Table 1: Copper, iron, and zinc content of diets.

Diet	Cu (µg/g)	Fe (µg/g)	Zn (µg/g)
Control TD.08584	8.83 ± 0.96	84.0 ± 2.01	41.1 ± 0.19
CuD TD.08585	0.46 ± 0.03	78.3 ± 1.50	43.1 ± 0.68
FeD TD.08586	7.20 ± 0.60	5.29 ± 0.12	41.2 ± 1.57

Metal content of diets was determined by wet ashing and flame AAS and represents the mean ± SEM of 3 separate diet samples.

1 **Table 2: Diet Formulations**

Control Diet Formula (TD.08584)	g/kg
Casein, low Cu & Fe	200.0
L-Cystine	3.0
Corn Starch[2]	390.6843
Maltodextrin	132.0
Sucrose	107.7
Soybean Oil	70.0
Cellulose	50.0
Vitamin Mix, AIN-93-VX (94047)	10.0
Choline Bitartate	2.5
TBHQ, antioxidant	0.014
Calcium Carbonate	15.75
Potassium Phosphate, monobasic	10.2
Potassium Citrate, monohydrate	2.4773
Sodium Chloride	2.59
Potassium Sulfate	1.631
Magnesium Oxide	0.8505
Zinc Carbonate	0.0578
Manganous Carbonate	0.0221
Potassium Iodate	0.0004
Sodium Selenate	0.0004
Ammonium Paramolybdate, tetrahydrate	0.0003

Sodium Meta-Silicate, nonahydrate	0.0508
Chromium Potassium Sulfate, dodecahydrate	0.0096
Lithium Chloride	0.0006
Boric Acid	0.0029
Sodium Fluoride	0.0022
Nickel Carbonate Hydroxide, tetrahydrate	0.0011
Ammonium Meta-Vanadate	0.0002
Cupric Carbonate[2]	0.0145
Ferric Citrate[2]	0.44

All diets contain 3.7 kcal/g (19% from protein, 63.7% from carbohydrate, 17.3% from fat). The Cu-deficient (CuD) and Fe-deficient (FeD) diet formulas are the same as the control diet except the CuD diet (TD.08585) does not contain cupric carbonate and the FeD diet (TD.08586) does not contain ferric citrate, with the amount of corn starch adjusted to account for the mass of cupric carbonate or ferric citrate removed.

Table 3: qRT-PCR primer sequences

Gene	PCR Primer Sequences	Position	Product length (bp)
Dio2	F: 5'-GAAGGACTACGCTGTGTCTGGAAC-3' R: 5'-TCGCTGAACCAAAGTTGACCAC-3'	172-195 385-364	214
Pvalb	F: 5'-AGCCTTTACTGCTGCAGACTCCTT-3' R: 5'-AGCTCATCCTCCTCAATGAAGCCA-3'	51-74 191-168	141
Mbp	F: 5'-GACTCACACACGAGAACT-3' R: 5'-CCAGCTAAATCTGCTGAG-3'	405-422 608-590	204
Mobp	F: 5'-TTCACCTTCCTCAACTCCAAGCGT-3' R: 5'-TGCTCCTCCTTCTCAATCTGGTCT-3'	88-111 223-200	136
Hr	F: 5'-TGGCCCTTGTAGGAAATGTCAGGA-3' R: 5'-TTTCAGCTTGGTGTGATGGCTTGG-3'	1092-1115 1215-1192	124
Rc3	F: 5'-TGCTCCAAGCCAGACGACGATATT-3' R: 5'-GGCCACACTCTCCACTCTTTATCT-3'	25-48 157-134	133
Fth1	F: 5'-TGATGTGGCCCTGAAGAACTTTGC-3' R: 5'-AGTGCACACTCCATTGCATTCAGC-3'	135-158 314-291	180
Cox IV	F: 5'-GCACATGGGAGTGTTGTGAAGAGT-3' R: 5'-TTTCTCATCCTGGAAAGGCTGCT-3'	67-90 234-211	168
Gapdh	F: 5'-TTCCTACCCCCAATGTATCCG-3' R: 5'-ACCACCCTGTTGCTGTAGCCA-3'	698-718 968-948	271
Ftl	F: 5'-TTTGATCGGGATGACGTGGCTTTG-3' R: 5'-ATGGCTTCTGCACATCCTGGAAGA-3'	112-135 256-233	145

Genes: Type II deiodinase, *Dio2*; Parvalbumin, *Pvalb*; Myelin basic protein, *Mbp*; Myelin-associated oligodendrocyte basic protein**,** *Mobp*; Hairless, *Hr*; Neurogranin, *Rc3*; Ferritin H, *Fth1*; Cytochrome c oxidase subunit IV, *CoxIV;* Glyceraldehyde 3-phosphate dehydrogenase, *Gapdh;* Ferritin L, *Ftl.* F = Forward, R = Reverse