Supporting Information

Metabolomics Analysis of Effects of Commercial Soy-based Protein Products in Red Drum (*Sciaenops ocellatus*)

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SUPPORTING INFORMATION

TABLE OF CONTENTS

Part I: Experimental Procedures

Animal Husbandry

Captive, wild red drum broodstock were volitionally spawned at the Marine Resources Research Institute (MRRI) in Charleston, South Carolina, by the South Carolina Department of Natural Resources (SCDNR). Larval fish grown from a single unique genetic family were transported and stocked into earthen ponds at the Waddell Mariculture Center (WMC, Bluffton, SC), harvested at a mean length of 30 mm and transported and held at the Marine Resources Research Institute (MRRI) in eight, 1,600 L recirculating culture tanks at 21 °C and constant salinity (30 mg/L to 32 mg/L). During this holding period, fish were fed to apparent satiation twice daily using a standard commercial feed containing 40 % crude protein and 10 % crude lipid. At the end of the holding period, fish were selected based on comparable weights and transported to an indoor, semi-recirculating seawater system where they were distributed into 24 x 1,100 L 1.52 m diameter experimental tanks at a density of 35 fish per tank. Subsequently, fish were fed twice daily to satiation on a pelleted soy-free conditioning diet (Table S1) for one month prior to the start of the experiment. Water temperature was increased by four degrees to 25 °C over a two-week period to minimize stress.

Limited water exchanges were performed as needed based on water quality parameters utilizing settled, polished seawater from the Charleston Harbor. Water temperature, salinity, dissolved oxygen, and pH were recorded two times per week using a YSI Pro Plus handheld meter (YSI, Inc., Yellow Springs, OH, USA) and total ammonia, nitrite

and nitrate were monitored weekly using a Hach spectrophotometer and reagents (Hach Company, Loveland, CO, USA) on a subset of tanks.

Plasma Collection and Metabolite Extraction for NMR analysis

Using a syringe equipped with a 22-gauge needle, 1 ml to 2 mL of blood from the fish caudal vasculature were collected into lithium heparin collection tubes and gently inverted eight times. The collection tubes were rapidly placed on ice. Blood samples were then centrifuged at 2,000 x g for 6 min at 4 °C. The top layer (plasma) was transferred into pre-labeled cryovials, flash frozen in liquid nitrogen and stored at -80 °C, until further processing.

Frozen plasma samples were thawed on ice for approximately 2 h. 400 µL of plasma per sample were loaded onto Nanosep 3 kDa molecular weight cutoff spin filters (Pall Life Sciences, Pall Corporation, Port Washington, NY, USA) that had been previously washed with Millipore DI water overnight to remove glycerol present in the filters. Filters were then centrifuged at 10000 g for 90 min at 4 °C and for up to two times an additional 30 min for samples that provided less than 200 µL of filtrate. 200 µL of filtrate were transferred into Eppendorf tubes (Eppendorf). 400 µL of NMR buffer (100 mmol/L phosphate buffer in D_2O, pH 7.3, with 1.0 mmol/L TMSP as an internal NMR chemical shift standard) were added to each sample to a final volume of 600 µL, the samples were then vortexed for a few seconds and centrifuged. A total of 550 µL of the resulting solution was transferred into 5-mm NMR tubes (Bruker Biospin) for NMR analysis.

Metabolomics Quality Control Findings

The median % RSD for LCM (n = 34) was 7.1 % with an interquartile range from 4.5 % to 10.8 %, while the % RSD for SRM 1946 (for liver; n = 33) was 8.9 % with an interquartile range from 4.5 % to 17.1 % (Figure S2). The median % RSD for MCM (n = 36) was 7.6 % with an interquartile range from 3.5 % to 13.9 %, while the % RSD for SRM 1946 (for muscle; n = 33) was 7.3 % with an interquartile range from 3.7 % to 13.3 % (Figure S3). The median % RSD for CP (n = 22) was 5.0 % with an interquartile range from 2.7 % to 10.2 %, while the % RSD for SRM 1950 (n = 19) was 5.1 % with an interquartile range from 2.6 % to 10.6 % (Figure S4).

NMR Spectroscopy Data Acquisition Details

All NMR experiments were performed at 298 K on a Bruker Avance II 700 MHz spectrometer (Bruker Biospin) equipped with a 5 mm triple-resonance, z-gradient TCI cryoprobe. 5 mm sample tubes were placed in 96-well racks for the refrigerated holding stage SampleJet sample changer (Bruker Biospin). Spectra were collected under full automation using ICON-NMR (Bruker Biospin) with water suppression using a three-pulse sequence based on a standard one-dimensional (1D) nuclear Overhauser effect spectroscopy (NOESY) pulse sequence with presaturation (noesygppr1d). The NMR protocol included 10 min for temperature equilibration, automated shimming with on-axis and off-axis shims, automated probe tuning and pulse calibration on each individual sample. 1D ^{1}H spectra were acquired with a spectral width of 20 ppm, a 3 s relaxation delay, 80 transients and 8 steady-state scans, collected into 65536 real data points. A

60 ms mixing period was used for solvent suppression and an acquisition time of 2.34 s for a total repetition time (D1 + AQ) of 5.34 s. The resulting spectra were processed by zero-filling to 65536 complex points and by multiplying the free induction decay by an exponential line broadening function of 0.3 Hz prior to Fourier transformation. The spectra were manually phased using Topspin 3.2 (Bruker Biospin), the baseline was automatically corrected by applying a fifth order polynomial and the chemical shift was calibrated by setting the standard TMSP peak at 0.00 ppm (also using Topspin 3.2 (Bruker Biospin)). An additional 2D homonuclear ^{1}H-^{1}H *J*-resolved (JRES) spectrum was collected resulting in a total NMR experiment time of approximately 45 min per sample. Samples that showed inadequate water suppression or which showed overly broad linewidth were re-run to achieve better results.

Two-dimensional edited ^{1}H,^{13}C-HSQC spectra with adiabatic ^{13}C decoupling (hsqcedetgpsisp2.2) were collected on selected samples to aid metabolite identification. In general, 2048 data points with 128 scans and 512 increments were acquired with spectral widths of 11 ppm in F2 and 180 ppm in F1 (^{13}C). A relaxation delay equal to 1.5 s was used between acquisitions and a refocusing delay corresponding to a 145 Hz $^{1}J_{\text{C-H}}$ coupling was used. The FIDs were weighted using a shifted sine-square function in both dimensions. Manual two-dimensional phasing was applied; all spectra were referenced to the TMSP internal standard at 0.00 ppm for ^{1}H and ^{13}C.

Part II: Supporting Figures

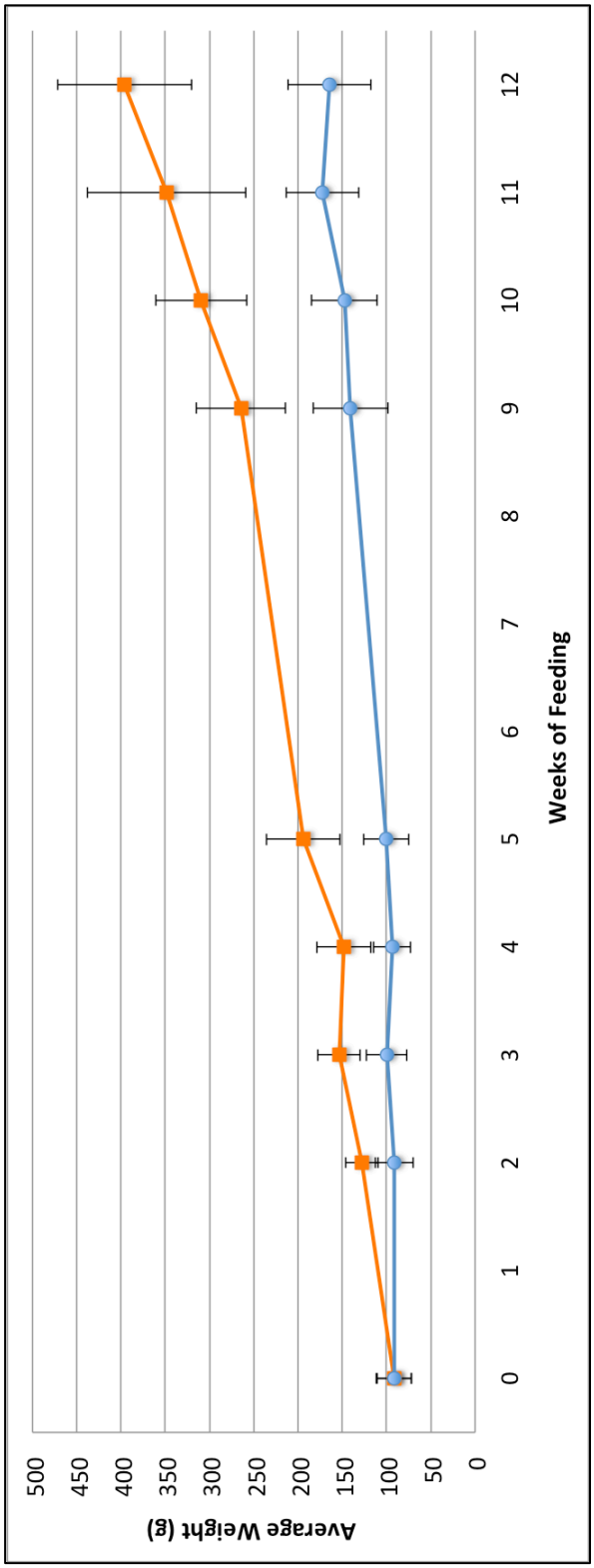

Figure S1. Average weights per time point of red drum fish fed either the soy-based diets (blue) or the natural diet (orange). Single data points for the soy diets are an average of the fish weights for diets #1 to #5 at each time point. Error bars represent mean ± SD.

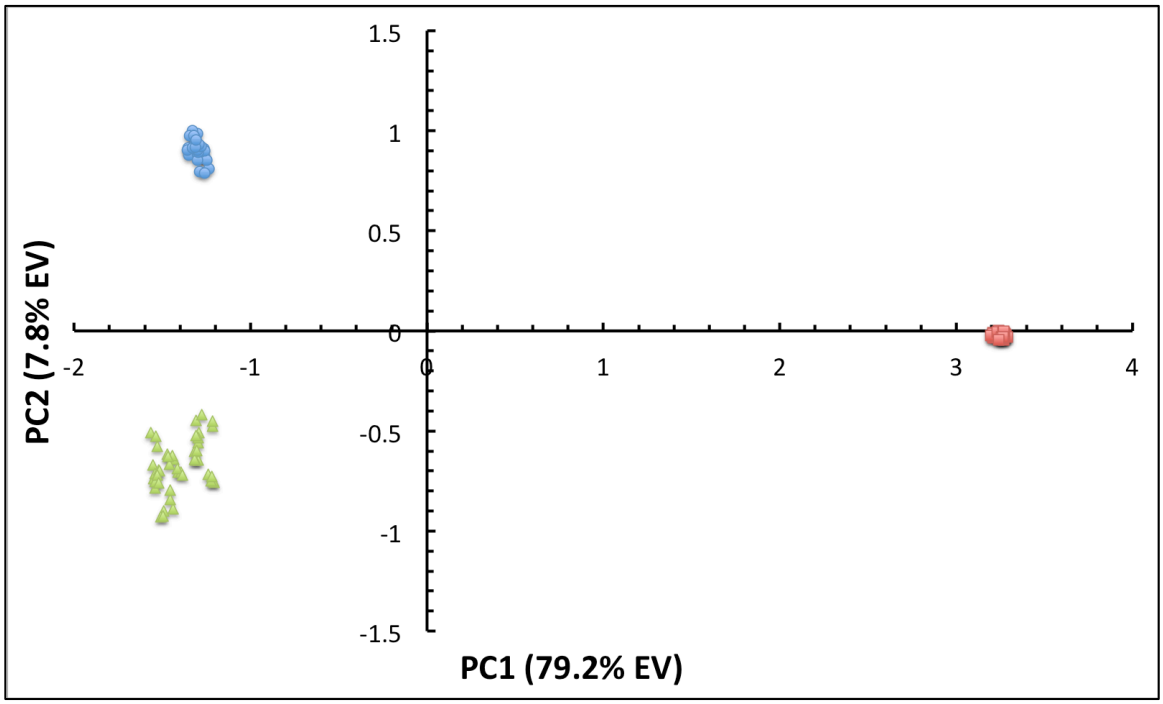

Figure S2. Liver QC sample PCA score plot. LCM, liver control material (blue circles; n = 34); NIST SRM 1946, standard reference material (red squares; n = 33). Technical replicate samples are displayed as green triangles.

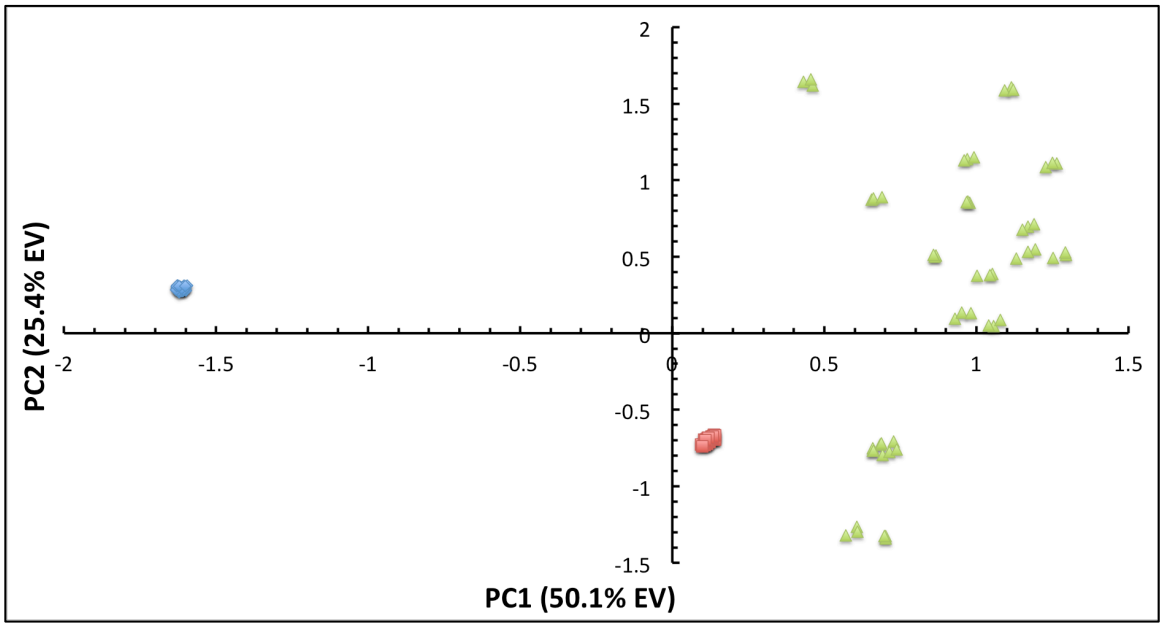

Figure S3. Muscle QC sample PCA score plot. MCM, muscle control material (red squares; n = 36); NIST SRM 1946, standard reference material (blue diamonds; n = 33). Technical replicate samples are displayed as green triangles.

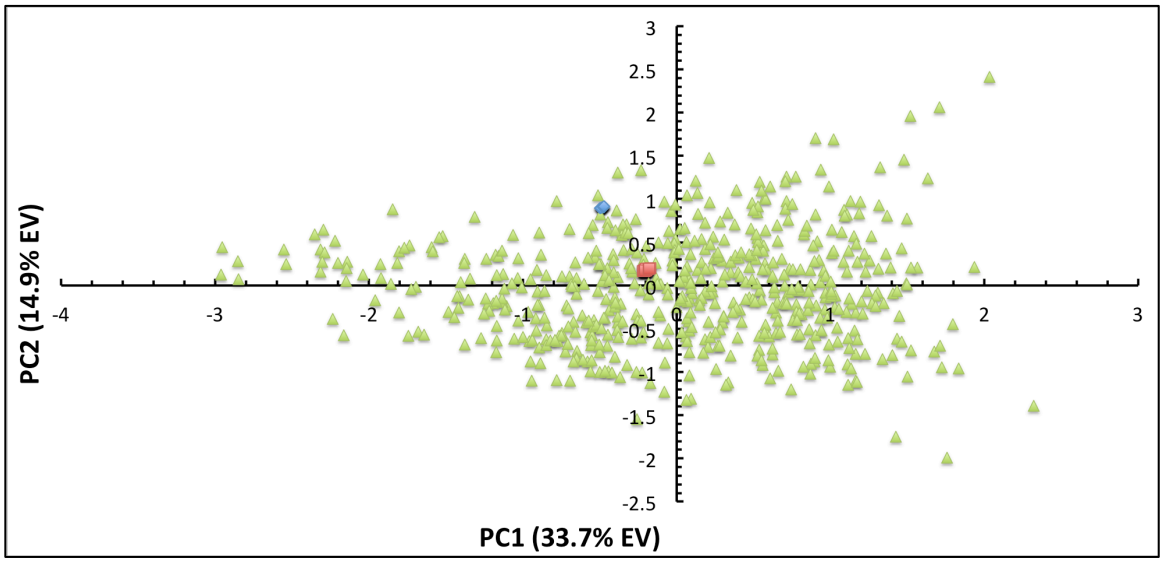

Figure S4. Plasma QC sample PCA score plot. CP, control plasma (red squares; n = 22); NIST SRM 1950, standard reference material (blue diamonds; n = 19). Experimental samples (green triangles; n = 571) are also displayed.

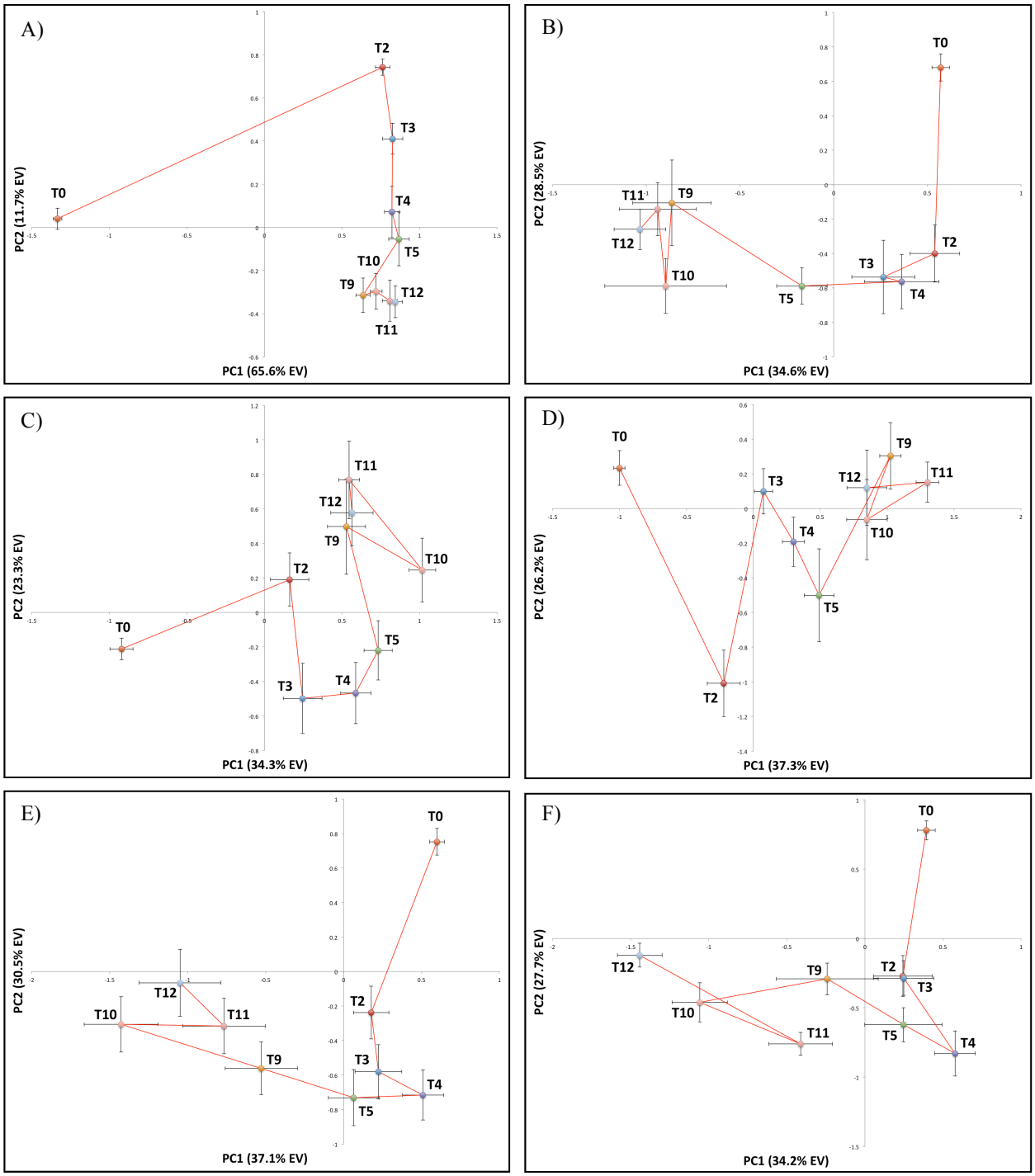

Figure S5. Unsupervised PCA score plots derived from ^{1}H NOESY 1D NMR spectra from red drum muscle tissue (independent models). A) Natural diet; B) diet #1 (60 %

soybean meal); C) diet #2; D) diet #3; E) diet #4; F) diet #5. Sampled time points were T_0 (at the end of the conditioning period), T_2 to T_4 and T_9 to T_{12} for sampling at week 2 to week 4 and week 9 to week 12, respectively. Error bars represent the mean ± 1 SEM.

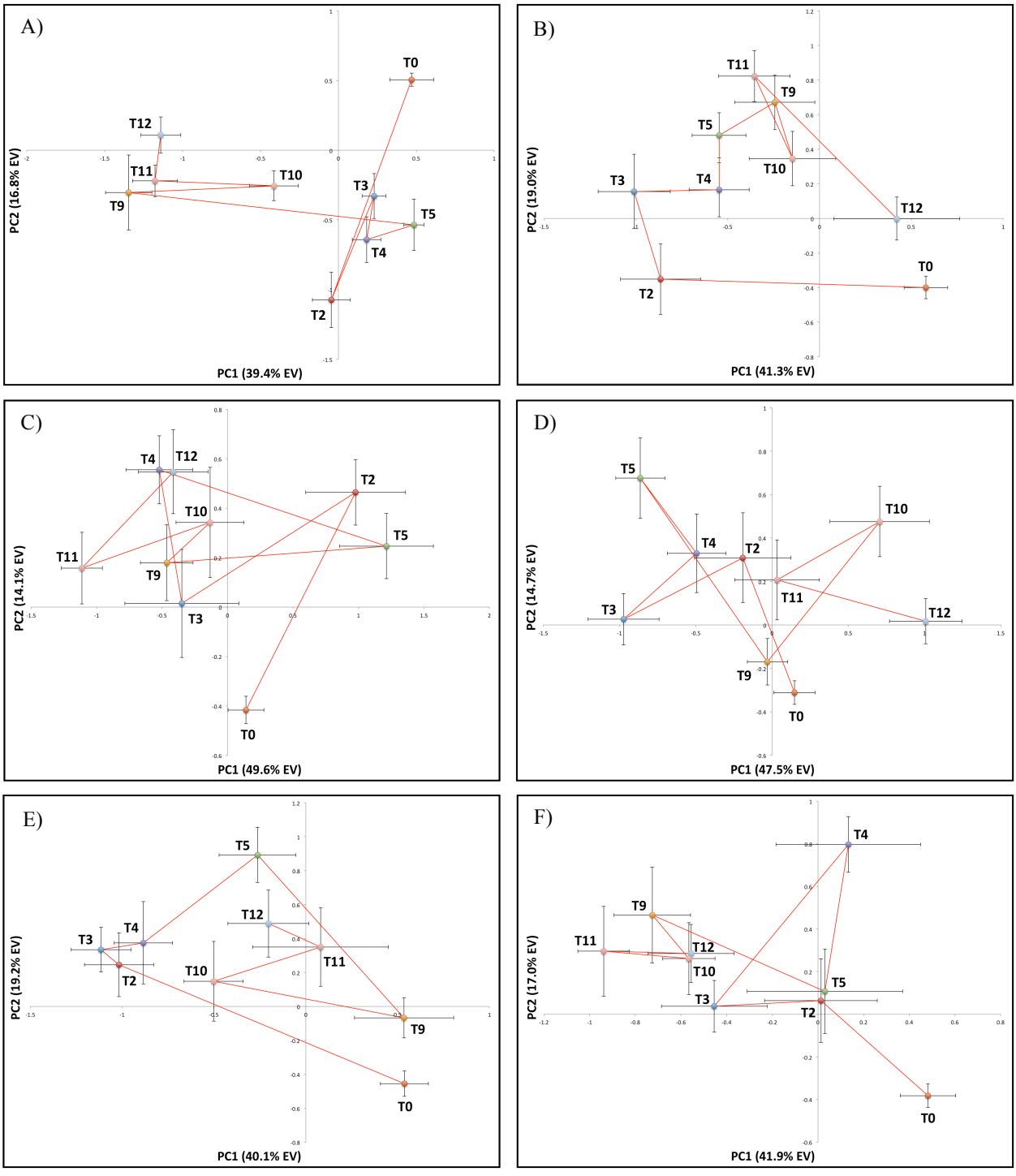
A)
PC2 (16.8% EV)
PC1 (39.4% EV)
T0
T12
T11
T10
T9
T3
T5
T4
T2
B)
PC2 (19.0% EV)
PC1 (41.3% EV)
T11
T9
T5
T10
T3
T4
T12
T0
T2
C)
PC2 (14.1% EV)
PC1 (49.6% EV)
T4
T12
T2
T10
T5
T11
T9
T3
T0
D)
PC2 (14.7% EV)
PC1 (47.5% EV)
T5
T10
T4
T2
T3
T11
T12
T9
T0
E)
PC2 (19.2% EV)
PC1 (40.1% EV)
T5
T12
T3
T4
T11
T10
T2
T9
T0
F)
PC2 (17.0% EV)
PC1 (41.9% EV)
T4
T9
T11
T12
T10
T5
T3
T2
T0

Figure S6. Unsupervised PCA score plots derived from ^{1}H NOESY 1D NMR spectra from red drum plasma (independent models). A) Natural diet; B) diet #1 (60 % soybean meal); C) diet #2; D) diet #3; E) diet #4; F) diet #5. Sampled time points were T_0 (at the end of the conditioning period), T_2 to T_4 and T_9 to T_{12} for sampling at weeks 2 to week 4 and week 9 to week 12, respectively. Error bars represent the mean ± 1 SEM.

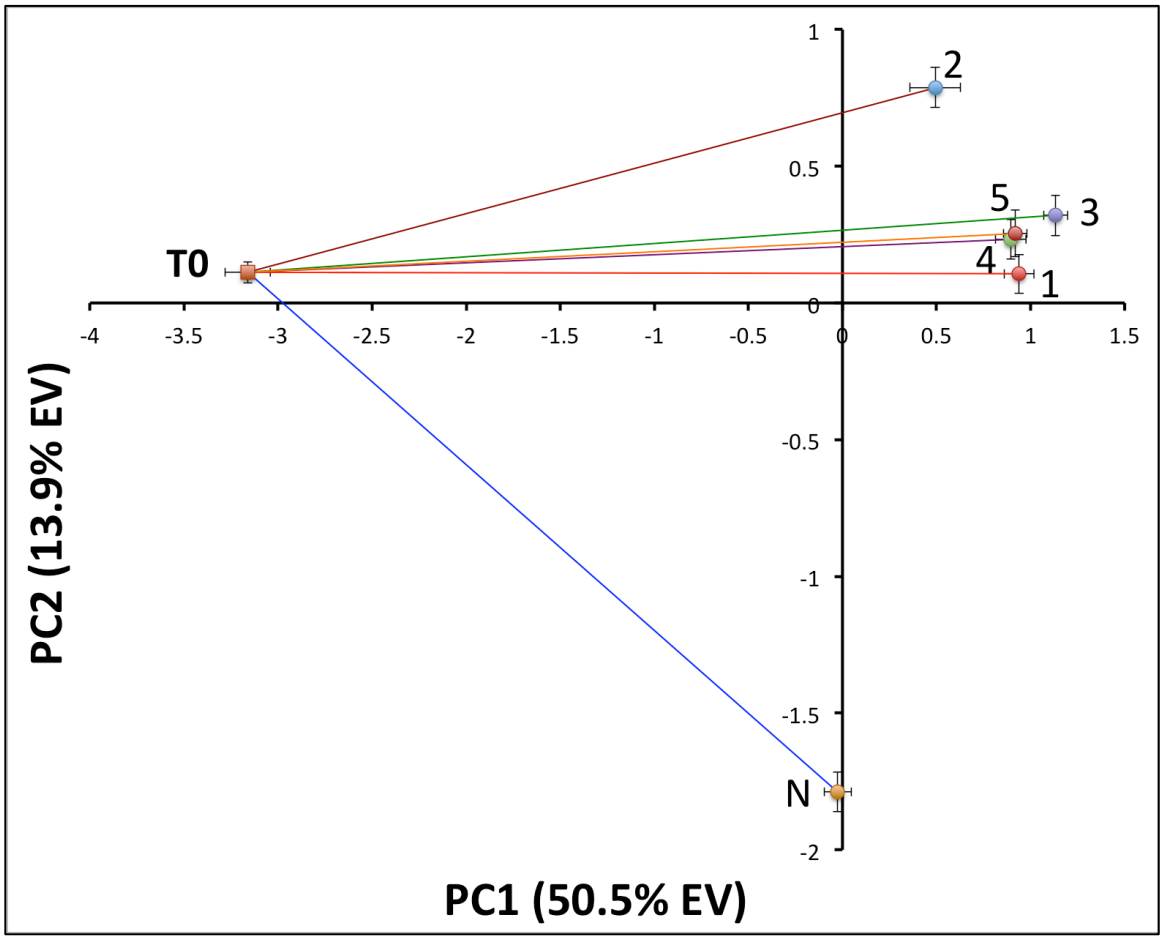

Figure S7. Liver PCA score plots for the five soy-based experimental diets (diet #1 to diet #5) and the natural diet (N) comparing T_0 and T_{end} time points. Error bars represent the mean ± 1 SEM.

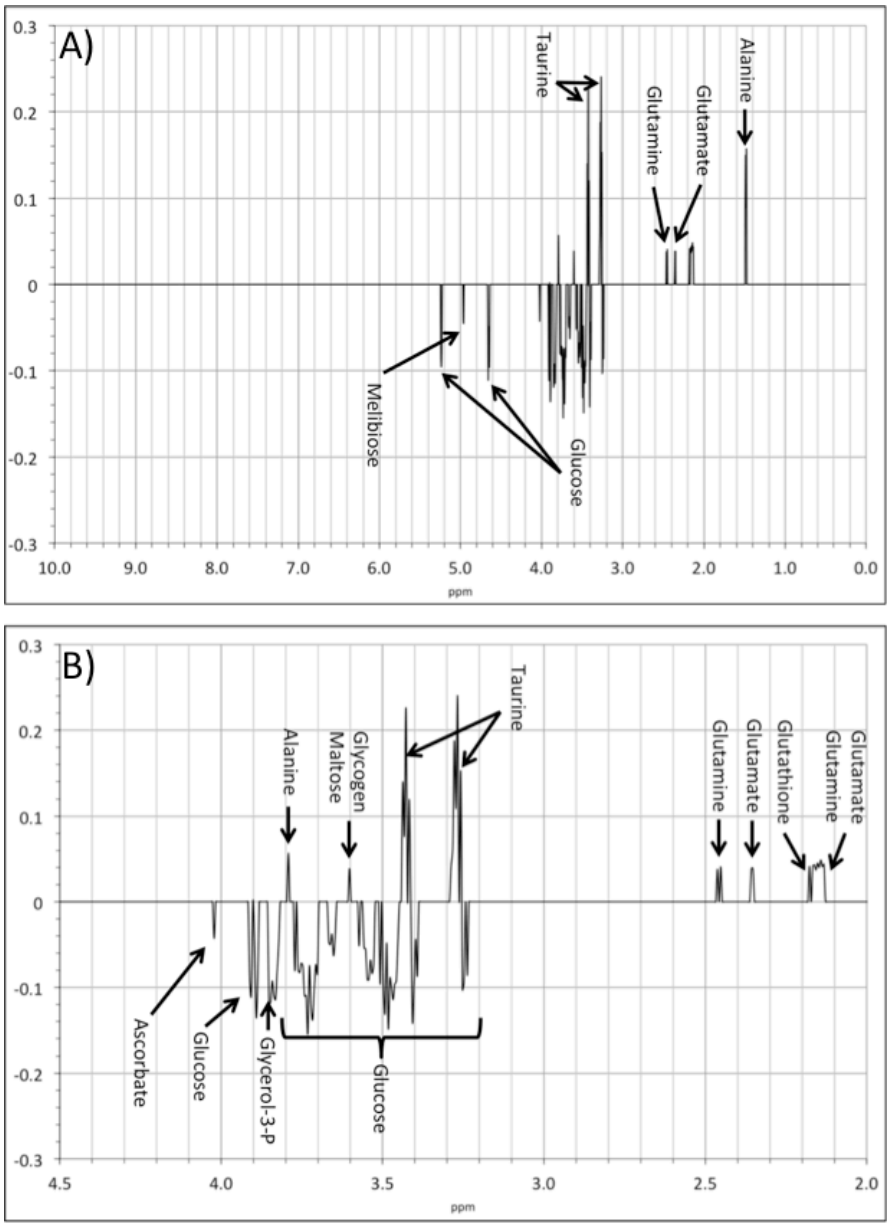

Figure S8. (A) Liver T_0-T_{end} PC1 loading plot (95th percentile) for the five experimental diets (diet #1 to diet #5) and the natural diet. (B) Expansion of the region 2.0 ppm to 4.5 ppm. Loadings with a negative sign indicate metabolites that are present at higher levels at T_0 and lower at T_{end} and vice versa.

Part III: Supporting Tables

Table S1. Composition of experimental diets for this study.

Grams/100 grams	Conditioning	Diet #1	Diet #2	Diet #3	Diet #4	Diet #5
Soy Protein Concentrate 3	0.00	0.00	0.00	55.35	0.00	0.00
Soy Protein Concentrate 4	0.00	0.00	0.00	0.00	47.20	0.00
Soy Protein Concentrate 5[a]	0.00	0.00	0.00	0.00	0.00	50.41
Soy Protein Concentrate 2[b]	0.00	0.00	43.10	0.00	0.00	0.00
Soybean meal[c]	0.00	59.00	0.00	0.00	0.00	0.00
Wheat flour[d]	36.42	11.38	27.93	13.48	22.42	20.32
Wheat gluten meal	0.00	5.90	3.80	5.30	4.57	4.46
Poultry by-product meal	18.10	0.00	0.00	0.00	0.00	0.00
Corn protein concentrate	18.10	0.00	0.00	0.00	0.00	0.00
Blood meal	5.00	0.00	0.00	0.00	0.00	0.00
Menhaden oil[e]	10.20	12.75	14.20	14.35	14.45	13.42
Squid meal, CSF	4.08	4.08	4.08	4.08	4.08	4.08
Lysine HCl	2.40	1.68	1.61	1.95	1.60	1.87
Methionine	0.60	0.71	0.72	0.77	0.72	0.77
Threonine	0.80	0.30	0.26	0.47	0.26	0.47
Mono-Dical phosphate	2.40	2.30	2.40	2.35	2.40	2.30
Vitamin premix[f]	1.00	1.00	1.00	1.00	1.00	1.00
Choline CL	0.60	0.60	0.60	0.60	0.60	0.60
Vitamin C[g]	0.20	0.20	0.20	0.20	0.20	0.20
Trace min premix[h]	0.10	0.10	0.10	0.10	0.10	0.10
Formulated Composition, % as-is						
Crude Protein	41.08	40.06	40.08	40.06	40.03	40.10
Lipid	14.08	15.01	15.05	15.05	15.04	15.02
Phosphorus	0.92	0.90	0.90	0.91	0.91	0.92

[a] 560 g/kg crude protein.
[b] 693 g/kg crude protein.
[c] ADM, 468 g/kg crude protein
[d] Manildra Milling, 120 g/kg crude protein.
[e] Omega Proteins Inc., Virginia Prime menhaden oil .
[f] ARS 702; contributed, per kg diet; vitamin A 9650 IU; vitamin D 6600 IU; vitamin E 132 IU; vitamin K3 1.1 gm: thiamin mononitrate 9.1 mg; riboflavin 9.6 mg; pyridoxine hydrochloride 13.7 mg; pantothenate DL-calcium 46.5 mg; cyancobalamin 0.03 mg; nicotinic acid 21.8 mg; biotin 0.34 mg; folic acid 2.5 mg; inostitol 600 mg.
[g] Stay-C, 35%, DSM Nutritional Products.

Table S2. Proximate analyses for whole body. ANOVA (P = 0.05) to test for significant differences between dietary treatments (natural diet excluded). Values reported as mean ± 1 S.D. Values with different superscripts are significantly different from one another.

Diet	Dry matter (%)	Protein (%)	Fat (%)	Ash (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Fe (ppm)	S (ppm)	Na (ppm)
Diet #1	24.35 ± 0.01^{a}	73.18 ± 2.07	7.43 ± 2.29	16.53 ± 2.47	27200 ± 2690^{a,b}	12600 ± 370^{a,b,c}	49800 ± 4820^{a,b}	1510 ± 149	48.4 ± 3.0	24.6 ± 12.9	20.1 ± 4.0	53.6 ± 10.7	9460 ± 906	6000 ± 663
Diet #2	25.73 ± 0.01^{a,b}	70.85 ± 4.29	9.77 ± 1.11	14.05 ± 1.58	24400 ± 1380^{a}	12000 ± 528^{a}	43100 ± 4480^{a}	1430 ± 82	43.8 ± 3.6	12.0 ± 4.5	16.6 ± 2.5	44.7 ± 5.9	9580 ± 329	5480 ± 299
Diet #3	26.13 ± 0.01^{b}	76.35 ± 6.43	8.46 ± 1.85	15.54 ± 1.26	26800 ± 1690^{a,b}	12900 ± 497^{b,c}	46100 ± 4700^{a,b}	1500 ± 58	48.2 ± 3.0	18.3 ± 9.7	15.1 ± 1.7	46.2 ± 6.8	10200 ± 433	5480 ± 342
Diet #4	25.08 ± 0.00^{a,b}	76.69 ± 5.47	8.41 ± 1.26	15.05 ± 1.48	26100 ± 2420^{a,b}	13200 ± 674^{c}	45100 ± 6400^{a,b}	1430 ± 99	46.5 ± 5.4	15.7 ± 3.2	18.9 ± 2.6	49.8 ± 12.9	10300 ± 354	5840 ± 307
Diet #5	26.03 ± 0.01^{b}	70.88 ± 3.43	10.61 ± 1.52	15.96 ± 2.47	29500 ± 2040^{b}	12400 ± 571^{a,b}	52500 ± 6590^{b}	1590 ± 123	49.2 ± 2.8	16.6 ± 5.8	15.5 ± 3.6	50.8 ± 9.8	9650 ± 548	5560 ± 656
Natural	28.15 ± 0.00	66.49 ± 2.84	12.34 ± 1.26	15.74 ± 1.00	29000 ± 1740	11100 ± 582	49800 ± 4070	1540 ± 67	43.6 ± 2.7	15.5 ± 6.8	6.7 ± 0.9	37.9 ± 5.2	8290 ± 422	4220 ± 215
P	0.007	0.059	0.059	0.316	0.006	0.001	0.041	0.080	0.175	0.176	0.059	0.450	0.055	0.364

Table S3. Proximate analyses for fillets. ANOVA (P = 0.05) to test for significant differences between dietary treatments (natural diet excluded). Values reported as mean ± 1 S.D. Values with different superscripts are significantly different from one another.

Diet	Dry matter (%)	Protein (%)	Fat (%)	Ash (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Fe (ppm)	S (ppm)	Na (ppm)
Diet #1	24.62 ± 0.04	87.59 ± 1.31[a]	1.00 ± 0.12	4.77 ± 0.21[a]	9400 ± 276	19110 ± 348	784 ± 408	1370 ± 66	28.4 ± 4.4	50.1 ± 101	1.99	64.3 ± 107	11700 ± 426	1700 ± 467
Diet #2	22.79 ± 0.04	87.56 ± 2.07[a]	1.10 ± 0.27	4.55 ± 0.21[b]	7310 ± 3440	14300 ± 6905	542 ± 250	1070 ± 502	20.4 ± 9.7	6.15 ± 5.53	< 1.00	25.4 ± 19.0	8410 ± 3930	1230 ± 586
Diet #3	24.31 ± 0.06	95.41 ± 1.72[b]	0.62 ± 0.22	5.14 ± 0.17[a,b]	9960 ± 564	18700 ± 652	876 ± 262	1470 ± 72	29.2 ± 4.2	7.59 ± 2.99	1.27 ± 0.13	41.8 ± 13.4	11800 ± 1160	1750 ± 115
Diet #4	21.93 ± 0.05	86.65 ± 2.07[a]	0.82 ± 0.04	5.20 ± 0.19[a]	10000 ± 535	19200 ± 504	1610 ± 1240	1430 ± 92	30.2 ± 10.1	13.5 ± 6.6	1.41 ± 0.01	53.6 ± 24.2	12600 ± 190	1930 ± 416
Diet #5	23.89 ± 0.02	86.36 ± 1.12[a]	1.02 ± 0.28	5.56 ± 1.58[a,b]	9520	18300	438	1280	22.2	9.13	2.10	71.1	10800	1340
Natural	23.82 ± 0.01	85.86 ± 2.18	1.60 ± 0.58	5.16 ± 0.18	9540 ± 257	17700 ± 720	911 ± 726	1250 ± 37	21.6 ± 3.6	16.0 ± 6.3	2.62 ± 1.63	63.4 ± 36.8	10700 ± 382	1280 ± 174
P	0.865	0.0001	0.588	0.011	0.274	0.328	0.132	0.273	0.259	0.727	n/a	0.867	0.143	0.267

Table S4. Production characteristics from the feeding trial. ANOVA (P = 0.05) to test for significant differences between dietary treatments. Natural diet feed consumption is wet weight and excluded from the ANOVA analysis. Values for the different parameters represent the mean ± SD of the fish sampled. Values with different letter superscripts are significantly different from one another.

Diet	Feed consumption (g/fish)	Weight gain (g)	Final weight (g)	Final length (mm)	FCR[1]	PER[2]	SGR[3]	Condition factor[4]
Diet #1	$152 \pm 6^{a,b}$	55 ± 17	145 ± 16	241 ± 26	2.99 ± 1.06	0.82 ± 0.23	0.57 ± 0.15	0.99 ± 0.12
Diet #2	123 ± 17^{a}	74 ± 24	164 ± 30	239 ± 22	1.80 ± 0.50	1.36 ± 0.32	0.70 ± 0.16	1.06 ± 0.13
Diet #3	$140 \pm 12^{a,b}$	89 ± 24	179 ± 25	258 ± 16	1.64 ± 0.35	1.37 ± 0.29	0.81 ± 0.15	1.08 ± 0.06
Diet #4	157 ± 16^{b}	84 ± 39	177 ± 38	247 ± 27	2.20 ± 0.94	1.23 ± 0.46	0.74 ± 0.27	1.03 ± 0.05
Diet #5	$137 \pm 13^{a,b}$	75 ± 18	166 ± 18	255 ± 14	1.91 ± 0.46	1.18 ± 0.28	0.71 ± 0.14	1.05 ± 0.07
Natural	1201 ± 3	308 ± 28	398 ± 28	319 ± 18	1.30 ± 0.13[5]	1.05 ± 0.09[5]	1.78 ± 0.09	1.21 ± 0.05
P	0.021	0.449	0.432	0.169	0.117	0.174	0.479	0.150

[1]Feed conversion ratio (FCR, *dry feed/gain*) = I /(Wf – Wi), where W_f = final body weight (g), and W_i = initial body weight (g) of red drum; I (g) is the total amount of dry feed fed.
[2]Protein efficiency ratio (PER) = W gain (g) /protein intake (g).
[3]Specific growth rate (SGR) = [(ln (W_f) − ln (W_i)) x 100/ t], where ln (W_f) = natural log of the final wet weight of red drum, ln (W_i) = natural log of the initial wet weight of red drum, and t is the duration of the feeding trial in days.
[4]Condition factor (K, g/cm^3) = 100 x (W_f/L_f^3), where W_f (g) and L_s (cm) are the final body weight and body length, respectively.
[5]FCR and PER for natural diet calculated using dry weight of natural feed items (assuming 67% water content – derived from average weight of oven dried natural feed items).

Table S5. Eviscerated fish weight (g) and hepatosomatic index (HSI) at final sampling. ANOVA (P = 0.05) to test for significant differences between dietary treatments (natural diet excluded). Values reported as mean ± 1 S.D. Values with different letter superscripts are significantly different from one another.

Diet	Eviscerated Weight (g)	Hepatosomatic Index[1]
Diet #1	132.50 ± 52.64	0.94 ± 0.23[a]
Diet #2	137.17 ± 46.93	1.38 ± 0.44[b]
Diet #3	170.33 ± 32.22	1.04 ± 0.22[a]
Diet #4	147.33 ± 49.42	1.01 ± 0.21[a]
Diet #5	161.17 ± 31.13	1.25 ± 0.26[a,b]
Natural	366.00 ± 71.37	1.14 ± 0.18
P	0.183	0.002

[1]Hepatosomatic index (HSI) = [liver W (g)/ body W (g)] x 100.

Table S6. Quantiles of % RSD derived from QC sample NMR spectra. CP, control plasma; LCM, liver control material; MCM, muscle control material; SRM, standard reference material.

Level	Minimum	10%	25%	Median	75%	90%	Maximum
CP[1]	0.528	1.694	2.712	5.043	10.218	17.453	39.438
LCM[2]	1.479	3.403	4.504	7.061	10.837	16.025	41.351
MCM[3]	1.114	2.243	3.490	7.641	13.938	20.492	137.594
SRM[4] 1946 (Liver)	0.777	2.732	4.513	8.913	17.136	23.134	67.503
SRM[4] 1946 (Muscle)	0.701	2.122	3.662	7.275	13.306	21.100	96.500
SRM[4] 1950 (Plasma)	0.312	1.495	2.539	5.109	10.607	18.369	49.332

[1]CP = Control Plasma.
[2]LCM = Liver Control Material.
[3]MCM = Muscle Control Material.
[4]SRM = Standard Reference Material.

Table S7. Significant metabolites identified in the PCA liver and muscle models for the five experimental diets (diets #1 to diet #5) and the natural diet by comparing the T_0 and T_{end} time points (see liver the score plot (Figure S7) and related loading plot (Figures S8 and S9)). Compound identity was confirmed using ^{1}H, 2D JRES and ^{1}H, ^{13}C HSQC spectra.

Metabolites	^{1}H and ^{13}C Chemical shift (ppm), multiplicities and J_{HH} couplings (Hz)	Tissue
Alanine	(1.48 (d, J = 7.2 Hz), 19.0), (3.78 (q, J = 7.2 Hz), 53.3)	L
Ascorbate	(3.74 (m), 65.3), (4.02 (m), 72.4), (4.52 (d, J = 2.3 Hz), 81.3)	L
Glucose	(3.25 (dd, J_1 = 9.3 Hz, J_2 = 7.9 Hz), 77.0), (3.41 (m), 72.5), (3.46 (m), 78.7), (3.48 (t, J = 9.3 Hz), 78.7), (3.54, (dd, J_1 = 10.0 Hz, J_2 = 3.9 Hz), 74.3), (3.72 (m), 75.6), (3.72 (m), 63.6), (3.78 (dd, J_1 = 14.4, J_2 = 7.0 Hz), 63.4), (3.84 (m),74.3), (3.84 (m), 63.4), (3.90 (dd, J_1 = 12.4 Hz, J_2 = 2.4 Hz), 63.6), (4.65 (d, J = 7.7 Hz), 98.8), (5.24 (d, J = 3.8 Hz), 94.9)	L
Glutamate	(2.06 (m), 29.8), (2.13 (m), 29.8), (2.35 (m), 36.3), (3.76 (dd, J1 = 6.9 Hz, J2 = 4.7 Hz), 57.4)	L
Glutamine	(2.15 (m), 29.1), (2.46 (m), 33.7), (3.78 (t, J = 6.3 Hz), 57.0)	L
Glutathione	(2.17 (m), 29.0), (2.56 (m), 34.2), (2.96 (m), 28.3), (3.30, 41.6), (3.32, 41.6), (3.78 (m), 46.2), (3.79 (m), 57.0), (4.57 (dd, J_1 = 7.3 Hz, J_2 = 5.1 Hz), 58.6), (4.76, 55.5)	L
Glycerol 3-phosphate	(3.62 (dd, J_1 = 11.6 Hz, J_2 = 5.9 Hz), 65.0), (3.68 (dd, J_1 = 11.5 Hz, J_2 = 4.7 Hz), 65.1), (3.78 (m), 73.9), (3.80 (m), 67.7), (3.83 (m), 67.7), (3.84 (m), 74.0), (3.88 (m), 74.0), (3.89 (m), 74.0)	L
Glycogen	(3.47 (m), 72.4), (3.66 (m), 79.7), (3.77 (m), 75.8), (3.87 (m), 63.3), (3.96 (dd, J_1 = 10.0 Hz, J_2 = 8.6 Hz), 76.1), (3.98 (dd, J_1 = 10.8 Hz, J_2 = 8.5 Hz), 76.1), (5.40 (d, J = 3.8 Hz), 102.4), (5.41 (d, J = 4.5 Hz), 102.4)	L
4-Hydroxyproline	(2.15 (m), 40.2), (2.44 (m), 40.2), (3.37 (t, J = 1.9 Hz), 55.7), (3.38 (t, J = 1.9 Hz), 55.7), (3.48 (dd, J_1 = 11.5 Hz, J_2 = 3.8 Hz), 55.8), (3.50 (dd, J_1 = 12.4 Hz, J_2 = 3.6 Hz), 55.8), (4.35 (ddd, J_1 = 10.8 Hz, J_2 = 7.7 Hz, J3 = 1.1 Hz), 62.6), (4.67 (m), 72.9)	M
Lactate	(1.33 (d, J = 7.0 Hz), 22.8), (4.11 (q, J = 7.0 Hz), 71.3)	M
Maltose	(3.23, 56.9), (3.28, 77.0), (3.43, 72.6), (3.58, 74.3), (3.60, 77.4), (3.65, 79.5), (3.72, 75.3), (3.75, 63.4), (3.78, 79.0), (3.80, 63.3), (3.84, 63.3), (3.90, 63.4), (3.94 (m), 72.8), (3.98 (t, J = 9.1 Hz), 76.0), (4.64 (d), 98.6), (5.24 (d), 94.8), (5.42 (d, 3.8 Hz), 102.3)	L
Melibiose	(3.49, 78.7), (3.50, 72.3), (3.53, 72.3), (3.54, 75.5), (3.64, 77.2), (3.77, 68.5), (3.82, 70.7), (3.96 (m, 68.5), (4.96 (m), 100.8)	L
Proline	(2.01 (m), 26.6), (2.08 (m), 31.8), (2.35 (m), 31.8), (3.34 (m), 48.9), (3.42, 48.8), (4.14 (dd, J1 = 8.1 Hz, J2 = 5.8 Hz), 64.0)	M
Taurine	(3.27 (t, J = 6.7 Hz), 50.4), (3.42 (t, J = 6.7 Hz), 38.1)	L, M

Chemical shifts were referenced to the internal standard TMSP $\delta^{1}H$ 0.00. Key: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet. L, Liver; M, Muscle. Underlined chemical shifts indicate well-isolated signals used in metabolite level determination via bin intensities.