The Journal of Experimental Biology 215, 1627-1632

doi:10.1242/jeb.059873

METHODS & TECHNIQUES

Evaluation of a tandem gas chromatography/time-of-flight mass spectrometry metabolomics platform as a single method to investigate the effect of starvation on whole-animal metabolism in rainbow trout (*Oncorhynchus mykiss*)

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Accepted 1 February 2012

SUMMARY

This study was conducted to evaluate the use of a two-dimensional gas chromatography/time-of-flight mass spectrometry (GC×GC/TOF-MS) metabolomic platform to comprehensively analyze the effect of starvation on whole-animal metabolism in rainbow trout (*Oncorhynchus mykiss*). Trout were either fed a commercial diet at 2% body mass twice daily or starved for 4 weeks. Metabolomic analysis was conducted on serum, liver and muscle tissue from each fish. Database searching and statistical analysis revealed that concentrations of more than 50 positively identified molecules changed significantly ($P<0.05$) as a result of starvation. Our results indicate that starving rainbow trout for 4 weeks promotes increased utilization of select tissue fatty acids in liver and muscle. However, starvation did not significantly affect protein catabolism in peripheral tissues, as indicated by reductions in the level of serum amino acids in starved fish. In contrast, starvation appears to promote protein catabolism in liver as the level of methionine, proline and lysine metabolite 2-piperidine carboxylic acid increased significantly. Also, starvation resulted in significant changes in the level of numerous xenobiotics that could indicate the origin of particular feed ingredients and selective retention of these molecules in tissues. We suggest that metabolomic analysis using GC×GC/TOF-MS is an effective tool in studying whole-animal metabolism and the fate of important xenobiotic compounds in rainbow trout as numerous polar and non-polar metabolites were rapidly and accurately profiled using a single method.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/215/10/1627/DC1

Key words: starvation, rainbow trout, metabolomics, amino acids, fatty acids, xenobiotics, GC×GC/MS.

INTRODUCTION

The potential for global metabolomic methods to revolutionize research on nutritional metabolism is well documented (Gibney et al., 2005; Kussman et al., 2006; Zeisel, 2007). Metabolite profiles have also been regarded as important indicators of normal phenotype and pathology, and offer the possibility of identifying surrogate biomarkers of disease states that affect metabolism (Whitfield et al., 2004; Wu et al., 2009). Nuclear magnetic resonance (NMR) has been successfully utilized to analyze the effect of starvation and temperature on energy metabolism in teleost fish species (Viant et al., 2003; Kullgren et al., 2010). However, mass spectrometry (MS)-based platforms are more sensitive than NMR at detecting shifts in low abundance metabolites. A gas chromatography (GC)/MS-based platform has significantly improved the ability of investigators to monitor shifts in metabolic fuel utilization as well as low abundance novel metabolites associated with changes in specific dietary components (Noguchi et al., 2003). The advent of two-dimensional chromatography allows a much greater number of compounds to be separated compared with standard GC/MS (Dallüge et al., 2003), providing a more sensitive and effective platform for comprehensive metabolomic analysis (Adahchour et al., 2006).

The purpose of this study was to evaluate the capability of a two-dimensional gas chromatography/time-of-flight mass spectrometry (GC×GC/TOF-MS) platform to analyze the global metabolome of serum, liver and muscle tissue from fed and starved rainbow trout. Our data reveal significant shifts in the utilization of select tissue fatty acids due to short-term in starvation. Changes in amino acid levels indicate that starvation did not result in increased protein degradation in peripheral tissue but may have resulted in greater protein catabolism in liver of starved trout. In addition, we found significant changes in the levels of numerous xenobiotic compounds due to starvation. Here we discuss the biological significance of these results and highlight the benefits of the analytical technique employed in this study.

MATERIALS AND METHODS

Experimental animals and design

A group of 12 mixed sex rainbow trout (*Oncorhynchus mykiss* Walbaum 1792; 139.2±5.9 g, mean ± s.d.) were stocked individually in 30 liter tanks supplied with continuous flow-through well water (16±2°C). All fish were fed a ration of ~2% of their mean total body mass with a 45% protein/16% fat commercial diet (Silver Cup Steelhead Diet, Nelson & Sons Inc., Murray, UT, USA) for 14 days prior to the beginning of the trial. This diet met the NRC (National Research Council, 1993) guidelines for nutritional requirements of rainbow trout. After the end of this acclimation period the mean (±s.d.) mass of all 12 fish was 209.4±21.8 g. All

fish were randomly assigned to two treatment groups. Six fish were fed twice daily with same feed and ration as before. The other six fish were starved for the duration of the trial. All fish were maintained on a 12 h light/12 h dark photoperiod cycle during the acclimation period and during the trial. The experimental trial lasted 4 weeks, at the end of which all fish were killed in a water bath containing 100 p.p.m. tricaine methanesulfonate (Argent Laboratories Inc., Redmond, WA, USA). Each fish was then weighed and sampled for serum, liver and muscle. Approximately 2 ml of blood was drawn from the caudal vein of each fish and allowed to coagulate at room temperature for 10 min, then centrifuged for 15 min at 10,000***g*** for serum collection. Approximately 1 g of white muscle tissue was removed from the left side of each fish, 2.5 cm below the dorsal fin. Whole liver, serum and muscle tissue were flash frozen in liquid nitrogen and stored at –80°C.

Metabolite extraction and derivatization

For each fish, 200 mg liver, 200 mg muscle and 200 µl serum were added to three separate 2 ml microcentrifuge tubes. To each tube, 400 µl of methanol was added. Each sample was mechanically homogenized, treated with ultrasonic disruption and mixed for 1 h prior to centrifugation at 12,200***g*** for 10 min at room temperature. Supernatants were removed and added to new 2 ml microcentrifuge tubes. Chloroform (200 µl) was added to the remaining pellets for each sample. All samples were again sonicated, mixed and centrifuged as before. Supernatants from chloroform extractions were combined with their respective methanol extraction and vacuum centrifuged for ~90 min at 65°C. Samples were stored at –80°C upon completion.

Derivatization was conducted immediately prior to analysis to improve compound volatility. Each sample was reconstituted in 50 µl of anhydrous pyridine, after which a 20 µl aliquot was removed and added to a fresh microcentrifuge tube. Each 20 µl sample was derivatized by adding 20 µl of *N*-tert-butyldimethylsilyl-*N*-methyltrifluoroacetamide (TBDMS, Sigma-Aldrich, St Louis, MO, USA), followed by heating for 1 h at 60°C, to generate a dimethyl tertbutylsilane (DMTBS) analog. Analysis was performed using a Pegasus 4D GC×GC/TOF-MS (LECO Corporation, St Joseph, MI, USA), utilizing conditions similar to those reported elsewhere (Ralston-Hooper et al., 2008). Briefly, the first dimension (1D) column was a non-polar DB-5 capillary column (J&W Scientific, 30 m×0.25 mm×0.25 µm), coupled to a second dimension (2D) medium DB-17 capillary column (J&W Scientific, 1 m×0.10 mm×0.10 µm) by a glass union. High purity helium was used for the carrier gas (1.0 ml min^{-1}). An injection volume of 2 µl was used, with a 20:1 split ratio. The temperature program for the 1D column began at 80°C with a hold time of 0.2 min and then increased at a rate of 8°C min^{-1} to 300°C with a hold time of 20 min. The 2D column was held in a separate oven and maintained at a 10°C higher temperature than the 1D oven. The 2D separation run time was 4 s and was controlled with an internal cryogenic modulator. The injection inlet temperature and mass spectrometer transfer line were held at 280°C. The electron impact (EI) ion source was held at 200°C, with a filament bias of –70 V. Mass spectra were collected from 60 to 1000 *m/z* at 100 spectra s^{-1}.

Metabolite identification and peak normalization

Metabolites were identified using the procedure of Oh et al. (Oh et al., 2008). Briefly, raw data from the instrument (Fig. 1) were processed using LECO Corporation ChromaTOF software version 4.0 to generate a peak table. The ChromaTOF software uses the US

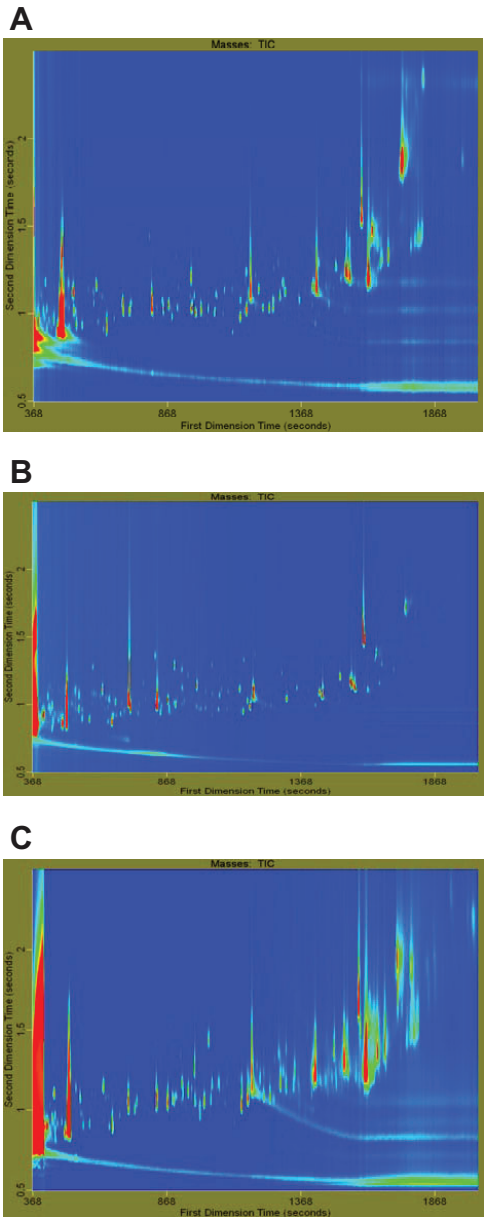

Fig. 1. Representative two-dimensional gas chromatography/time-of-flight mass spectrometry (GC×GC/TOF-MS) total ion chromatograms of rainbow trout (A) serum, (B) muscle and (C) liver. The first dimension column was a non-polar DB-5 capillary column and the second dimension column was a medium polarity DB-17 capillary column. The blue color represents low signal intensity and the red color represents chromatographic peaks of high intensity. The line across the lower region is the pyridine solvent front in the second dimension.

National Institutes of Standards and Technology (NIST) MS database (NIST MS Search 2.0, NIST/EPA/NIH Mass Spectral Library) for peak compound identification (Fig. 2). The peak table contained the features of each identified peak, such as peak name (if identified), registry number, peak area, 1D retention time, 2D retention time, fragment spectrum and similarity value (SV) for metabolite identification. The SV measures the goodness of fit between a deconvoluted sample peak spectrum and a hit in the NIST chemical database. Scores range from 0 to 999, with a score of 999 being an exact match. Peaks returning a SV of ≥700 were determined to be positive structural identifications and named with the

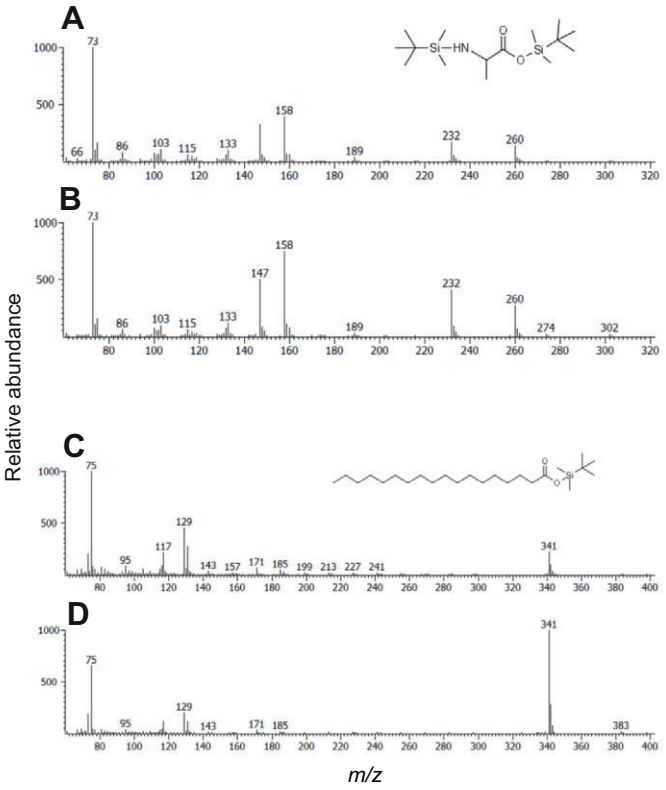

Fig. 2. Example of MS spectra for (A) a deconvoluted serum sample spectrum for the peak identified as alanine, (B) a National Institutes of Standards and Technology (NIST) library reference spectrum for derivatized alanine, (C) a deconvoluted serum sample spectrum for the peak identified as octadecanoic acid and (D) a NIST library reference spectrum for derivatized octadecanoic acid.

corresponding library metabolite (Hope et al., 2005). Peaks were normalized across samples using a constant mean approach where the total ion current was assumed equal across samples (Ralston-Hooper et al., 2008). Normalization was executed using R (v. 2.6.0, www.r-project.org) statistical software. Normalized peaks were then subjected to statistical analysis using software (MSort®) to align, match and compare metabolite peaks from various samples and groups (Ralston-Hooper et al., 2008).

Data analysis

Mass gain was calculated as a percentage of initial body mass (M_b) gained during the experiment (initial M_b/final $M_b \times 100$). Specific growth rate (SGR) was calculated as $100\times$(ln final mean M_b – ln initial mean M_b)/trial days. Data analysis using MSort® can only be conducted in a pair-wise manner. Therefore, a paired *t*-test ($P<0.05$) was used to analyze differences in mean peak area for all identified metabolites that were present in all sample replicates. Conducting multiple pair-wise comparisons can increase the likelihood of making type I errors (Benjamini and Hochberg, 1995). Therefore, to ensure we did not report potential false positives, false discovery rate (FDR) analysis was conducted (Strimmer et al., 2008). Here, we report only those metabolites that were statistically significant between treatment groups and that were determined as unlikely to be type I errors.

RESULTS AND DISCUSSION

To determine the effect of short-term starvation on whole-organism metabolism in rainbow trout we conducted metabolite analysis of multiple tissues from fed and starved rainbow trout using a state of the art GC×GC/TOF-MS platform. Fig. 1 presents GC×GC/TOF-MS chromatograms of rainbow trout serum, liver and muscle. The horizontal axis shows the 1D time separation using a non-polar separation column. The vertical axis is the additional separation obtained by the second GC column, utilizing a mid-polarity separation. The 1D separation is in minutes, whereas the 2D separation occurs in seconds. Within the chromatogram, the blue represents the baseline and the red represents chromatographic peaks of interest, as detected by the mass spectrometer. Note the many instances where multiple compounds would have suffered from co-elution in the first dimension alone, but are separated as a result of employing 2D chromatography. The peak capacity of GC×GC/TOF-MS is far greater than could be obtained by conventional GC/MS. As a result, GC×GC/TOF-MS is a superior technique for the metabolite profiling of complex biological samples. A mass spectrum was generated for each compound observed, two of which are shown in Fig. 2A,C. All empirical spectra were compared with the NIST library for compound identification. Fig. 2B,D shows the library spectra that yielded the highest SV scores for Fig. 2A,C, respectively. As shown by the structures, both library matches corresponded to a DMTBS derivatized compound. Across all tissues, an average of 1857 compounds were detected in each sample. The mean number of positive identifications (SV of ≥700) per sample was 240. Therefore, approximately 13% of the compounds detected were positively identified across all three tissues. A complete list of positively identified metabolites is provided in supplementary material Tables S1–S6. In many instances multiple peaks were identified as the same compound in a given sample. In this scenario each of these peaks was ranked by their SV, with the peak having the highest SV considered the correct identification. The remaining duplicates were removed and not considered for further analysis. This reduced the mean number of positively identified compounds per sample to 143. To reduce the data set to a manageable level and to provide a more robust analysis, we selected only those metabolites that were present in all six sample replicates of both treatment groups for further analysis. This step reduced the mean number of compounds per sample to 23. The remaining compounds were subjected to FDR analysis. Table 1 lists all compounds that were found to be both statistically significant and unlikely to be type I errors.

Starvation results in the utilization of select tissue fatty acids

Many teleost fishes must tolerate extended periods of starvation (e.g. a complete absence of dietary intake) as part of their natural life cycle. Because teleost fishes are poikilothermic organisms, their metabolic rate is dependent on the temperature of their aqueous environment. Therefore, the response of a given teleost fish species to extended periods of starvation depends on the temperature of the water in which the animal is being maintained. In this study, we maintained rainbow trout at a temperature of 16±2°C with the intention of promoting a high metabolic rate to facilitate anabolic conditions in fed fish and catabolic conditions in starved animals. At the end of the trial, fed fish had gained an average of 78.5% of their initial M_b, with a final mean mass of 377.3±48.9 g. At the same time, starved fish lost 5% of their initial M_b resulting in a final mean (±s.d.) mass of 202.2±28.6 g. This reduction in M_b was most likely attributable to increased utilization of tissue lipid reserves. Serum analysis revealed 13 fatty acids with significantly different concentrations (Table 1). Eleven were significantly reduced in the serum of starved fish. The most

Table 1. List of positively identified metabolites whose concentrations were significantly altered by starvation in serum, liver and muscle of rainbow trout

Tissue	Metabolite	CAS no.	Starved/fed	*P*-value
Serum	Heptadecanoic acid	506-12-7	26.7	0.038
	3-Penten-2-one, 4-[(dimethyl-2-propenylsilyl)oxy]	88641-47-8	4.80	0.017
	Nonanoic acid	112-05-0	4.40	0.030
	Heptacosane	593-49-7	3.60	0.030
	Oxalate	13425-25-7	2.40	0.017
	2-Octyn-1-ol, 7-[(tetrahydro-2H-pyran-2-yl)oxy]	125483-30-9	2.00	0.017
	Squalene	7683-64-9	2.00	0.004
	N,*N*,*N*′,*N*′-Tetraethyl-1,2-di-furan-2-yl-ethane-1,2-diamine	110-18-9	1.70	0.009
	Glycidol stearate	7460-84-6	1.60	0.030
	1-Hydroxycyclohexane-1-carboxylic acid	1123-28-0	0.67	0.004
	2,4-Hexadienoic acid	110-44-1	0.63	0.009
	2-Pentenoic acid	13991-37-2	0.63	0.017
	Decanoic acid	334-48-5	0.59	0.030
	Acetamide, 2,2,2-trifluoro-*N*-methyl	685-27-8	0.53	0.017
	Tridecane	629-50-5	0.53	0.030
	1-Pentamethyldisilyloxybutane	NA	0.48	0.017
	Pentanoic acid	109-52-4	0.48	0.004
	L-Valine	516-06-3	0.45	0.004
	2-Propenoic acid	79-10-7	0.42	0.030
	L-Leucine	61-90-5	0.40	0.009
	Hexadecane	544-76-3	0.38	0.017
	Glycine	56-40-6	0.37	0.030
	L-Serine	56-45-1	0.33	0.004
	Butanoic acid	107-92-6	0.30	0.017
	4,7,10,13,16,19-Docosahexanoic acid	6217-54-5	0.24	0.017
	L-Glutamine	56-85-9	0.22	0.030
	Propane	74-98-6	0.21	0.009
	Heptadecane, 2,6,10,15-tetramethyl	54833-48-6	0.20	0.004
	Propanoic acid	79-09-4	0.15	0.009
	L-Alanine	56-41-7	0.13	0.004
	Arachidonic acid	506-32-1	0.03	0.009
	Octadecanoic acid	57-11-4	0.01	0.004
	Tetradecanoic acid	544-63-8	0.01	0.009
Liver	L-Methionine	63-68-3	7.80	0.019
	L-Proline	609-36-9	5.40	0.036
	2,2-Dimethyl-5-[1,3]dioxolane-4-carboxaldehyde	5736-03-8	5.40	0.024
	Oleic acid	112-80-1	3.00	0.036
	Tetradecanoic acid	544-63-8	2.80	0.038
	2-Piperidinecarboxylic acid	4043-87-2	1.50	0.038
	Tris(trimethylsilyl)borate	4325-85-3	0.67	0.038
	2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one	1135-66-6	0.63	0.010
	Pentanoic acid	109-52-4	0.59	0.010
	4-Pyridinecarboxylic acid	55-22-1	0.50	0.010
	Docosanoic anhydride	55726-23-3	0.42	0.010
	1-Octanol, 2-butyl	3913-02-8	0.38	0.024
	L-Threonine	72-19-5	0.31	0.019
	5-Eicosene, (E)	NA	0.31	0.038
	Octadecanoic acid	57-11-4	0.11	0.001
Muscle	Octadecanoic acid	57-11-4	154.4	0.041
	Di-*n*-octyl phthalate	117-84-0	6.00	0.004
	Carbonimidodithioic acid, methyl-, dimethyl ester	53687-90-4	3.40	0.004
	2-(1,1-Dimethylethyl)-5-oxohexanal	NA	3.00	0.002
	Tetradecanoic acid	544-63-8	2.10	0.026
	Ethanedioic acid, bis(cyclohexylidenehydrazide)	370-81-0	1.80	0.015
	Carbonimidodithioic acid	34318-05-3	1.60	0.026
	Acetamide	60-35-5	1.50	0.015
	Benzenemethanol, α-[1-(ethylmethylamino)ethyl]	NA	0.59	0.026
	4H-Pyran-4-one	29943-42-8	0.42	0.026
	Sarcosine	107-97-1	0.24	0.004
	Oxalate	13425-25-7	0.14	0.002
	L-Aspartic acid	56-84-8	0.11	0.026
	Malonate	141-82-2	0.06	0.002
	Hexadecanoic acid	57-10-3	0.04	0.009

significant were reduced concentrations of the saturated long chain fatty acids (LCFAs) octadecanoic acid and tetradecanoic acid and the polyunsaturated fatty acids (PUFAs) arachidonic acid and 4,7,10,13,16,19-docosahexanoic acid (4,7,10,13,16,19-DHA). In addition to LCFAs, concentrations of numerous short chain fatty acids (SCFAs) were reduced significantly in the serum of starved fish. These included propanoic acid, butanoic acid, 2-propenioc acid, pentanoic acid, decanoic acid and 2-pentenoic acid. The significant reduction in serum concentrations of these molecules may represent increased clearance and utilization of circulating fatty acids for cellular energy metabolism in starved trout. However, starvation did result in an ~27-fold increase in the concentration of the LCFA heptadecanoic acid and a 4-fold increase in the concentration of the SCFA nonanoic acid in the serum of starved trout. This could indicate selective utilization of certain fatty acids early in the starvation process, reflected by their drastic reduction in concentration, and utilization of other fatty acids later in starvation as reflected by their minor reduction or increased concentration in the serum of starved fish. Alternatively, the increased serum concentrations of these two fatty acids may reflect a completely different metabolic origin and potential utilization of these compounds, one that is unrelated to utilizing tissue fatty acid reserves to meet cellular energy demands.

The liver analysis results support the notion that starvation promotes the utilization of select tissue fatty acids, as oleic acid and tetradecanoic acid concentrations increased significantly in livers of starved fish (Table 1). In contrast, levels of octadecanoic acid and pentanoic acid were significantly reduced in livers of starved fish. This may suggest that starved fish rely on hepatic octadecanoic acid and pentanoic acid earlier in the starvation process, while hepatic tetradecanoic acid and oleic acid concentrations may have increased as a result of greater mobilization and utilization of these fatty acids near the end of the 4 week period.

This was also the case for muscle fatty acid profile as hexadecanoic acid concentration was significantly reduced in muscle of starved fish. Notably, however, octadecanoic acid increased by more than 150-fold in muscle of starved fish. This significant increase in octadecanoic acid is notable given the reduced concentration of this fatty acid in serum and liver of starved fish. The results for muscle fatty acid analysis would seem to suggest preservation of octadecanoic acid and perhaps tetradecanoic acid at the cost of depleting hexadecanoic acid levels in starved fish muscle.

Starvation promotes increased protein catabolism in liver but not in peripheral tissues

Starvation resulted in a significant reduction in concentrations of circulating amino acids (Table 1). Perhaps most importantly, the gluconeogenic amino acids alanine and glutamine were approximately 8- and 5-fold lower, respectively, in the serum of starved fish. Glycine and serine concentrations were also significantly reduced in the serum of starved fish. This likely indicates that starved fish were not relying significantly on catabolism of peripheral tissues and hepatic gluconeogenesis to maintain an adequate blood glucose level (Aikawa et al., 1973; Odessey et al., 1974). Overall, the profile of serum amino acids seems to indicate that starvation did not result in greater levels of protein catabolism in peripheral tissues of starved fish and agrees with those of previous studies that examined the effect of starvation on energy metabolism in rainbow trout (Cowey et al., 1977; Morata et al., 1982).

In contrast, the amino acid profile of liver may be indicative of increased protein catabolism of hepatic tissue in starved trout (Table 1). Most notably, methionine was nearly 8-times greater in the liver of starved fish. Methionine is an essential amino acid for carnivorous fish species, required for protein synthesis and sulfur metabolism (Espe et al., 2008). Also, concentrations of proline increased by ~5-fold in livers of starved fish. In addition, the concentration of the lysine metabolite 2-piperidine carboxylic acid (2-PCA) was 1.5-times greater in the liver of starved fish. Although 2-PCA is produced during lysine synthesis and degradation, it is unlikely that this increase is related to greater synthesis as lysine is an essential amino acid used for protein synthesis. This finding is consistent with that of Higgins et al. (Higgins et al., 2005), who determined that starving rainbow trout for 2 weeks resulted in a 50% increase in the activity of the lysine degradation enzyme lysine α-ketoglutarate reductase in livers of starved fish. Therefore, based on the increased methionine, proline and 2-PCA concentrations it seems logical to conclude that, in the current study, starving rainbow trout for 4 weeks promoted increased catabolism of cellular protein in liver.

Effect of starvation on biologically relevant xenobiotics

The most abundant group of xenobiotics identified in serum samples was *n*-alkanes (Table 1). Alkanes have been successfully utilized to estimate diet composition and intake of grazing herbivores (Bezabih et al., 2011). The use of *n*-alkanes as markers for determination of intake and digestibility of fish feeds has been reviewed (Gudmundsson and Halldorsdottir, 1995). In this study, short-term starvation resulted in increased concentrations of serum heptacosane, but decreased concentrations of tridecane, hexadecane, propane and 2,6,10,15-tetramethyl-heptadecane in the serum of starved fish. As these molecules are likely indicative of plant-based ingredients in feeds, their overall reduction in the serum of starved fish is not surprising given their absence in the fish diet. However, the increase in serum heptacosane, as well as the varying decreases in other *n*-alkanes in the serum of starved fish may be indicative of retention of these molecules in various tissues and may also indicate increased mobilization of tissue energy reserves. Depending on the selective retention of various *n*-alkanes in specific tissues, serum alkane levels may prove to be useful for determining how lipid, glycogen and other energy stores are mobilized during periods of prolonged food deprivation in rainbow trout.

Starvation also resulted in significant changes in the concentration of xenobiotics that could be of great importance to human health. Most notably, di-*n*-octyl phthalate (DnOP) concentrations increased 6-fold in muscle tissue of starved fish. This molecule has proven to be toxic in rats, primarily affecting the liver, kidneys, thyroid and possibly immune function when administered in longer multiple doses (Lhuguenot, 2009). DnOP is a plasticizer that is commonly used in the commercial production of plastic products, such as plastic tanks and feed containers. Therefore, storage of commercially prepared diets in plastic containers may have resulted in an increased exposure of rainbow trout to DnOP. Further, the increased concentration of DnOP in skeletal muscle of starved trout may indicate that it is not lipid or glycogen soluble and may have been retained in muscle tissue of starved fish. As a result, when an equal mass of tissue was analyzed, there was a greater concentration of DnOP per gram of muscle tissue in starved *vs* fed trout. Many additional xenobiotic molecules were found to have changed significantly as a result of starvation (Table 1). Thus far, however, little information is available on their toxicity and biological importance. Further studies are needed to determine the impact of these xenobiotics on the health of fish and humans alike.

CONCLUSIONS

In this study, we have demonstrated the capability of GC×GC/TOF-MS metabolomics to rapidly and accurately detect changes in numerous polar and non-polar molecules. Using this single technique we were able to determine that starving rainbow trout for 4 weeks resulted in the utilization of select tissue fatty acids and increased catabolism of cellular proteins in liver but not in peripheral tissues. Also, we have shown that GC×GC/TOF-MS is useful for analyzing dynamic changes in numerous xenobiotic compounds that may be of great importance to animal and human health. The primary advantage of this metabolomic platform is its sensitivity and accuracy for detecting changes in the concentration of such a dynamic group of chemical compounds from a single sample. Future work should utilize complementary techniques, such as HPLC/MS or NMR, to verify and support the metabolites identified in this screening study.

ACKNOWLEDGEMENTS

The authors thank Dr Paul B. Brown, Jeff Weber and Robert Rode of the Purdue University Aquaculture Research Laboratory for their technical assistance. The authors also thank Douglas Sweet of the Ohio Department of Natural Resources London Fish Hatchery for kindly providing the animals for this study.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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