68% orthophosphoric acid) was added to the samples (Vanillin, ReagentPlus®, 99% obtained from Sigma-Aldrich). The microplate was shaken for 15 minutes at room temperature and the absorbance was read at 525 nm and compared to the calibration standards.