

## Supplementary Information

Supplementary Table 1

|                              | NIH 07*                                |             | NTP-2000*                              |             | AIN76**           |             | AIN93G   |             | AIN93M   |             |
|------------------------------|--|-------------|--|-------------|-------------------|-------------|--|-------------|--|-------------|
| Proximate Analysis           | Energy (kcal/g)***                     | 3.44        | Energy (kcal/g)                        | 3.4         |                   |             | Energy (kcal/g)                                  | 3.77        | Energy (kcal/g)                                  | 3.6         |
|                              | <u>Nutrient</u>                        | <u>% wt</u> | <u>Nutrient</u>                        | <u>% wt</u> |                   |             | <u>Nutrient</u>                                  | <u>% wt</u> | <u>Nutrient</u>                                  | <u>% wt</u> |
|                              | Protein                                | 22.9        | Protein                                | 14.6        |                   |             | Protein  | 17.9        | Protein  | 12.6        |
|                              | Carbohydrate                           | 51          | Carbohydrate                           | 52          |                   |             | Carbohydrate                                     | 59.6        | Carbohydrate                                     | 68          |
|                              | Fat                                    | 5.4         | Fat                                    | 8.2         |                   |             | Fat  | 7           | Fat  | 4           |
|                              | Fiber                                  | 3.4         | Fiber                                  | 9.3         |                   |             | Fiber  | 5           | Fiber  | 5           |
|                              | <u>Ingredient</u>                      | <u>% wt</u> | <u>Ingredient</u>                      | <u>% wt</u> | <u>Ingredient</u> | <u>% wt</u> | <u>Ingredient</u>                                | <u>% wt</u> | <u>Ingredient</u>                                | <u>% wt</u> |
| Primary Protein Sources      | Dried Skim Milk                        | 5           | Fish meal (60% protein)                | 4           | Casein            | 20          | Casein (>85%protein)                             | 20          | Casein (>85%protein)                             | 14          |
|                              | Fish meal (60% protein)                | 10          | Soybean meal (49% protein)             | 5           | DL-Methionine     | 0.3         | L-cystine  | 0.3         | L-cystine  | 0.18        |
|                              | Soybean meal (49% protein)             | 12          | Methionine                             | 0.2         |                   |             |  |             |  |             |
|                              | Alfalfa meal (dehydrated, 17% protein) | 4           | Alfalfa meal (dehydrated, 17% protein) | 7.5         |                   |             |  |             |  |             |
|                              | Corn gluten meal (60% protein)         | 3           |  |             |                   |             |  |             |  |             |
| Primary Carbohydrate Sources | Ground No. 2 yellow shelled corn       | 24.5        | Ground corn                            | 22.18       | Cornstarch        | 15          | Cornstarch                                       | 39.75       | Cornstarch                                       | 46.57       |
|                              | Ground hard winter wheat               | 23          | Ground wheat                           | 22.26       |                   |             | Dextrinized cornstarch (90-94% tetrasaccharides) | 13.2        | Dextrinized cornstarch (90-94% tetrasaccharides) | 15.5        |

|                              |                            |       |                            |      |                              |              |  |              |  |              |
|------------------------------|----------------------------|-------|----------------------------|------|------------------------------|--------------|--|--------------|--|--------------|
|                              | Wheat middlings            | 10    | Wheat middlings            | 15   | Sucrose                      | 50           | Sucrose                                      | 10           | Sucrose                                      | 10           |
|                              | Dry molasses               | 1.5   | Oat hulls                  | 8.5  | Sucrose, finely powdered     | add to 1000g | Sucrose, finely powdered                     | add to 1000g | Sucrose, finely powdered                     | add to 1000g |
|                              |                            |       | Purified cellulose         | 5.5  | Fiber (Cellulose-type fiber) | 5            | Fiber (Solka-Floc 200FCC powdered cellulose) | 5            | Fiber (Solka-Floc 200FCC powdered cellulose) | 5            |
| Primary Lipid Sources        | Soybean Oil                | 2.5   | Soybean Oil                | 3    | Corn Oil                     | 5            | Soybean oil                                  | 7            | Soybean oil                                  | 4            |
|                              |                            |       | Corn Oil                   | 3    |                              |              |  |              |  |              |
| Micronutrients and Additives | Brewers dried yeast        | 2     | Brewers dried yeast        | 1    |                              |              |  |              |  |              |
|                              | AIN77 Mineral mix          | 0.125 | AIN77 Mineral mix          | 0.5  | AIN77 Mineral mix            | 3.5          | Mineral mix (AIN-93G-MX)                     | 3.5          | Mineral mix (AIN-93G-MX)                     | 3.5          |
|                              | AIN77 Vitamin mix          | 0.125 | AIN77 Vitamin mix          | 0.5  | AIN77 Vitamin mix            | 1            | Vitamin mix (AIN-93-VX)                      | 1            | Vitamin mix (AIN-93-VX)                      | 1            |
|                              | Sodium chloride            | 0.5   | Sodium chloride            | 0.3  |                              |              | tert-butylhydroquinone                       | 0.014        | tert-butylhydroquinone                       | 0.008        |
|                              | Calcium phosphate, dibasic | 1.25  | Calcium phosphate, dibasic | 0.4  |                              |              |  |              |  |              |
|                              | Calcium carbonate          | 0.5   | Calcium carbonate          | 0.9  |                              |              |  |              |  |              |
|                              | Choline chloride           | 0.09  | Choline chloride           | 0.26 | choline bitartrate           | 0.2          | choline bitartrate                           | 0.25         | choline bitartrate                           | 0.25         |

\*Mean nutrient contents shown in the unrefined diets are not necessarily representative, as unrefined ingredient composition is subject to significant lot-to-lot variability.

\*\* Refers to the original AIN76 formulation; no proximate analysis was provided for this formulation. A reformulation, AIN76A, was recommended in March 1980 to increase Vitamin K 10-fold, include an antioxidant to prevent oxidation of the corn oil, and suggested replacing sucrose with starch if sucrose complicated experimental plans.

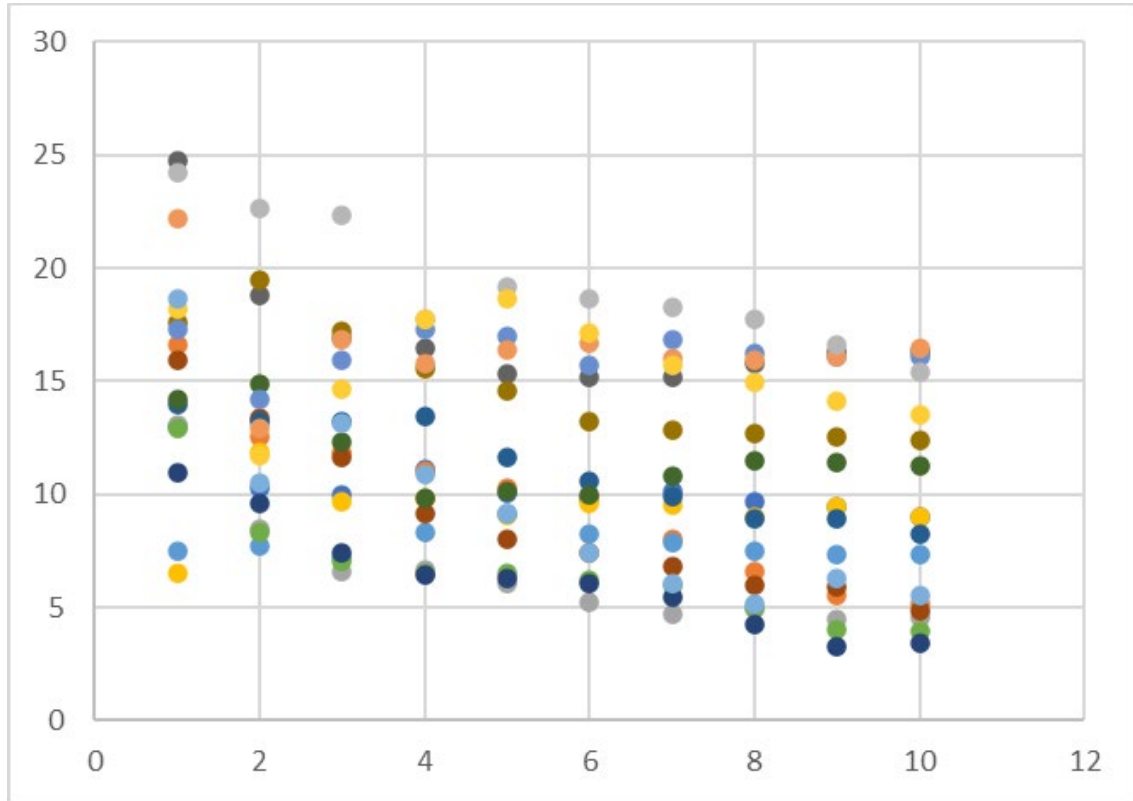
\*\*\* Energy values derived from proximate analyses assuming no caloric contribution from fiber.

### **Sample size and power analysis:**

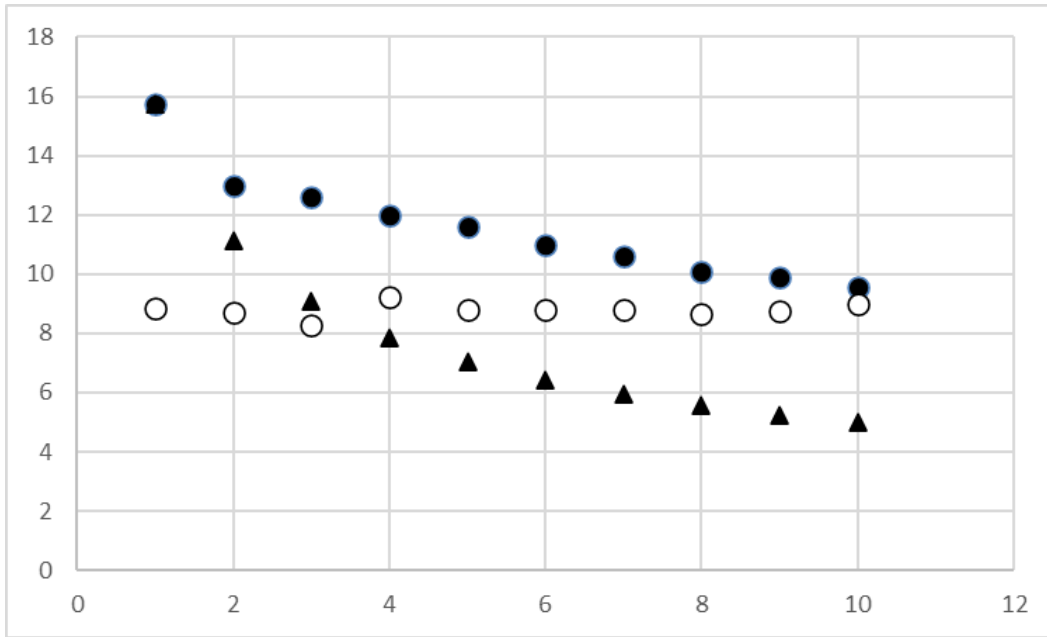
We modeled the overall coefficient of variation in food intake using data from a cohort of 17 individually housed ad libitum fed C57BL/6 mice where food intake was measured daily for a period of 103 days (control groups from Mitchell et al 2015). We calculated the average intake for each individual averaged over 2,3,4,5...10 days. We then selected the 1<sup>st</sup>, 11<sup>th</sup>, 21<sup>st</sup> ..etc measurements giving 10 completely independent measures of food intake averaged across different periods for each individual. The coefficient of variation in these measurements in relation to the period over which the intake was averaged is shown for each individual in Figure S1. As anticipated the within individual error declined as the number of days over which the average was calculated increased. The average of these values for any duration of measurement represents the total (within and between individual variation) and is shown in Figure S2 (black circles). We calculated the between individual variation by calculating the average and standard deviation of intakes across all 17 individuals, each averaged across the 10 measurements for that individual at each duration. The between individual variation was independent of the number of days over which the measurement was averaged (Figure S2 open circles) and averaged 9%. In theory, the within individual variation should decline as a function of the initial variation divided by the square root of the sample size. This theoretical curve is also shown in Figure S2 (black triangles). The decline in the total variation (within and between individuals) in relation to the number of days sampled is less than the theoretical anticipated improvement, because the variation between individuals becomes the limiting factor on further reduction in the total variation. In this instance the variation when sampling only single days averaged 15.8%. In theory taking the average over 10 days should reduce this to 5% ( $15.8/\sqrt{10}$ ) but in fact the CV only declined to 9% to match the between individual variation.

**Supplementary Table 2:** Sample sizes required to detect different effect sizes in a standard two-sample t-test in relation to the coefficient of variation in intake. Diets typically generate coefficients of variation in intake between 10 and 17% depending on diet composition. Values around the typically used sample size of about  $n = 10$  are highlighted in red.

| Effect size(%) | CV(10%) | CV(12%) | CV(15%) | CV(17%) |
|----------------|---------|---------|---------|---------|
| 1              | 1571    | 2262    | 3533    | 4538    |
| 3              | 176     | 253     | 394     | 506     |
| 5              | 64      | 92      | 143     | 183     |
| 10             | 17      | 24      | 37      | 47      |
| 15             | 9       | 12      | 17      | 22      |
| 20             | 6       | 8       | 10      | 13      |
| 25             | 4       | 5       | 7       | 9       |



**Supplementary Figure 1:** Coefficient of variation in food intake ( $CV = \text{mean}/\text{sd}$ ) for 17 individual mice (individually colored) where food intake was measured daily for 103 days. The plot shows the CV in relation to the number of days over which intake was averaged (1 to 10 days). The sample size for all durations was 10 completely independent measurements. As anticipated CV declined as the duration over which intake was averaged increased.



**Supplementary Figure 2:** Average CV across all the individuals illustrated in figure A (black circles) which reflects the total within and between individual variation, and the theoretical expectation in the reduction (black triangles) as the number of days over which the average is calculated increases (assuming the variation stemmed only from within individual effects).. The between individual CV in the average intake, calculated across all 17 individuals for the different durations of measurement, is shown as open circles. The total variation (within and between individuals: black circles) homes in on the between individual variation (white circles) which becomes limiting on further reduction in the CV by increasing the sample duration over which intake is measured.