Supplemental Materials

An NMR-based metabolomics assessment of the effect of combinations of natural feed items in juvenile red drum, *Sciaenops ocellatus*

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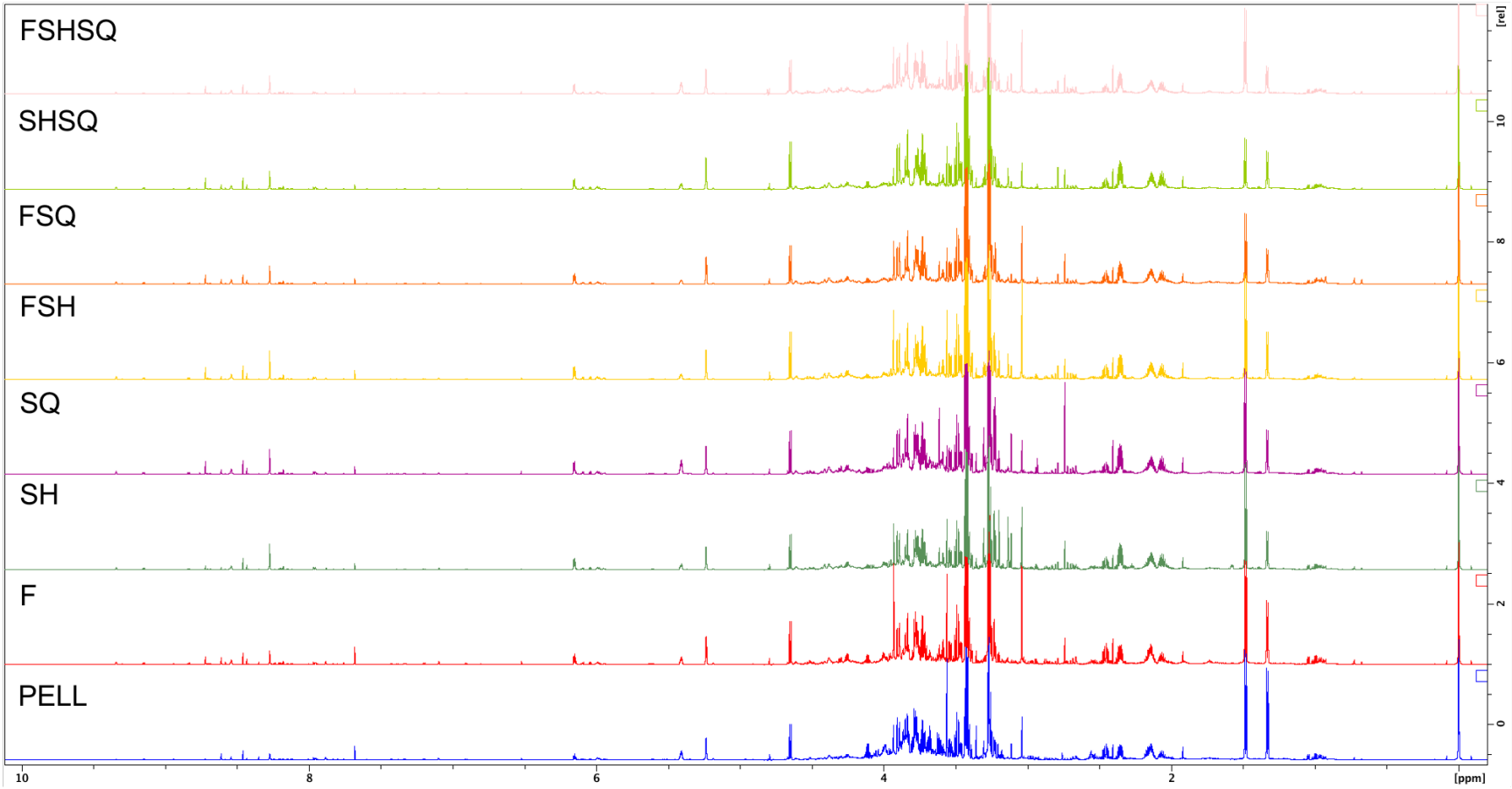

Figure S1. Representative 1D ^{1}H NMR spectra with water signal suppressed using the Bruker pulse sequence "noesygppr1d" of juvenile red drum liver extracts for fish fed the 7 experimental diets and the commercial pelleted diet. Spectra were acquired using a 700 MHz Bruker NMR spectrometer at 298K. Solvent: D_2O. Chemical shift reference: TMSP. PELL, pelleted commercial fishmeal-based diet; F, fish diet; SH, shrimp diet; SQ, squid diet; FSH, fish + shrimp diet; FSQ, fish + squid diet; SHSQ, shrimp + squid diet; FSHSQ, fish + shrimp + squid diet.

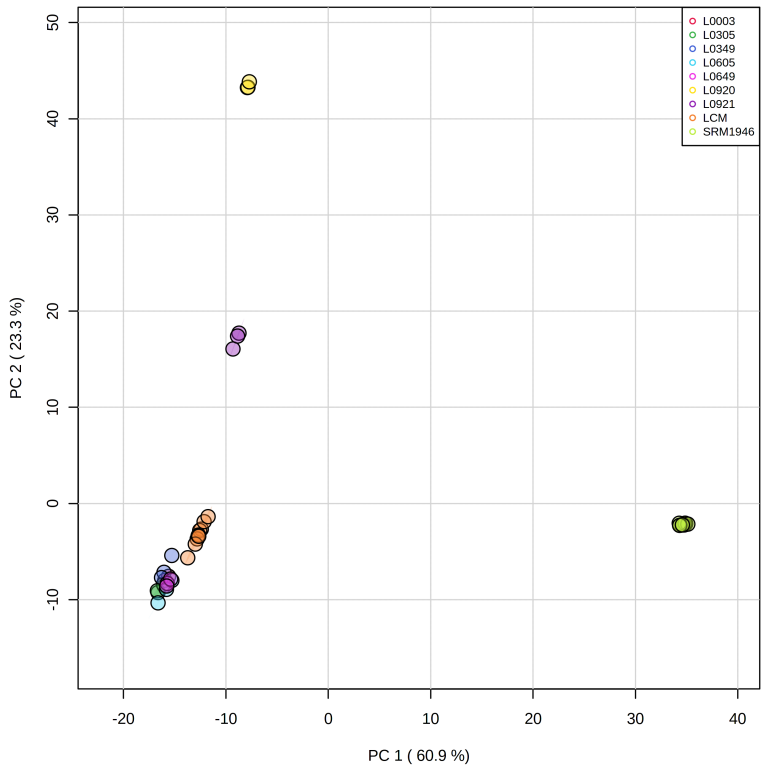

Figure S2. Liver QC samples PCA score plot. LCM, liver control material (orange circles; n=12; RSD=7.3%); NIST SRM 1946, standard reference material (light green circles; n=12; RSD=6.7%). Technical replicate samples (n=3/sample) are labeled with their respective sample IDs.

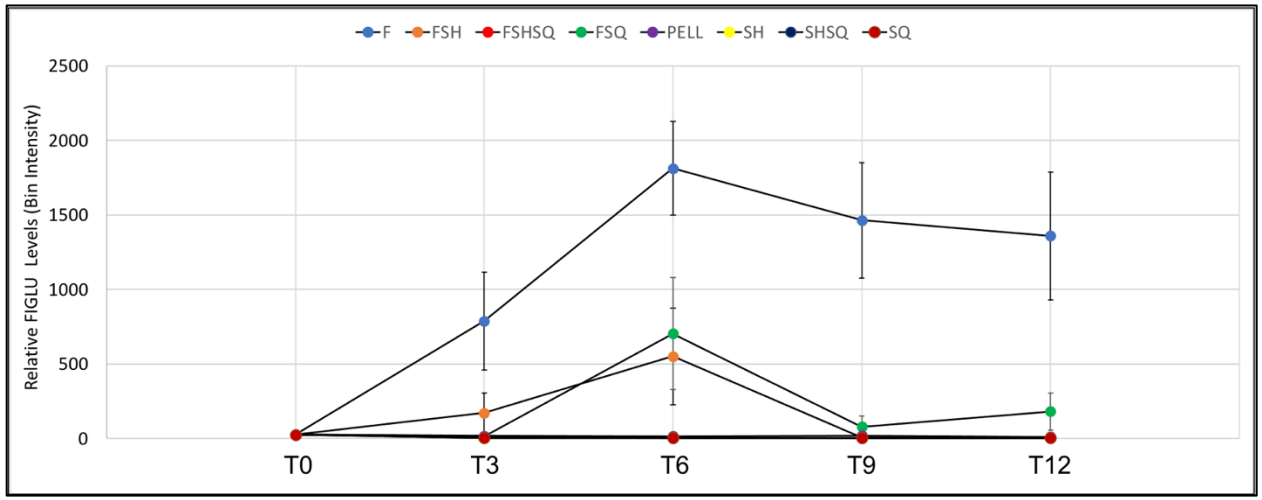

Figure S3. Relative hepatic FIGLU levels (bin intensity, 7.83 ppm) measured over the course of the 12-week juvenile red drum feeding trial at T0, T3, T6, T9 and T12. Each data point represents the mean ± SEM (*n*=12, T0; *n*=9, T3, T6, T9, T12). FIGLU, N-formimino-L-glutamate. F, fish diet; FSH, fish + shrimp diet; FSHSQ, fish + shrimp + squid diet; FSQ, fish + squid diet; PELL, pelleted commercial fishmeal-based diet; SH, shrimp diet; SHSQ, shrimp + squid diet; SQ, squid diet.

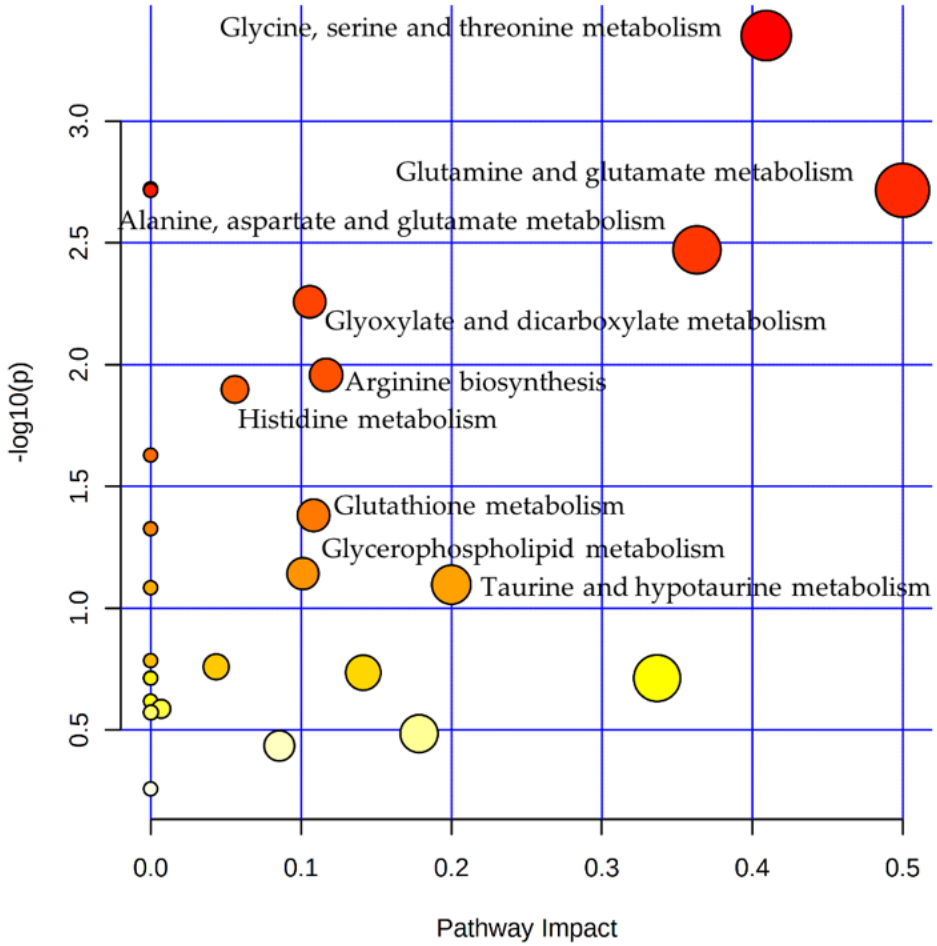

Figure S4. Metabolomic pathway analysis overview showing the metabolic pathways that are mostly impacted by the different dietary treatments. The diameter of the circles (nodes) indicates the pathway impact; the color of the nodes is graded from white to red with increasing *p* value derived from enrichment analysis.