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Postprandial hepatic protein expression in trout Oncorhynchus mykiss a proteomics examination

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ABSTRACT

Following a meal, a series of physiological changes occurs in animals as they digest, absorb and assimilate ingested nutrients, the kinetics of these responses depends on metabolic rate and nutrient quality. Here we investigated the hepatic proteome in the ectothermic teleost, the rainbow trout, following a single meal to define the postprandial expression of hepatic proteins. The fish were fed a high marine fishmeal/fish oil single meal following a period of 24 h without feeding. Liver protein profiles were examined by 2D gel electrophoresis just before feeding (time 0 h) and at 6 and 12 h after feeding. Of a total of 588 protein spots analysed in a temporal fashion, 49 differed significantly in abundance between the three time groups (ANOVA, $p < 0.05$), before and after feeding, 15 were increased and 34 were decreased in abundance after feeding. Amino acid metabolism-regulated proteins such as phenylalanine-4-hydroxylase and proliferating cell antigen were increased in abundance 12 and 6 h following the meal, suggesting by this time that the fish were increasing their protein turnover to utilize efficiently their dietary protein consumption. Overall, these results highlight some specificity of the trout metabolism and identify postprandial response of metabolism-related proteins 6–12 h after feeding a single meal.

1. Introduction

Aquaculture production has increased dramatically in recent years and it is predicted to grow from 67 M tonnes in 2012 to 140Mt by 2050 [1], this reflects the increased demand for fish protein and growing global human population [2]. One major limitation to the expansion of aquaculture is its reliance on wild fish for aquafeed production to fulfil the demands of aquaculture. Salmonids similar to other carnivorous fish require a high protein diet to attain maximum growth rates. Fishmeal is used in high-protein feed for carnivorous fish but climate change and the overexploitation of fisheries resources have resulted in a reduced supply and, consequently, alternative protein sources such as plant protein meal are used in aquafeeds [2]. However, the long term metabolic consequences of feeding fish with a low fishmeal high plant protein diet is still under consideration and further knowledge is required regarding the way that dietary composition influences protein metabolism in fish. In addition, it is necessary to obtain a better

understanding on the potential interactions between dietary protein sources and the way that they may regulate the processes involved in protein metabolism. Protein metabolism and dietary amino acid profiles differ depending on the protein source in the diet, with essential and non-essential amino acids appearing synchronously in the plasma in juvenile rainbow trout (Oncorhynchus mykiss) fed a fishmeal diet, while the appearance was less synchronised in fish fed a plant meal diet [3]. Thus, amino acid uptake patterns are influenced by the protein source in the feed, with different dynamics of protein digestion and amino acid uptake between fishmeal and plant protein-rich ingredients. Developing a better understanding of the molecular mechanisms controlling nutrient utilization will help us to generate sustainable and functional diets and improve the efficiency of aquaculture.

Recent research shows an improvement in understanding the regulation of physiological functions of fish under conditions of restricted or excessive food intake and in understanding the biochem-

ical processes occurring in the fish tissues due to feeding. After a meal, a sequence of physiological changes occurs in animals in response to digestion, absorption and assimilation of the ingested nutrients [4]. Studies have investigated the post-prandial changes in protein synthesis in fish [5,6]. The liver responds early to the arrival of a single meal as liver protein mass increases and protein synthesis rates rise sharply 4–6 h and then fall in 24 h [4]. Postprandial free amino acid (FAA) levels indicate availability for muscle growth and provide a link between feed intake and anabolic processes. Several researchers have studied postprandial concentrations of FAA in fish tissues such as Atlantic cod (*Gadus morhua*), koi carp (*Cyprinus carpio*), rainbow trout [3,7–10] and Atlantic salmon (*Salmo salar*) [6,11,12]. A study by [13] on postprandial regulation of hepatic microRNAs in rainbow trout in relation to insulin signalling and target genes contributed to an improved understanding of the nutritional regulation of intermediary metabolism in this tissue. Gene expression in relation to diet in trout fed a single meal showed a triggering of the signalling pathway of insulin/amino acid after a single meal [14], indicating that trout use amino acids both as an energy source and probably as a substrate for lipogenesis. A study of muscle gene expression revealed that growth-related genes in juvenile Atlantic salmon were up-regulated within one hour of refeeding following fasting [15].

Proteomic studies have improved the understanding of the relationships between diet composition, protein metabolism and nutrient utilization in aquatic animals [16,17]. Two-dimensional proteomics is a robust tool for examining protein responses to environmental or dietary stimulations [18–21] and has greatly enhanced knowledge of the metabolic pathways influenced by dietary changes in both liver and muscle in rainbow trout [18,22–25], Atlantic salmon [26,27], gilthead sea bream and white sea bream [28–30]. The only reported postprandial proteomic examination of liver has been in the warm water zebrafish which were fed diets containing different protein and carbohydrates levels [31]. Ammonia excretion rates in rainbow trout reached a peak values at 5–7 h post-feeding a single meal, suggesting that the rates of dietary protein digestion occurs at that time [23]. Using this approach we examined the effect of a single meal on the postprandial expression of proteins related to hepatic metabolism in trout fed on a diet containing high level of marine protein derived protein. The liver was chosen as the target organ in view of its importance in fish metabolism. We show temporal changes in the trout liver proteome following a single meal. The identification of the proteins by mass spectrometry has been facilitated by the recent publication of the rainbow trout genome and *in silico* derived transcriptome and proteome [32].

2. Material and methods

2.1. Ethics statement

The experiment was carried out strictly in line with EU legal frameworks related to the welfare and protection of animals used for scientific purposes (Directive 2010/63/EU) and the guidelines of legislation in the UK that governs the ethical treatment of animals (Animal Scientific Procedures Act, 1986, UK). No regulated procedures were carried out during this trial as defined by the Animal Scientific Procedures Act, 1986 and Schedule 1 method (SFI), UK. The study was approved under the University of Aberdeen's Code of Practice on the Use of Animals in Research, which takes account of ethical, health and safety considerations.

2.2. Experimental diets

The diet was formulated as a high marine fishmeal /fish oil diet (560 fishmeal/120 fish oil g.kg^{-1}). It used only organic trimmings from a sustainable certified Peruvian fishmeal, organic fish oil, organic peas and organic soya cake (which also supplied a small amount of protein)

Table 1
Chemical composition of the experimental diet.

Chemical composition (g kg^{-1} DM)	
Moisture (g kg^{-1})	577
Crude protein	430
Total lipid	230
Crude fibre	10
Ash	100
CHO[a]	240
GE (kJ.kg^{-1}DM)[b]	21.5

[a] CHO, carbohydrate calculated by difference (100% - (% crude protein +% total lipid +% ash)
[b] GE, Gross energy calculated from nutrients assuming gross energy content of 23.6 kJ g^{-1} for protein, 39.5 kJ g^{-1} for lipid, 17.2 kJ g^{-1} for carbohydrate (Bradfield 1985).

and did not contain any GM ingredients or synthetic amino acids. It was prepared based on a commercial organic fish diet at BIOMAR (Scotland, UK). The chemical composition of the diet (Table 1) was analysed using the Kjeldahl method for determining the protein content and the Soxtherm method for determining fat content [33]. Dry matter was measured after drying at 105 °C for 24 h.

2.3. Experimental procedures

Juvenile immature rainbow trout of mixed sex, weighing 44.98 ± 1.08 g (n =140), were acquired from a commercial fish farm and reared in 250 l tanks at the freshwater aquarium facilities in the School of Biological Sciences, University of Aberdeen. The fish were randomly distributed in three tanks with 40 individuals in each tank. The temperature was maintained at 12 °C, pH at 7.60 and oxygen saturation at 90% under a natural photoperiod and the fish were acclimatised for two weeks to the tanks prior to feeding the organic diet. Fish were fed by hand *ad libitum* twice daily at 09:00 and 15:00 for 5 weeks. The amount of feed per tank was measured in order to ensure that feed intake was equally distributed between the tanks (around 2% of fish body weight). At the end of the experiment fish remained unfed for 24 h. They were used to examine the liver proteome prior to feeding (t=0 h) and at two times after feeding (6 h and 12 h after feeding) based on previous data, which examined the postprandial response of proteins to feeding a meal in trout [23]. A total of 18 fish were sampled using two from each tank before feeding (0 h, n=6) and at 6 h and 12 h after a single meal. Fish were sacrificed by anaesthesia overdose (phenoxyethanol) followed by destruction of the brain, wet weight was measured and the liver tissues immediately were dissected out, samples weighed, frozen in liquid nitrogen and kept at −80 °C. Wet weight was measured in all remaining fish not used for proteomics analysis.

2.4. Protein extraction and gel analysis

Protein extraction and analysis were performed in line with Cash et al., 1995 [34] and Martin et al., 2003 [23]. Briefly, the samples of the liver (approximately 100 mg each, n =6 for each time point) were homogenized in 2-D lysis buffer (0.5 ml 0.5 M Tris–HCl pH 6.8, 0.125 ml 0.2 M EDTA, 12*g* urea (8 M), 2.5 ml 0.5 M DTT, 2.5 ml glycerol (10%), 1.25 ml NP-40 (5%), 3.7 ml pH 3–10 ampholytes (40%) 6%, 5 ml MilliQ water) using a pestle, kept cooled and lysis buffer was added in a 10:1 ratio (e.g. 1.0 ml added to a 100 mg sample). The homogenates were centrifuged at 11,000 *g* for 10 min, and the supernatants were decanted and stored at −80 °C. Proteins from the supernatants were precipitated by using a ReadyPrep™ 2-D Cleanup kit (Bio-Rad Laboratories, Hercules USA) following the manufacturer's instructions. The precipitate was solubilized in 200 μL IPG buffer (2.01*g* UREA (7 M), 0.76*g* Thiourea (2 M), 0.2*g* CHAPS (4%), 0.015*g* DTT (0.3%), 3 ml MilliQ water, 50 _l pH 4–7 IPG buffer

(GE Healthcare) and in sufficient bromophenol blue in order to provide to the solution a blue colour. The protein solution was sonicated with 3 bursts each of 5 s. It was then incubated with one part DNase solution (0.05 ml 1 M MgCl2, 0.5 ml 1 M Tris–HCl pH 8.0 and 0.1 ml 20,000 U ml−1) to two parts protein solution for 10 min on ice. The protein samples (2 μL) were first analysed by 1-dimensional SDS PAGE to check protein quality and amount. A volume of between 30 and 35 μL of protein sample was applied to the first dimension IPG strips (11 cm, pH 4–7) (Bio-Rad) to equalise the total protein load, as judged from the 1-D profiles. Following isoelectric focussing, IPG strips were applied to the second dimension SDS-PAGE (Criterion Any kD™, Bio-Rad), electrophoresed and the resolved proteins detected using Colloidal Coomassie Blue G250 staining. The gels were dried then scanned in an ImageScannerTMIII (GE Healthcare, UK) with LabScan software (GE Healthcare, UK). 16 bit images were obtained in a resolution of 600 dpi. The digitised images were transferred to the Progenesis SameSpots, version 4.5 (Non-linear Dynamics, Newcastle upon Tyne, UK). A reference gel from the control samples at time 0 h was selected using a combination of manual and automatically generated vectors. The remaining gel images were aligned to the reference gel. Spots on the 2D dried gel profiles were detected automatically and normalised spot volumes calculated. The SameSpots normalisation factors ranged from 0.88 to 1.45, demonstrating that similar amounts of protein had been loaded. Then, they were reviewed manually using the software and spots showing a significant difference (p≤0.05) between treatments were selected.

2.5. Identification of protein spots that are altered following meal

Comparisons of the 2D protein profiles of fish before feeding (0 h) and at 6 h and 12 h after a single meal (n=6 biological replicates). The 2D protein profiles for each time point for each fish were matched to the 2D reference gel using Progenesis SameSpots software [35]. The 2D gel images were grouped per time point; thus there were three groups: 0 h, 6 h and 12 h and protein spots that showed statistically significant differences by ANOVA (p < 0.05) in abundance between the three groups were selected. These spots were further analysed by using Principal Component Analysis (PCA). Both spot and gel data were plotted as a "biplot".

2.6. Protein identification by mass spectrometry (LC–MS/MS)

Ten protein spots were chosen according to several criteria. They represent changes that increased and decreased in abundance at 6 h or 12 h following a single meal. They also had an intensity enough to allow trypsin digest fingerprinting. In addition, they were not attached to a second spot or other spots. Selected proteins were excised in 1.5-mm diameter gel plugs from Coomassie Blue stained 2-D gels. They were processed as described by Nøstbakken et al. [36]. Proteins in the gel plugs were digested with trypsin. Peptide solutions were analysed using an HCTultra PTM Discovery System (Bruker Daltonics Ltd., Coventry, UK) combined to an UltiMate 3000 LC System (Dionex Ltd., Camberley, Surrey, UK). Peptides were separated on a Monolithic Capillary Column (200 μm i.d. ×5 cm; Dionex) at a flow rate of 2 μL/min using a gradient of acetonitrile (6–38% over 12 min) in 0.04% (aq.) formic acid. Peptide fragment mass spectra were acquired in data-dependent AutoMS(2) mode with a scan range of 300–1500 *m/z*, 3 averages, and up to 3 precursor ions selected from the MS scan (100–2200 *m/z*). Precursors were rejected within a 1.0 min window, and all singly charged ions were excluded. Peptide peaks were identified and deconvoluted automatically using DataAnalysis software (Bruker). Mass lists in the form of Mascot Generic Files were created automatically and used as the input for Mascot MS/MS Ions searches of the rainbow trout proteome [32] using Mascot Server version 2.2. The default search parameters used were: enzyme=trypsin; max. missed cleavages =1; fixed modifications=carbamidomethyl (C); variable mod-

ifications=oxidation (M); peptide tolerance ± 1.5 Da; MS/MS tolerance ± 0.5 Da; peptide charge =2+ and 3+; instrument=ESI-TRAP. The proteins from the rainbow trout proteome are not annotated and to assign annotated proteins a BLASTP search was performed against all proteins on NCBI; following this all searches defined a significant protein match.

2.7. Statistical analysis

Means with their standard error (SE) are presented. The growth performance of the fish (initial and final weights) was analysed using Student's *t*-tests. One-way ANOVA and if necessary, Tukey multiple-range tests was used to analyse the relationship between time after feeding and proteome analysis. Homogeneity was confirmed using Levene's test. Database searching for protein identities was performed. All statistical analyses were carried out using IBM SPSS Statistics, version 22.

3. Results

3.1. Growth performance

At the beginning of the trial there were no significant differences among tanks in the initial weight of the fish, which was 44.98 ± 1.08 g ($p > 0.05$). Fish weight gain was 32.60 ± 1.91 g. The specific growth rate (SGR, %/day) and feed efficiency was 1.55 ± 0.08, 1.06 ± 0.17 respectively.

3.2. Detection of differentially expressed proteins

The effects of refeeding on the expression of hepatic proteins were analysed by comparing fish, which were fasted for 24 h (0 h) and 6 and 12 h after refeeding. The protein profile from a fish at 0 h (Fig. 1) shows a representative sample of the liver proteins separated by 2DE. This was chosen as a reference gel. All the other gel profiles were matched against the reference with each protein assigned a reference number, molecular weight, pI and abundance. The gels showed good resolution of the cellular proteins with *pI* of 4–7 and molecular masses of 10–250 kDa (Fig. 1).

The number of protein spots identified on each gel varied from 500–800. Following quality control and editing 588 spots were obtained for statistical analysis in all gels, among which forty-nine spots were found to differ significantly (ANOVA, P < 0.05) in abundance (Table 2) after refeeding. We found fifteen spots that increased in abundance, of which 10 protein spots increased at 6 h after feeding and 5 increased at 12 h after feeding (Table 2). The fold differences between the protein abundances for significant spots in the three time groups ranged between 1.2 and 2.05. Thiry-four proteins were found decreased in abundance with 22 lower at 6 h and 12 lower at 12 h following the meal
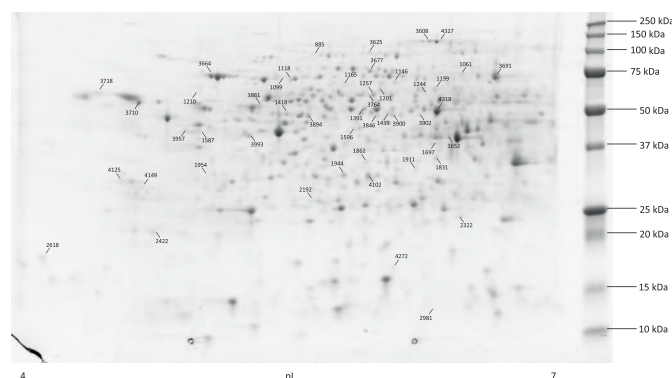

Fig. 1. A representative two-dimensional gel of rainbow trout (*Oncorhynchus mykiss*) liver proteins after 24 h of fasting.

Table 2
Protein spots that were increased or decreased following a single meal (mean values and their standard deviations, n=4 or 5).

Spots reference no	q value	Fold difference	Time after feeding	PI	MW (kDa)
Upregulated*					
4272	0.379	1.6	12 h	6.03	17
3902	0.364	1.6	12 h	6.19	47
3677	0.293	1.5	12 h	5.86	70
1944	0.316	1.6	12 h	5.71	31
1418	0.201	1.3	12 h	5.33	47
Upregulated**					
4149	0.379	1.4	6 h	4.48	29
4125	0.156	1.6	6 h	4.32	30
3957	0.379	1.7	6 h	4.74	42
3718	0.156	1.5	6 h	4.21	58
2981	0.379	1.5	6 h	6.5	13
2322	0.329	1.4	6 h	6.47	23
2192	0.377	1.4	6 h	5.52	26
1862	0.384	1.4	6 h	5.85	33
1652	0.382	1.3	6 h	6.4	39
1210	0.440	1.4	6 h	4.79	59
Downregulated***					
1099	0.271	1.4	12 h	5.31	68
1165	0.184	1.8	12 h	5.69	63
1199	0.244	1.2	12 h	6.29	60
1201	0.201	1.6	12 h	5.93	59
1596	0.26	1.6	12 h	5.78	42
1587	0.356	1.4	12 h	4.82	42
1697	0.383	1.5	12 h	6.32	37
1911	0.384	1.4	12 h	6.19	32
1954	0.271	1.5	12 h	4.86	31
3710	0.316	1.7	12 h	4.47	53
3861	0.382	1.4	12 h	5.16	50
4102	0.244	2.0	12 h	5.87	31
Downregulated****					
885	0.44	1.9	6 h	5.78	89
1061	0.364	1.2	6 h	4.99	69
1118	0.184	1.3	6 h	5.36	67
1146	0.383	1.2	6 h	6.02	64
1257	0.244	1.2	6 h	5.89	56
1244	0.356	1.9	6 h	6.24	57
1391	0.364	1.3	6 h	5.83	48
1439	0.316	1.6	6 h	5.99	47
1831	0.244	1.3	6 h	6.32	34
2422	0.244	2.0	6 h	4.57	21
2618	0.383	1.8	6 h	5.84	17
3608	0.384	1.5	6 h	6.24	106
3625	0.271	1.5	6 h	5.85	93
3664	0.385	1.5	6 h	4.9	70
3691	0.364	1.3	6 h	6.65	70
3764	0.349	1.7	6 h	5.84	56
3846	0.364	1.2	6 h	5.91	46
3894	0.344	1.3	6 h	5.47	47
3900	0.293	1.3	6 h	6.03	47
3993	0.364	1.3	6 h	5.11	40
4318	0.384	1.2	6 h	5.32	13
4327	0.271	1.7	6 h	5.66	12

MW: experimentally obtained molecular mass from the gel.

* Significant difference (upregulated) at 12 h compared to 0 h and 6 h (One-way ANOVA followed by post-hoc Tukey HSD, $p < 0.05$)

** Significant difference (upregulated) at 6 h compared to 0-h and 12 h (One-way ANOVA followed by post-hoc Tukey HSD, $p < 0.05$)

*** Significant difference (downregulated) at 12 h compared to 0 h and 6 h (One-way ANOVA followed by post-hoc Tukey HSD, $p < 0.05$)

**** Significant difference (downregulated) at 6 h compared to 0 h and 12 h (One-way ANOVA followed by post-hoc Tukey HSD, $p < 0.05$)

(Table 2). We found six protein spots that were increased at 6 h but were then decreased in expression following 12 h after the meal and four proteins that were increased at both 6 h and 12 h (Fig. 2).

Further analysis of the 49 spots by PCA characterized the spots according to their abundance levels amongst the three groups (Fig. 2). According to the PCA, 28 spots (57%) were significantly more abundant in the 6-h cluster compared to the abundance of 23 spots (47%) in the 12 h group and the 19 spots (38%) in the 6–12 h group. The time 0 h cluster expressed different abundance of protein spots that distinguishes it from the other two groups (6 and 12 h) (Fig. 2).

3.3. Spot identification by mass spectrometry

Ten protein spots were selected for peptide mass mapping, which were significantly different at 6 h and 12 h after feeding. All proteins that were identified showed fold changes between 1.3 and 1.7 and 3 were significantly up-regulated at 6 h (4125,4149,3718), 2 were significantly up-regulated at 12 h (1418, 1944), 1 was up regulated at 6 h and down regulated at 12 h (2322), 2 were significantly down-regulated at 6 h (3900, 3993), 1 was significantly up regulated at 12 h (3677) and 1 was significantly down regulated at 12 h (3710). Details of protein homologies for trypsin digest fingerprinting are shown in Table 3. The following proteins were successfully identified: *phenylalanine hydroxylase*, which catalyses the hydroxylation of the aromatic side-chain of phenylalanine to generate tyrosine; *3-hydroxyanthranilate 3,4-dioxygenase*, which participates in tryptophan metabolism; *94 kD glucose-regulated protein* and *calreticulin* that may have a role in transcription regulation and *type II keratin E3*, a structural protein, *flotillin-1* involved in cellular metabolism and cell to cell communication, *succinyl-CoA,* a carbohydrate metabolism protein*, subunit beta proliferating cell nuclear antigen,* involved in amino acid metabolism and *14-3-3 protein epsilon* which controls gene expression.

4. Discussion

Nutritional proteomics studies the relationship between nutrients as controlling or regulatory factors and metabolic proteins. It analyses the expression of a network of metabolic proteins in relation to nutrients. The liver is a vital organ for the numerous metabolic pathways and other functions that are taking place. Studies using fasting and refeeding protocols have been used to investigate the molecular mechanisms that regulate protein metabolism in fish. This study identified differentially expressed proteins and changes in protein abundances in the liver at two times after feeding a single meal in rainbow trout fed on a high marine protein diet. Therefore, this work investigates the proteomic changes resulting from altering metabolic activity as a result of feeding. Proteomic research on salmonid fish and especially in the liver give us the ability to analyse increased or decreased abundance of different possible unrelated metabolic pathways without prior assumptions of biological pathways affected [18]. Although fasting and refeeding have been examined [22,23,37–39] the postprandial response of hepatic proteins has not previously been explored in salmonids. In this study, a total number of 588 spots were maintained from 2DE gel analysis for differential protein abundance. PCA analysis clearly defined the protein patterns with three distinguishable groups, the unfed group for 24 h and the 6 h and 12 h after a meal, indicating that consistent hepatic protein changes were occurring following the meal. The changes in fish liver after a single marine protein meal are documented with a rise in protein synthesis in 5–7 h and then a fall until the next meal [4,23]. This agrees with the findings of this study, which showed that a single meal caused a response to the hepatic proteins in 6–12 h after the meal.

Following the single meal we identified proteins involved in a number of key biological processes. An interesting finding was that amino acid metabolism-regulated proteins were increased in abundance 12 h following the meal, suggesting by this time that the fish were increasing their protein turnover to utilize efficiently their dietary protein consumption. Furthermore, phenylalanine-4-hydroxylase, which catalyses the hydroxylation of the aromatic side-chain of

Principal Components Analysis
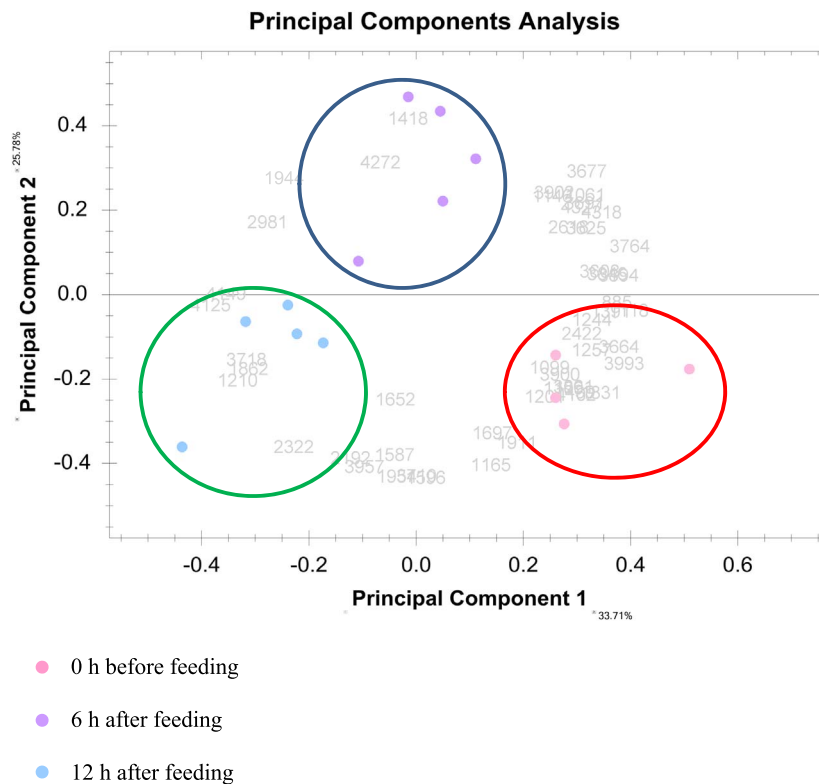
Fig. 2. PCA changes in abundance of proteins spots. The 2D gel images were grouped per time point and three groups before feeding (0 h) and at 6 h and 12 h after feeding a single meal are shown. The locations of the significantly different protein spots (expressed as mean normalised values) from 4 or 5 gels images per time point were used.

phenylalanine to generate tyrosine and 3-hydroxyanthranilate 3,4-dioxygenase which participates in tryptophan metabolism and elongation factor 1-beta, 14-3-3 protein beta/alpha-1 were increased in response to feeding, 12 h and 6 h after a single meal, indicating an increase in metabolic activity. This demonstrates an altered regulation of protein synthesis due to the arrival of amino acids from a meal that initiated the synthesis of new proteins. This agrees with the findings of Martin et al. [23], which showed that protein synthesis in trout liver rose in 6 h after a single high protein meal and fell in 24 h until the next meal. A single meal triggered the signalling pathway of insulin/amino acid indicating that trout use amino acids both as an energy source and probably as a substrate for lipogenesis [14]. Transferrin was found to be upregulated at 12 h after the meal, this protein binds iron and members of this protein family are involved in immune response in fish [40]. The 14-3-3 protein was upregulated 6 h after the meal which shows that at this time ubiquitous molecules are involved in a variety of biologic events, such as transduction pathway modulation, cell cycle control, and apoptosis [41]. The structural protein keratin E3 type II protein, was increased at 12 h after the meal, which would suggest that at this time there is still active translation of structural proteins in the liver [42]. The result may reflect decreased requirements for energy metabolism in the trout, thus, more energy available to synthesize structural proteins 12 h after a single meal.

The recent availability of the rainbow trout genome and associated resources has ensured that proteomic research approaches on salmonids ensure much improved protein spot detection. The eighteen identified proteins found by Enyu [43] of hepatic mitochondrial proteome of zebrafish revealed that the fifteen days starvation caused a reduction in glycolysis and an increase in gluconeogenesis which returned to normal levels following seven days feeding. During starvation energy was obtained by the utilization of non-carbohydrate resources as suggested by the expression pattern of several proteins associated with amino acid and fatty acid metabolism. In addition in starved conditions glucose-regulated, heat-shock and paraxonase pro-

tein known for their chaperoning and antioxidative roles were also up-regulated. Heat shock proteins (Hsps) are ubiquitous chaperone proteins that are conserved evolutionarily in most organisms. In this study, glucose-related protein 94 (GRP94), a member of the heat shock protein, was decreased in abundance 12 h after a meal. This protein is a member of the HSP90 family, which is expressed in the endoplasmic reticulum (ER), assists cells maintain protein integrity, regulate protein quality control and degradation and may be an indicator of stability of recently synthesised proteins [44]. The results from this work confirm that protein turnover is maximal at 6 h and then reduced by 12 h following the meal.

Changes in protein abundance in the fish are affected by the dietary quality of consumed proteins. Proteins were expressed in a differential manner due to altered metabolism in trout that were fed soy protein extracts. Martin et al. [23] indicated a lower level of non-structural protein expression in trout fed a diet containing fishmeal and plant ingredients in comparison to a diet containing higher proportion of soy protein. Martin et al. [23] and Vilhelmson et al. [18] showed the effect of various plant proteins on hepatic metabolism in rainbow trout (*O. mykiss*). Thus, this protein profile analysis is a characteristic of a specific proteomic phenotype, which provides further insights in the biology of the fish. In this study a high marine protein meal diet was used and succinyl-CoA ligase [GDP-forming] subunit beta protein was decreased in abundance at 6 h after the meal suggesting a low carbohydrate metabolism. The findings of Kolditz et al. [24] demonstrated the effects of long term feeding of a high-PUFA diet on the expression of genes and proteins involved in anti-oxidant metabolism and in fatty acid desaturation in a similar fashion to that in mammals. The liver transcriptome and proteome of rainbow trout identified several molecules that respond to an increase in dietary energy and lipid supply [24]. The results of this study showed that calreticulin was up-regulated 6 h after a single meal. Calreticulin is associated with lipid droplets and it plays an important role in embryonic development, calcium homeostasis and immune function although there is limited

Table 3
Identities of protein spots based on peptide mass fingerprinting.

| Spots reference no. | Identities of protein spots based on peptide mass fingerprinting | | Identities by Mascot followed by BLAST prot | | | | | | |
	Mascot score	Protein hit from trout proteome	NCBI BLAST_Accession no.	Species	Protein	emPAI*	Mass	E value	Queries matched
1418	997	GSONMT00070809001	XP010902162.1	*Esox lucius*	phenylalanine–4-hydroxylase	2.17	52573	0	23
1944	259	GSONMT00045985001	ACI69087.1	*Salmo salar*	3-hydroxyanthranilate 3,4-dioxygenaseE	1.12	20743	9×10^{-110}	9
2322	88	GSONMT00017042001 GSONMT00031882001	ACI67383.1	*Salmo salar*	proteasome subunit beta type-6 precursor	0.28	25293	3×10^{-134}	2
3677	787	GSONMT00028984001	XP007254286.1	*Astyanax mexicanus*	type II keratin E3	0.58	76972	0	12
3710	986	GSONMT00046981001	NP001181938.1	*Oncorhynchus mykiss*	94 kD glucose-regulated protein	1.11	90766	0	29
3718	605	GSONMT00075686001	NP001117950.1	*Oncorhynchus mykiss*	calreticulin	0.94	48321	0	10
3900	429	GSONMT00052662001	ACN10783.1	*Salmo salar*	flotillin–1	0.71	41366	0	7
3993	667	GSONMT00029009001	CBN81077.1	*Dicentrarchus labrax*	succinyl-CoA ligase [GDP-forming] subunit beta	1.5	38101	0	12
4125	447	GSONMT00001563001	NP001290698.1	*Esox lucius* *Oncorhynchus*	proliferating cell nuclear antigen	1.14	28960	5×10^{-179}	7
4149	544	GSONMT00028209001	NP001117945.1	*Oncorhynchus mykiss*	14–3–3 protein epsilon	1.64	29216	3×10^{-178}	10

**Mass is theoretically calculated
* Exponentially modified protein abundance index (emPAI): relative quantitation of the proteins.

knowledge about this protein with respect to its many functions in fish and mammals [45].

Proteomic analysis extended our knowledge of postprandial changes in the liver of rainbow trout and ectothermic vertebrate. This is of relevance with changing dietary sources that farmed fish are now fed and understanding the kinetics of liver responses to different dietary nutrients will aid in understanding performance on new feeds. Integration with genomics will facilitate identification of the key proteins that function to regulate metabolic pathways and are affected by specific nutrients and also allow for future comparative proteomics to be performed.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2016.10.012.

References

[1] R. Waite, M. Beveridge, R. Brummett, S. Castine, N. Chaiyawannakarn, S. Kaushik, R. Mungkung, S. Nawapakpilai, M. Phillips, Improving Productivity and Environmental Performance of Aquaculture. Working Paper, Instalment 5 of Creating a Sustainable Food Future, World Resources Institute, Washington, DC, 2014, p. 53.

[2] FAO, The State of World Fisheries and Aquaculture: Opportunities and Challenges, Food and Agriculture Organization of the United Nations, Rome, 2014.

[3] B.K. Larsen, J. Dalsgaard, P.B. Pedersen, Effects of plant proteins on postprandial, free plasma amino acid concentrations in rainbow trout (Oncorhynchus mykiss), Aquaculture 326–329 (2012) 90–98.

[4] C.G. Carter, D.F. Houlihan, A. Kiessling, F. Medale, M. Jobling, Physiological effects of feeding, in: D.F. Houlihan, T. Boujard, M. Jobling (Eds.), Food Intake in Fishes, Blackwell Scientific, Oxford, 2001, pp. 297–331.

[5] D.F. Houlihan, E. Mathers, A.R. Foster, Biochemical correlates of growth rate in fish, in: J.C. Rankin, F.B. Jensen (Eds.), Fish Ecophysiology, Chapman and Hall, London, 1993, pp. 45–70.

[6] I.D. McCarthy, L.A. Fuiman, Post-prandial changes in protein synthesis in red drum (Sciaenops ocellatus) larvae, J. Exp. Biol. 214 (2011) 1821–1828.

[7] S.J. Kaushik, P. Luquet, Influence of dietary amino acid patterns on the free amino acid contents of blood and muscle of rainbow trout (Salmo gairdneri R.), Comp. Biochem. Physiol. B. (1979) 175–180.

[8] A.R. Lyndon, I. Davidson, D.F. Houlihan, Changes in tissue and plasma free amino acid concentrations after feeding in Atlantic cod, Fish. Physiol. Biochem. 10 (1993) 365–375.

[9] C.G. Carter, Z.Y. He, D.F. Houlihan, I.D. McCarthy, I. Davidson, Effect of feeding on tissue free amino acid concentrations in rainbow trout (Oncorhynchus mykiss Walbaum), Fish. Physiol. Biochem. 14 (2) (1995) 153–164.

[10] K. Kwasek, Y. Zhan, K. Dabrowski, Utilization of dipeptide/protein based diets in larval and juvenile Koi carp — post-prandial free amino acid levels, J. Anim. Physiol. Anim. Nutr. 94 (2010) 35–43.

[11] C.G. Carter, D.F. Houlihan, Z.Y. He, Changes in tissue free amino acid concentrations in Atlantic salmon, Salmo salar L., after consumption of a low ration, Fish. Physiol. Biochem. 23 (2000) 295–306.

[12] E. Mente, S. Deguara, M.B. Santos, Df Houlihan, White muscle free amino acid concentrations following feeding a maize gluten dietary protein in Atlantic salmon (Salmo salar L.), Aquaculture 225 (2003) 133–147.

[13] J.A. Mennigen, S. Pancerat, M. Larquier, E. Plagnes-Juan, F. Medale, I. Seiliez, S. Skiba-Cassy, Postprandial regulations of hepatic microRNAs predicted to target the insulin pathway in rainbow trout, PLoS One. 7 (6) (2012) e38604.

[14] I. Seiliez, S. Panserat, M. Lansard, S. Polakof, E. Plagnes-Juan, A. Surget, K. Dias, M. Larquier, S. Kaushik, S. Skiba-Cassy, Dietary carbohydrate-to-protein ratio affects TOR signaling and metabolism-related gene expression in the liver and muscle of rainbow trout after a single meal, Am. J. Physiol. Regul. Integr. Comp. Physiol. 300 (2011) R733–R743.

[15] L.M.P. Valente, N.I. Bower, I.A. Johnston, Postprandial expression of growth-related genes in Atlantic salmon (Salmo salar L.) juveniles fasted for 1 week and fed

a single meal to satiation, Br. J. Nutr. 108 (2012) 2148–2157.

[16] P. Gomez-Requeni, L.E.C. Conceicao, A.E. Jordal Olderbakk, I. Rønnestad, A reference growth curve for nutritional experiments in zebrafish (Danio rerio) and changes in whole body proteome during development, Fish. Physiol. Biochem. J. 36 (2010) 1199–1215.

[17] P.M. Rodrigues, T.S. Silva, J. Dias, F. Jessen, Proteomics in aquaculture: applications and trends, J. Proteom. 3 (2012) 4325–4345.

[18] O.T. Vilhelmson, S.A.M. Martin, F. Médale, S.J. Kaushik, D.F. Houlihan, Dietary plant-protein substitution affects hepatic metabolism in rainbow trout (Oncorhynchus mykiss), Br. J. Nutr. 92 (2004) 71–80.

[19] D.R. Jury, S. Kaveti, Z.H. Duan, B. Willard, M. Kinter, R. Londraville, Effects of calorie restriction on the zebrafish liver proteome, Comp. Biochem Physiol. Part D. Genom. Proteom. 3 (2008) 275–282.

[20] A. Bohne-Kjersem, A. Skadsheim, A. Goksoyr, B.E. Grosvik, Candidate biomarker discovery in plasma of juvenile cod (Gadus morhua) exposed to crude North Sea oil, alkyl phenols and polycyclic aromatic hydrocarbons (PAHs), Mar. Environ. Res. 68 (2009) 268–277.

[21] L. Bradley, A. Baumgarner, A.S. Bharadwaj, D. Inerowicz, A.S. Goodman, P.B. Brown, Proteomic analysis of rainbow trout (Oncorhynchus mykiss) intestinal epithelia: physiological acclimation to short-term starvation, Comp. Biochem. Physiol., Part D. 8 (2013) 58–64.

[22] S.A.M. Martin, P. Cash, S. Blaney, D.F. Houlihan, Proteome analysis of rainbow trout (Oncorhynchus mykiss) liver proteome during short-term starvation, Fish. Physiol. Biochem. 24 (2001) 259–270.

[23] S.A.M. Martin, O. Vilhelmsson, F. Medale, P. Watt, S. Kaushik, D.F. Houlihan, Proteomic sensitivity to dietary manipulations in rainbow trout, Biochim. Et. Biophys. Acta-Proteins Proteom. 2003 (1651) 17–29.

[24] C. Kolditz, G. Paboeuf, M. Borthaire, D. Esquerré, M. SanCristobal, F. Lefèvre, F. Médale, Changes induced by dietary energy intake and divergent selection for muscle fat content in rainbow trout (Oncorhynchus mykiss), assessed by transcriptome and proteome analysis of the liver. BMC, Genomics 9 (2008) 506.

[25] S. Keyvanshokooh, A. Tahmasebi-Kohyani, Proteome modifications of fingerling rainbow trout (Oncorhynchus mykiss) muscle as an effect of dietary nucleotides, Aquaculture 324 (2012) 79–84.

[26] N.H. Sissener, S.A.M. Martin, P. Cash, E.M. Hevroy, M. Sanden, G. Hemre, Proteomic profiling of liver from Atlantic salmon (Salmo salar) fed genetically modified soy compared to the near-isogenic non-GM line, Mar. Biotechnol. 12 (2010) 273–281.

[27] S. Morais, T. Silva, O. Cordeiro, P. Rodriques, D.R. Guy, J.E. Bron, J.B. Taggart, J.G. Bell, D.R. Tocher, Effects of genotype and dietary fish oil replacement with vegetable oil on the intestinal transcriptome and proteome of Atlantic salmon (Salmo salar), BMC Genom. 13 (2012) 448.

[28] E. Rufino-Palomares, F.J. Reyes-Zurita, C.A. Fuentes-Almagro, M. de la Higuera, J.A. Lupianez, J. Peragon, Proteomics in the liver of gilthead sea bream (Sparus aurata) to elucidate the cellular response induced by the intake of maslinic acid, Proteomics 11 (2011) 3312–3325.

[29] M. de Vareilles, L.E.C. Conceição, P. Gómez-Requeni, K. Kousoulaki, R.N. Rodrigues, K.E. Fladmark, I. Rønnestad, Dietary Lysine Imbalance Affects muscle proteome in zebrafish (Danio rerio): a comparative 2D-DIGE study, Mar. Biotechnol. 14 (2012) 643–654.

[30] E. Matos, T.S. Silva, T. Wulff, L.M.P. Valente, V. Sousa, E. Sampaio, A. Goncalves, J.M.G. Silva, M.T. Dinis, P. Guedes de Pinho, P.M. Rodrigues, J. Dias, Influence of supplemental maslinic acid (olive-derived triterpene) on the post-morterm muscle properties and quality traits of gilthead seabream, Aquaculture 396–399 (2013) 146–155.

[31] I. Seiliez, F. Medale, P. Aquirre, M. Larquier, L. Lanneretonne, H. Alami-Durante, S. Panserat, S. Skiba-Cassy, Postprandial regulation of growth and metabolism-related factors in zebrafish, Zebrafish 10 (2) (2013). http://dx.doi.org/10.1089/zeb.2012.0835.

[32] C. Berthelot, F. Brunet, D. Chalopin, A. Juanchich, M. Bernard, B. Noël, P. Bento, C. Da Silva, K. Labadie, A. Alberti, J.M. Aury, A. Louis, P. Dehais, P. Bardou, J. Montfort, C. Klopp, C. Cabau, C. Gaspin, G.H. Thorgaard, M. Boussaha, et al., The rainbow trout genome provides novel insights into evolution after whole-genome duplication in vertebrates. Nature, Communications 5 (2014) 3657.

[33] AOAC, Official methods of analysis of AOAC International, 16th Ed. 4th Rev. Association of Official Analytical Chemists International, Inc., Gaithersburg. Maryland, USA, 1988.

[34] P. Cash, E. Argo, K. Bruce, Characterisation of haemophilus influenza proteins by two-dimensional gel electrophoresis, Electrophoresis 16 (1995) 135–148.

[35] A. Smith, J.P. van Rooyen, E. Argo, P. Cash, Proteomic analysis of Escherichia coli associated with urinary tract infections, Proteomics (11) (2011) 2283–2293.

[36] O.J. Nøstbakken, S.A.M. Martin, P. Cash, B.E. Torstensen, H. Amlund, P.A. Olsvik, Dietary methylmercury alters the proteome in Atlantic salmon (Salmo salar) kidney, Aquat. Toxicol. 108 (2011) 70–77.

[37] R.E. Drew, K.J. Rodnick, M. Settles, J. Wacyk, E. Churchill, M.S. Powell, et al., Effect of starvation on trascriptomes of brain and liver in adult female zebrafish (Danio rerio), Physiol. Genom. 35 (2008) 283–295.

[38] M. Salem, J. Silverstein, C.E. Rexroad, J. Yao, Effect of starvation on global gene expression and proteolysis in rainbow trout (Oncorhynchus mykiss). BMC, Genomics 8 (2007) 328.

[39] S.A.M. Martin, A. Douglas, D.F. Houlihan, C.J. Secombes, Starvation alters the liver transcriptome of the innate immune response in Atlantic salmon (Salmo salar), BMC Genom. (2010) 11–418.

[40] C. Uribe, H. Folch, R. Enriquez, G. Moran, Innate and adaptive immunity in teleost fish: a review, Vet. Med. 56 (2011) 486–503.

[41] H. Koskinen, A. Krasnov, C. Rexroad, Y. Gorodilov, S. Afanasyev, H. Mölsä, The 14-3-3 proteins in the teleost fish rainbow trout (Oncorhynchus mykiss), J. Exp. Biol. 207 (19) (2004) 3361–3368.

[42] H.B. Hermann, D.G. Homberger, Structure and functions of keratin proteins in simple stratified, keratinized and cornified epithelia, J. Anat. 214 (2009) 516–559.

[43] Y.L. Enyu, A.C. Shu-Chien, Proteomics analysis of mitochondrial extract from liver of female zebrafish undergoing starvation and refeeding, Aquacult Nutr. 17 (2011) E413–E423.

[44] R.A. Stetler, Y. Gan, W. Zhang, A.K. Liou, Y. Gao, C. Guodong, J. Chen, Heat shock proteins: cellular and molecular mechanisms in the CNS, Prog. Neurobiol. 92 (2) (2010) 184–211.

[45] E. Szabo, Y. Qiu, S. Baksh, M. Michalak, M. Opas, Calreticulin inhibits commitment to adipocyte differentiation, J. Cell Biol. 182 (2008) 103–116. http://dx.doi.org/10.1083/jcb.200712078.