

Table S1. Control mouse diet (Ca:ratio = 1.5)

AIN-93M Maintenance Purified Diet (also known as #5801-M) 58M1

DESCRIPTION

TestDiet® AIN-93M Maintenance Purified Diet is the maintenance diet for rodents recommended by the American Institute of Nutrition. It is formulated to substitute for the previous version (AIN-76A) to improve animal performance.

Storage conditions are particularly critical to TestDiet® products, due to the absence of antioxidants or preservative agents. To provide maximum protection against possible changes during storage, store in a dry, cool location. Storage under refrigeration (2° C) is recommended. If long term studies are involved, store the diet at -20° C or colder. Be certain to keep in air tight containers.

Product Forms Available*	Catalog #
1/2" Pellet	7603
1/2" Pellet, Irradiated	1810541
3/16" Pellet	1812811
Meal	1810540
Meal, Irradiated	1810542

*Other Forms Available By Request

TYPICAL ANALYSIS

Protein.....	13.06%
Fat.....	4.0%
Fiber.....	5.0%
Carbohydrate.....	73.8%
Metabolizable Energy.....	3.83

INGREDIENTS (%)

Corn Starch	46.5692
Dextrin	15.5000
Casein - Vitamin Free	14.0000
Sucrose	10.0000
Powdered Cellulose	5.0000
Soybean Oil	4.0000
AIN 93M Mineral Mix	3.5000
AIN 93 Vitamin Mix	1.0000
Choline Bitartrate	0.2500
L-Cystine	0.1800
t-Butylhydroquinone	0.0008

FEEDING DIRECTIONS

Feed ad libitum to mice and rats. Plenty of fresh, clean water should be available at all times.

CAUTION:

Perishable, upon receipt store in a cool dry place, refrigeration recommended.

For laboratory animal experimental use only, NOT for human consumption.

2/15/2011

NUTRITIONAL PROFILE ¹

Protein, %	13.0	Minerals	
Arginine, %	0.49	Calcium, %	0.50
Histidine, %	0.36	Phosphorus, %	0.31
Isoleucine, %	0.67	Potassium, %	0.36
Leucine, %	1.21	Magnesium, %	0.05
Lysine, %	1.02	Sodium, %	0.13
Methionine, %	0.36	Chlorine, %	0.20
Cystine, %	0.23	Fluorine, ppm	1.0
Phenylalanine, %	0.67	Iron, ppm	39
Tyrosine, %	0.71	Zinc, ppm	35
Threonine, %	0.54	Manganese, ppm	11
Tryptophan, %	0.15	Copper, ppm	6.0
Valine, %	0.80	Cobalt, ppm	0.0
Alanine, %	0.39	Iodine, ppm	0.21
Aspartic Acid, %	0.90	Chromium, ppm	1.0
Glutamic Acid, %	2.86	Molybdenum, ppm	0.14
Glycine, %	0.27	Selenium, ppm	0.22
Proline, %	1.65		
Serine, %	0.77	Vitamins	
Taurine, %	0.00	Vitamin A, IU/g	4.0
		Vitamin D-3 (added), IU/g	1.0
		Vitamin E, IU/kg	78.8
		Vitamin K (as menadione), ppm	0.75
		Thiamin Hydrochloride, ppm	6.0
		Riboflavin, ppm	6.5
		Niacin, ppm	30
		Pantothenic Acid, ppm	16
		Folic Acid, ppm	2.1
		Pyridoxine, ppm	5.8
		Biotin, ppm	0.2
		Vitamin B-12, mcg/kg	28
		Choline Chloride, ppm	1,250
		Ascorbic Acid, ppm	0.0

Fat, %	4.1		
Cholesterol, ppm	0		
Linoleic Acid, %	2.04		
Linolenic Acid, %	0.31		
Arachidonic Acid, %	0.00		
Omega-3 Fatty Acids, %	0.31		
Total Saturated Fatty Acids, %	0.60		
Total Monounsaturated Fatty Acids, %	0.88		
Polyunsaturated Fatty Acids, %	2.16		
Fiber (max), %	5.0		
Carbohydrates, %	73.0		
Energy (kcal/g) ²	3.77		
From:	kcal	%	
Protein	0.518	13.7	
Fat (ether extract)	0.368	9.8	
Carbohydrates	2.922	77.5	

1. Formulation based on calculated values from the latest ingredient analysis information. Since nutrient composition of natural ingredients varies and some nutrient loss will occur due to manufacturing processes, analysis will differ accordingly. Nutrients expressed as percent of ration on an As Fed basis except where otherwise indicated.
2. Energy (kcal/gm) - Sum of decimal fractions of protein, fat and carbohydrate x 4,9,4 kcal/gm respectively.



Table S2. Experimental mouse diet (Ca:P ratio = 10.5)

Mod AIN93M w/ 0.11% Phosphorus and 1.5% Calcium

5BLX

DESCRIPTION

Modification of TestDiet® AIN-93M Semi-Purified Diet with 0.11% Phosphorus and 1.5% Calcium.

Storage conditions are particularly critical to TestDiet® products, due to the absence of antioxidants or preservative agents. To provide maximum protection against possible changes during storage, store in a dry, cool location. Storage under refrigeration (2° C) is recommended. Maximum shelf life is six months. (If long term studies are involved, storing the diet at -20° C or colder may prolong shelf life.) Be certain to keep in air tight containers.

Product Forms Available* Catalog #
 3/8" Pellet, Irradiated 1816464-203

**Other Forms Available On Request*
INGREDIENTS (%)

Corn Starch	44.0952
Maltodextrin	15.5000
Casein - Vitamin Tested	14.0000
Sucrose	10.0000
Powdered Cellulose	5.0000
Soybean Oil	4.0000
AIN-93M Min Premix/No phosphorus	3.5000
Calcium Carbonate	2.4740
AIN 93 Vitamin Mix	1.0000
Choline Bitartrate	0.2500
L-Cystine	0.1800
t-Butylhydroquinone	0.0008

FEEDING DIRECTIONS

Feed ad libitum. Plenty of fresh, clean water should be available at all times.

CAUTION:

Perishable - store properly upon receipt.
For laboratory animal use only; NOT for human consumption.

4/29/2014

NUTRITIONAL PROFILE ¹

Protein, %	12.9	Minerals	
Arginine, %	0.49	Calcium, %	1.50
Histidine, %	0.36	Phosphorus, %	0.11
Isoleucine, %	0.67	Potassium, %	0.35
Leucine, %	1.21	Magnesium, %	0.05
Lysine, %	1.02	Sodium, %	0.14
Methionine, %	0.36	Chloride, %	0.23
Cystine, %	0.23	Fluorine, ppm	1.0
Phenylalanine, %	0.67	Iron, ppm	39
Tyrosine, %	0.71	Zinc, ppm	35
Threonine, %	0.54	Manganese, ppm	11
Tryptophan, %	0.15	Copper, ppm	6.0
Valine, %	0.80	Cobalt, ppm	0.0
Alanine, %	0.39	Iodine, ppm	0.21
Aspartic Acid, %	0.90	Chromium (added), ppm	1.0
Glutamic Acid, %	2.86	Molybdenum, ppm	0.15
Glycine, %	0.27	Selenium, ppm	0.19
Proline, %	1.65		
Serine, %	0.77	Vitamins	
Taurine, %	0.00	Vitamin A, IU/g	4.0
		Vitamin D-3 (added), IU/g	1.0
Fat, %	4.1	Vitamin E, IU/kg	78.8
Cholesterol, ppm	0	Vitamin K, ppm	0.75
Linoleic Acid, %	2.04	Thiamin Hydrochloride, ppm	6.0
Linolenic Acid, %	0.31	Riboflavin, ppm	6.5
Arachidonic Acid, %	0.00	Niacin, ppm	30
Omega-3 Fatty Acids, %	0.31	Pantothenic Acid, ppm	16
Total Saturated Fatty A	0.60	Folic Acid, ppm	2.1
Total Monounsaturated		Pyridoxine, ppm	5.8
Fatty Acids, %	0.88	Biotin, ppm	0.2
Polyunsaturated Fatty Acids, %	2.16	Vitamin B-12, mcg/kg	28
		Choline Chloride, ppm	1,250
Fiber (max), %	5.0	Ascorbic Acid, ppm	0.0
Carbohydrates, %	70.8		
Energy (kcal/g) ²	3.72		
From:	kcal	%	
Protein	0.517	13.9	
Fat (ether extract)	0.368	9.9	
Carbohydrates	2.832	76.2	

1. Formulation based on calculated values from the latest ingredient analysis information. Since nutrient composition of natural ingredients varies and some nutrient loss will occur due to manufacturing processes, analysis will differ accordingly. Nutrients expressed as percent of ration on an As-Fed basis except where otherwise indicated.
 2. Energy (kcal/gm) - Sum of decimal fractions of protein, fat and carbohydrate x 4,9,4 kcal/gm respectively.



Table S3. Skull landmarks (cranium + midface)

Landmark	Description
1	Nasal bone's most anterior suture
2	Nasal bone's most posterior suture
3	Frontal bone's most posterior suture
4	Parietal bone's most posterior suture
5-6	Frontal-squamosal intersection at temporal crest
7-8	The intersection between parietal, occipital and squamosal bones
9-10	Joining of squamosal body to zygomatic process
11-12	Most anterior suture of jugal bone and the maxillary zygomatic process
13-14	Mid zygomatic bone
15-16	Intersection of frontal process of maxilla with frontal and lacrimal bones
17-18	Midpoint of the anterior zygomatic process (dorsal view)
19-20	Most inferior point of the zygomatic process
21-22	Anterior-most point at intersection of premaxillae and nasal bones
23-24	Most superior point of the incisor alveolus
25-26	Most inferior anterior point of the incisor alveolus
27-28	Most inferior lateral point on premaxilla-maxilla suture
29-30	Most anterior point of the first molar alveolus
31-32	Most posterior point of the third molar alveolus
33-34	Most anterior point of the anterior palatine foramen
35-36	Most posterior point of the anterior palatine foramen
37-38	Most inferior aspect of posterior tip of medial pterygoid process
39	Line point of the suture between occipital and basisphenoid bones
40	Line point of the suture between basisphenoid and presphenoid bones
41	Line point of the suture between palatine bones.
42	Foramen magnum most anterior point, basion
43	Foramen magnum most posterior point, bregma

Table S4. Descriptive statistics and unpaired t-test results of skull measurements

Variable		Control (Mean \pm SD)	Experiment (Mean \pm SD)	p-value
Cranial Centroid Size (μm)	Total	42480 \pm 2197	41017 \pm 3181	0.2491 ^{ns}
	Males	40102 \pm 186.9	44244 \pm 841.4	<0.0001 ^{****}
	Females	44064 \pm 1065	38691 \pm 1224	<0.0001 ^{****}
Midface Centroid Size (μm)	Total	14903 \pm 919.1	14370 \pm 1368	0.3221 ^{ns}
	Males	13918 \pm 133.6	15791 \pm 419.9	<0.0001 ^{****}
	Females	15560 \pm 465.9	13372 \pm 509.3	<0.0001 ^{****}
Cranial Length (CL) (μm)	Total	21792 \pm 1161	20878 \pm 1475	0.0892 ^{ns}
	Males	20473 \pm 253.3	22531 \pm 263.1	<0.0001 ^{****}
	Females	22671 \pm 260.3	19777 \pm 484.2	<0.0001 ^{****}
Midface Length (ML) (μm)	Total	7288 \pm 546.9	6958 \pm 684.5	0.2495 ^{ns}
	Males	6742 \pm 95.2	7727 \pm 193.3	<0.0001 ^{****}
	Females	7651 \pm 369	6445 \pm 180.2	<0.0001 ^{****}
Cranial Breadth (CB) (μm)	Total	10895 \pm 474.5	10390 \pm 777.1	0.1000 ^{ns}
	Males	10409 \pm 152.1	11254 \pm 234	0.0009 ^{***}
	Females	11218 \pm 278.1	9814 \pm 242.4	<0.0001 ^{****}
Midface Breadth (MB) (μm)	Total	4453 \pm 156.7	4165 \pm 240	0.0185 [*]
	Males	4289 \pm 56.04	4356 \pm 38.73	0.0985 ^{ns}
	Females	4562 \pm 81.02	4038 \pm 233	0.0022 ^{**}
Tissue Mineral Density (TMD) (arbitrary units)	Total	0.01626 \pm 0.0012	0.01427 \pm 0.002	0.016 [*]
	Males	0.01498 \pm 0.00034	0.01651 \pm 0.00054	0.0032 ^{**}
	Females	0.01711 \pm 0.00064	0.01277 \pm 0.0008	<0.0001 ^{****}

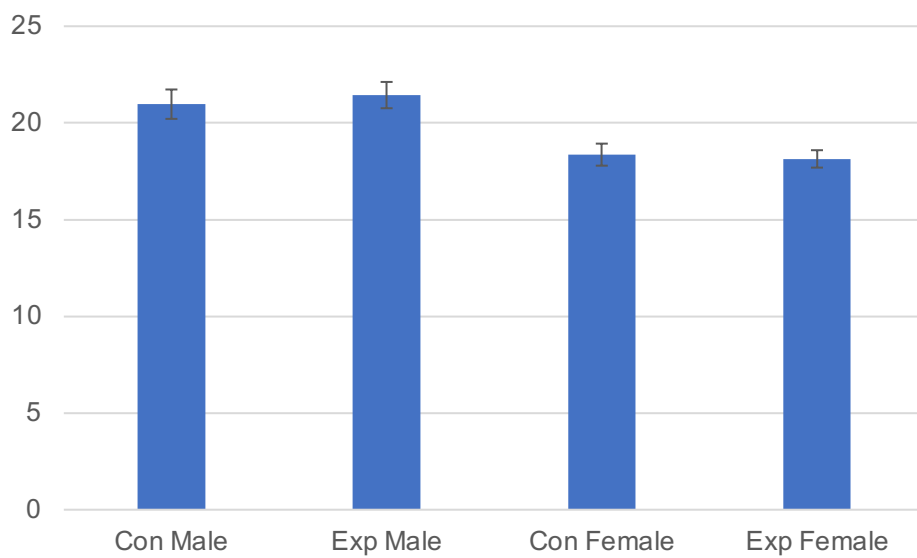
* $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$; **** $p < 0.0001$; ns (no significance), $p > 0.05$

Table S5. Descriptive statistics and unpaired t-test results of dental measurements

Variable		Control (Mean \pm SD)	Experiment (Mean \pm SD)	p-value
Molar Row Length (MRL) (μm)	Total	3789 \pm 122.7	3690 \pm 188.6	0.1810 ^{ns}
	Males	3684 \pm 59.99	3899 \pm 59.39	<0.0022**
	Females	3860 \pm 100.8	3550 \pm 57.46	<0.0001****
Anterior Alveolar Height (AAH) (μm)	Total	1172 \pm 97.21	1090 \pm 117.8	0.1230 ^{ns}
	Males	1118 \pm 6.242	1214 \pm 73.9	<0.0405*
	Females	1207 \pm 114.6	1007 \pm 31.99	<0.0020**
Middle Alveolar Height (MAH) (μm)	Total	697.6 \pm 76.22	675.1 \pm 76.06	0.5158 ^{ns}
	Males	677.4 \pm 38.31	750.7 \pm 25.39	<0.0188*
	Females	711.1 \pm 95.03	624.6 \pm 48.90	<0.0755 ^{ns}

* $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$; **** $p < 0.0001$; ns (no significance)

Figure S1. Weights (in grams) of 6-week-old mice at collection.



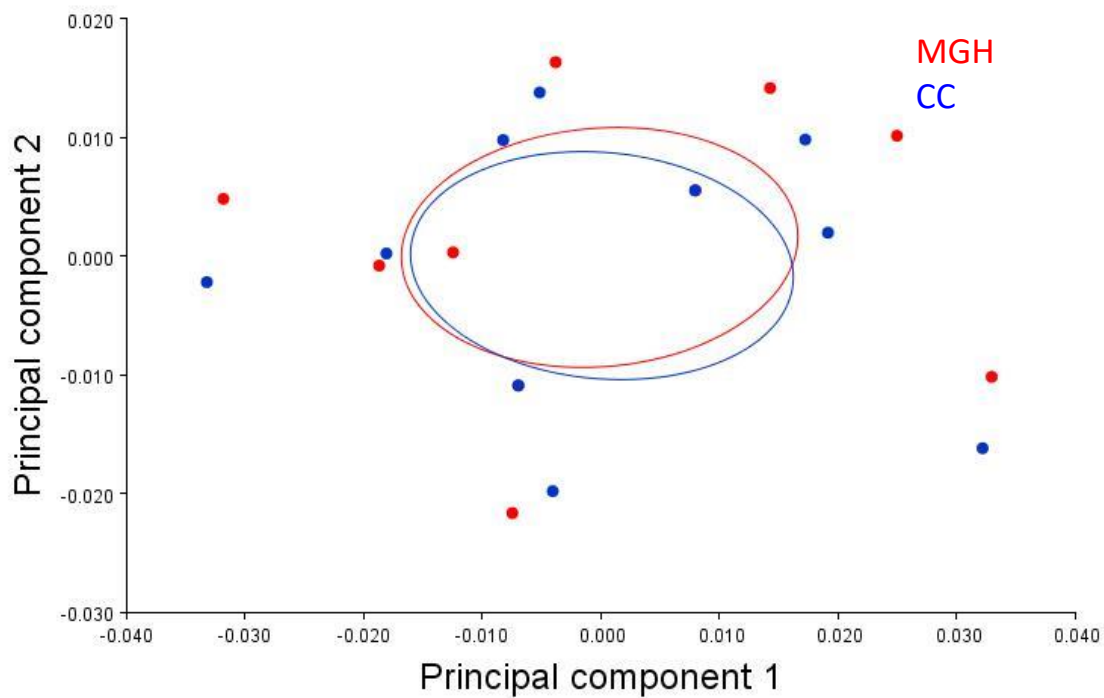


Fig. S2. Example of an inter-reliability test between observers MGH and CC demonstrating the consistency of landmarking (43 total landmarks) of the skull (16).