

RESEARCH ARTICLE

Regulation of metabolism by dietary carbohydrates in two lines of rainbow trout divergently selected for muscle fat content

Biju Sam Kamalam¹, Françoise Medale¹, Sadasivam Kaushik¹, Sergio Polakof^{1,2}, Sandrine Skiba-Cassy¹ and Stephane Panserat^{1,*}

¹Institut National de la Recherche Agronomique (INRA), Nutrition, Metabolism and Aquaculture Unit (UR1067), F-64310 Saint-Pée-sur-Nivelle, France and ²INRA, Unité de Nutrition Humaine (UMR 1019), Centre de Recherche en Nutrition Humaine d'Auvergne, F-63009 Clermont-Ferrand, France

*Author for correspondence (panserat@st-pee.inra.fr)

SUMMARY

Previous studies in two rainbow trout lines divergently selected for lean (L) or fat (F) muscle suggested that they differ in their ability to metabolise glucose. In this context, we investigated whether genetic selection for high muscle fat content led to a better capacity to metabolise dietary carbohydrates. Juvenile trout from the two lines were fed diets with or without gelatinised starch (17.1%) for 10 weeks, after which blood, liver, muscle and adipose tissues were sampled. Growth rate, feed efficiency and protein utilisation were lower in the F line than in the L line. In both lines, intake of carbohydrates was associated with a moderate post-prandial hyperglycaemia, a protein sparing effect, an enhancement of nutrient (TOR-S6) signalling cascade and a decrease of energy-sensing enzyme (AMPK). Gene expression of hepatic glycolytic enzymes was higher in the F line fed carbohydrates compared with the L line, but concurrently transcripts for the gluconeogenic enzymes was also higher in the F line, possibly impairing glucose homeostasis. However, the F line showed a higher gene expression of hepatic enzymes involved in lipogenesis and fatty acid bioconversion, in particular with an increased dietary carbohydrate intake. Enhanced lipogenic potential coupled with higher liver glycogen content in the F line suggests better glucose storage ability than the L line. Overall, the present study demonstrates the changes in hepatic intermediary metabolism resulting from genetic selection for high muscle fat content and dietary carbohydrate intake without, however, any interaction for an improved growth or glucose utilisation in the peripheral tissues.

Key words: fish nutrition, carbohydrates, genetic selection, metabolism, gene expression.

Received 23 January 2012; Accepted 27 March 2012

INTRODUCTION

The ability of fish to use dietary carbohydrates varies greatly among and within species in relation to their feeding habits. Carnivorous fishes are generally considered to be poor utilisers of dietary carbohydrates (Wilson, 1994; Moon, 2001; Hemre et al., 2002). For example, the rainbow trout (*Oncorhynchus mykiss*) exhibits persistent hyperglycaemia following ingestion of digestible carbohydrates or injection of glucose (Bergot, 1979; Wilson, 1994; Moon, 2001), without any clear increase in the provision of net energy (Hilton et al., 1987). Various assumptions were put forth to explain this poor utilisation, out of which insulin deficiency, lack of insulin-dependent glucose transporters and lack of an inducible hepatic glucokinase were proved to be false (Mommensen and Plisetskaya, 1991; Panserat et al., 2000a; Planas et al., 2000); low potency of glucose as an insulin secretagogue compared with amino acids, relatively low number of insulin receptors, lack of inhibition of endogenous glucose production and poor hepatic lipogenesis from glucose were proved to be true (Mommensen and Plisetskaya, 1991; Hemre and Kahrs, 1997; Navarro et al., 1999; Panserat et al., 2000b; Enes et al., 2009); reduced peripheral utilisation of glucose and inhibition of insulin secretion by other hormones requires more elaboration (Moon, 2001). Further, the presence of most of the key enzymes involved in carbohydrate metabolic pathways in fish indicate that the poor utilisation may be due to an aberrant hormonal

and nutritional regulation caused by evolutionary adaptation (Covey and Walton, 1989; Enes et al., 2009; Polakof et al., 2011a). For example, a carbohydrate-rich diet induces the hepatic glycolytic enzymes in rainbow trout very much as in mammals, but it does not affect the activity or mRNA levels of key enzymes of gluconeogenesis in complete contrast (Panserat et al., 2009). However, there are also reports of a better glycaemic profile in rainbow trout, which is genetically selected (Skiba-Cassy et al., 2009) or treated with anti-diabetic drug, linking enhanced *de novo* lipogenesis to better glucose homeostasis (Panserat et al., 2009; Polakof et al., 2011b).

From a practical perspective of salmonid aquaculture, the diminishing supply of fish meal and fish oil (the protein and energy source of choice in feeds, respectively) together with their escalating cost and demand emphasise the critical need to substitute them with ingredients of plant origin (naturally rich in carbohydrates) for the sustainability and expansion of production (Naylor, 2000; Tacon and Metian, 2008; Stone, 2003). Therefore, identifying and selecting new genotypes of fish with an improved capability to use the absorbed carbohydrate will be of economical advantage. Though some early studies do not indicate a promising prospect (Edwards et al., 1977; Austreng et al., 1977; Refstie and Austreng, 1981), others have revealed strains of salmonids exhibiting differences in the utilisation of dietary carbohydrates (Mazur et al., 1992; Blanc,

2002). Moreover, the large genetic variation for production traits in the farmed population of salmonids with medium to high heritabilities makes the fish family ideal for genetic selection (Gjedrem, 2000; Henryon et al., 2002).

Recently, two experimental lines of rainbow trout have been developed through divergent selection for low or high muscle fat content (Quillet et al., 2005). Metabolic studies suggest that the two lines differ in utilisation of energy sources when fed diets differing in lipid content but with a constant level (9–12%) of carbohydrates. Reduced hepatic fatty acid oxidation and enhanced glycolysis was observed in liver and muscle of the fat line (F) fish as compared with the lean line (L) fish (Kolditz et al., 2008a). The F line was also found to have lower plasma glucose levels 24 h after the last meal than the L line when fed a standard diet containing 10% starch (Skiba-Cassy et al., 2009). Based on these observations, we hypothesised that the F line has a higher ability to metabolise dietary carbohydrates than the L line. Our objective was to describe the metabolic response of both trout lines to diets with a high level of carbohydrates (17.1%) or without carbohydrates (<1%). We analysed the effects of genetic selection and dietary carbohydrate supply on growth, feed utilisation, body composition and post-prandial plasma metabolite levels. At the tissue level, we examined the signalling of intracellular energy sensors (AMPK), activation of insulin and nutrient pathway (Akt, TOR-S6) and gene expression of key enzymes involved in glycolysis, gluconeogenesis, lipogenesis, β -oxidation and fatty acid bioconversion in liver, which is the centre of intermediary metabolism. We also analysed gene expression in other tissues implicated in glucose utilisation, i.e. white adipose tissue and muscle.

MATERIALS AND METHODS

Experimental fish and diets

The study was performed with two lines of rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792), namely L (lean line) and F (fat line), obtained after four generations of divergent selection for high or low muscle fat content using a non-destructive method (Distell Fish Fatmeter, Fauldhouse, West Lothian, UK) as detailed by Quillet et al. (Quillet et al., 2005). Fish were reared in the INRA experimental facilities at Donzacq (Landes, France) at a constant water temperature of $17.5 \pm 0.5^\circ\text{C}$, under natural photoperiod during the months of July–September. They were fed a standard trout commercial diet (T-3P classic, Skretting, Fontaine-les-Vervins, France) during the acclimatisation period. Two experimental diets, namely 0C (without carbohydrate) and 20C (with carbohydrate), were prepared in our own facilities (INRA, Donzacq, Landes, France) as extruded pellets. Gelatinised starch was included as the carbohydrate source. The 0C diet contained <1% starch, whereas the 20C diet contained 17.1% starch (Table 1). The increase in dietary carbohydrate content was accompanied by a decrease in the proportion of protein in the 20C diet.

Feeding trial and sampling procedure

Fish of each line were distributed into six tanks of 35 animals (mean body mass $27 \pm 0.5\text{ g}$). Triplicate groups of each genotype were fed either the 0C or the 20C diet for 10 weeks, two times daily *ad libitum*. The fish were bulk weighed every 3 weeks and counted to calculate the mean body mass. Feed intake was recorded and feed efficiency was calculated. At the end of the trial, nine fish per group (three per tank) were randomly sampled at 2, 8 and 24 h after the meal. Trout were killed by a sharp blow to the head and individually weighed. Blood was removed from the caudal vein into heparinised syringes and centrifuged (3000 g, 5 min); the recovered plasma was

Table 1. Composition of diets

	0C	20C
Ingredient (%)		
Fish meal ^a	81	59
Gelatinised starch ^b	0	20
Fish oil ^c	16	18
Binder ^d	1	1
Mineral mix ^e	1	1
Vitamin mix ^f	1	1
Analytical composition		
Dry matter (%)	94.0	94.0
Protein (% DM)	58.6	44.7
Lipid (% DM)	25.4	22.6
Starch (% DM)	<1	17.1
Energy (kJ g ⁻¹ DM)	24.7	23.6
Ash (% DM)	11.3	9.1

0C, diet without carbohydrate; 20C, diet with carbohydrate; DM, dry matter.

^aFishmeal (Sopropeche, Boulogne-sur-Mer, France).

^bGelatinised starch (Lestrem 62, Roquette, France).

^cFish oil (North sea fish oil; Sopropeche, Boulogne-sur-Mer, France).

^dAlginate GF 150 (Louis François exploitation, Saint-Maur, France).

^eMineral mix – 2.15 g calcium carbonate (40% Ca), 1.24 g magnesium oxide (60% Mg), 0.2 g ferric citrate, 0.4 mg kg⁻¹ diet potassium iodide (75% I), 0.4 g zinc sulphate (36% Zn), 0.3 g copper sulphate (25% Cu), 0.3 g manganese sulphate (33% Mn), 5 g dibasic calcium phosphate (20% Ca, 18% P), 2 mg kg⁻¹ diet cobalt sulphate, 3 mg kg⁻¹ diet sodium selenite (30% Se), 0.9 g KCl, 0.4 g NaCl (UPAE, Jouy, Inra, France).

^fVitamin mix – 60 IU DL-a tocopherol acetate, 5 mg kg⁻¹ diet sodium menadione bisulphate, 15,000 IU retinyl acetate, 3000 IU DL-cholecalciferol, 15 mg kg⁻¹ diet thiamin, 30 mg kg⁻¹ diet riboflavin, 15 mg kg⁻¹ diet pyridoxine, 0.05 mg kg⁻¹ diet B12, 175 mg kg⁻¹ diet nicotinic acid, 500 mg kg⁻¹ diet folic acid, 1000 mg kg⁻¹ diet inositol, 2.5 mg kg⁻¹ diet biotin, 50 mg kg⁻¹ diet calcium pantothenate, 2000 mg kg⁻¹ diet choline chloride (UPAE).

immediately frozen and kept at -20°C until analysis. The gut content of each fish was systematically checked to confirm that the fish sampled had effectively consumed the diet. Liver, perivisceral white adipose tissue and a sample of dorso-ventral white muscle were dissected, weighed, immediately frozen in liquid nitrogen and kept at -80°C pending analysis. The mass of liver was used to calculate the hepatosomatic index [HSI (%) = $100 \times (\text{liver mass}/\text{body mass})$]. Six fish per genotype at the beginning of the feeding trial and three fish per tank at the end were killed and then frozen for analysis of whole-body composition. They were pooled per tank, ground and freeze-dried before being analysed. The experiments were carried out in accordance with the Guidelines of the National Legislation on Animal Care of the French Ministry of Research (decree no. 2001-464, 29 May 2001) and were approved by the Ethics Committee of INRA (according to INRA 2002-36, 14 April 2002).

Growth performance and nutrient utilisation traits were calculated for each tank with 35 fish, which represented one experimental unit. Final body mass was calculated as the final biomass divided by the number of fish in each tank at the end of the feeding trial. Daily growth coefficient was calculated as $100 \times (\text{mean final body mass}^{1/3} - \text{mean initial body mass}^{1/3})/\text{day}$. Daily feed intake was calculated as the total amount of ingested food (kg) divided by the mean biomass over the trial [(initial biomass + final biomass)/2, expressed in kg wet mass (WM)] and the number of days. Feed efficiency was estimated as the gain in total biomass [(final biomass – initial biomass) (kg WM)] divided by the amount of ingested dry matter (kg DM). Protein efficiency ratio was the gain in total biomass divided by the amount of ingested crude protein (kg). Nitrogen and fat gain were calculated

as (final carcass nutrient content – initial carcass nutrient content) divided by the mean biomass over the trial [(initial biomass + final biomass)/2 (kg WM)] and the number of days, where nutrient refers to nitrogen and fat. Protein and fat retention were calculated as $[100 \times (\text{final body mass} \times \text{final carcass nutrient content}) - (\text{initial body mass} \times \text{initial carcass nutrient content})] / \text{nutrient intake}$, where nutrient refers to protein and lipid.

Analytical methods

The chemical composition of the diets and the freeze-dried samples of whole body was analysed using the following procedures: dry matter after drying at 105°C for 24 h, fat by petroleum ether extraction (Soxtherm, Gerhardt, Königswinter, Germany), protein content (nitrogen content $\times 6.25$) by the Kjeldahl method after acid digestion, gross energy in an adiabatic bomb calorimeter (IKA, Heitersheim Grifzheimer, Germany) and ash content by incinerating the samples in a muffle furnace at 600°C for 6 h. Total starch content in the diets was estimated by the enzymatic method (InVivo Labs, Vannes, France). Total lipid content in the muscle was measured according to the method of Folch et al. (Folch et al., 1957), with dichloromethane instead of chloroform as the solvent. Tissue glycogen levels were determined using the amyloglucosidase method (Keppler and Decker, 1974). Plasma glucose (Glucose RTU, bioMérieux, Marcy l'Etoile, France), triglycerides (PAP 150, bioMérieux) and free fatty acid (NEFA C kit, Wako Chemicals, Neuss, Germany) levels were determined using commercial kits adapted to a microplate format, according to the recommendations of the manufacturer. Total plasma free amino acid levels were determined by the ninhydrin reaction according to the method of Moore (Moore, 1968), with glycine as standard.

Western blot analysis

Protein extraction and western blotting were performed on liver samples of fish sampled 2 h after the meal. Six individual samples per experimental condition were used as biological replicates. Frozen livers (200 mg) were homogenised on ice with an Ultraturrax homogeniser in 2 ml of buffer containing 150 mmol⁻¹ NaCl, 10 mmol⁻¹ Tris, 1 mmol⁻¹ EGTA, 1 mmol⁻¹ EDTA (pH 7.4), 100 mmol⁻¹ sodium fluoride, 4 mmol⁻¹ sodium pyrophosphate, 2 mmol⁻¹ sodium orthovanadate, 1% Triton X-100, 0.5% Nonidet P-40-IGEPAL and a protease inhibitor cocktail (Roche, Basel, Switzerland). Homogenates were centrifuged at 1000g for 15 min at 4°C and supernatants were again centrifuged at 20,000g for 30 min. The resulting supernatants were aliquoted and stored at –80°C. Protein concentrations were determined using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Munich, Germany) with bovine serum albumin as standard. Liver protein lysates (10 µg of protein for Akt and S6, and 20 µg for AMPK) were subjected to SDS-PAGE and western blotting using the appropriate antibody. Anti-phospho Akt (Ser473), anti-carboxyl terminal Akt, anti-phospho-S6 (Ser235/236), anti-S6 ribosomal protein, anti-phospho-AMPK (Thr172) and anti-AMPK antibodies were used (Cell Signaling Technology, Saint Quentin Yvelines, France). These antibodies have been shown to cross-react successfully with rainbow trout proteins of interest (Skiba-Cassy et al., 2009; Polakof et al., 2011c). After washing, membranes were incubated with an IRDye infrared secondary antibody (LI-COR Biosciences, Lincoln, NE, USA). Bands were visualised by infrared fluorescence using the Odyssey imaging system (LI-COR Biosciences) and quantified by Odyssey Infrared Imaging System software (version 1.2, LI-COR Biosciences).

mRNA levels analysis: quantitative RT-PCR

Analyses of mRNA levels were performed on samples from the liver, white muscle and perivisceral white adipose tissue of fish sampled 8 h after the last meal, the time interval corresponding to the post-prandial peak of nutrient absorption in juvenile rainbow trout reared at 17°C. Six individual samples per experimental condition were used as biological replicates. Total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendations, quantified by spectrophotometry (absorbance at 260 nm) and integrity was controlled using the Agilent 2100 bioanalyzer (Agilent Technologies, Kista, Sweden). One microgram of the resulting total RNA was reverse transcribed into cDNA using the SuperScript III RNaseH⁻ reverse transcriptase kit (Invitrogen) and random primers (Promega, Charbonnières, France) according to the instructions of each manufacturer. Target gene expression levels were determined by quantitative real-time PCR as previously described, using specific primers (Kolditz et al., 2008a; Skiba-Cassy et al., 2009; Panserat et al., 2009; Polakof et al., 2009), except for $\Delta 6$ desaturase (forward: AGGGTGCCTCTGCTAACTGG; reverse: TGGTGTGGTGATGGTAGGG; GenBank AF301910) and $\Delta 9$ desaturase (forward: GCCGTCCGAGGGTTCTTCTT; reverse: CTCTC-CCCACAGGCACCAAG; GenBank FP323026, *Sigenae* clone tba0028.c.12.5). The transcripts analysed were glucokinase (GK; EC 2.7.1.2), hexokinase 1 (HK1; EC 2.7.1.1), 6-phosphofructo-1-kinase (6PFK; EC 2.7.1.11) and pyruvate kinase (PK; EC 2.7.1.40) for glycolysis; glucose-6-phosphatase (G6Pase; EC 3.1.3.9), fructose 1,6-bisphosphatase (FBPase; EC 3.1.3.11) and phosphoenolpyruvate carboxykinase (PEPCK; EC 4.1.1.32) for gluconeogenesis; glucose 6-phosphate dehydrogenase (G6PD; EC 1.1.1.49), ATP citrate lyase (ACLY; EC 2.3.3.8), fatty acid synthase (FAS; EC 2.3.1.85) and the transcription factor sterol regulatory element binding protein 1-like (SREBP-1c) for lipogenesis; $\Delta 6$ desaturase (D6D; EC 1.14.19.3) and $\Delta 9$ desaturase (D9D; EC 1.14.19.1) for fatty acid bioconversion; and carnitine palmitoyl transferase 1 (CPT1; EC 2.3.1.21) and 3-hydroxyacyl-CoA dehydrogenase (HOAD; EC 1.1.1.35) for fatty acid oxidation. When different isoforms of a gene were known in rainbow trout (as for G6Pase and CPT1), gene expression analysis was performed on each isoform. The PCR run included quadruplicates for each sample (duplicates of reverse transcription and PCR amplification, respectively) and also negative controls (reverse transcriptase and RNA free samples). Elongation factor-1 alpha (EF1 α) was employed as a non-regulated reference gene (Olsvik et al., 2005) and it was found to be stably expressed in this study. Relative quantification of target gene expression was performed using the mathematical model described by Pfaffl (Pfaffl, 2001) with the Relative Expression Software Tool (REST).

Enzyme activity analysis

Enzyme activities were measured on liver samples of fish sampled 24 h after the meal. Six individual samples per experimental condition were used as biological replicates. Liver samples used to measure the lipogenic enzyme activities were homogenised by ultrasonic disruption in four volumes of ice-cold buffer (0.02 mol⁻¹ Tris-HCl, 0.25 mol⁻¹ sucrose, 2 mmol⁻¹ EDTA, 0.1 mol⁻¹ NaF, 0.5 mmol phenylmethyl sulphonyl fluoride, 0.01 mol⁻¹ β -mercaptoethanol, pH 7.4), centrifuged at 24,000g at 4°C for 20 min and the supernatant were used immediately for enzyme assays in pre-established conditions. G6PD (final substrate concentration 0.5 mmol⁻¹ glucose-6-phosphate) and FAS (final substrate concentration 50 µmol⁻¹ malonyl-CoA) were assessed following the method described in Figueiredo-Silva et al. (Figueiredo-Silva

Table 2. Growth performance and nutrient utilization of rainbow trout from L and F lines fed 0C and 20C diets for 10 weeks

	L line		F line		P		
	0C	20C	0C	20C	Diet	Line	Diet × Line
Final body mass (g)	78.5±4.5	79.8±3.2	70.9±5.5	68.4±3.7	0.75	0.005	0.49
Daily growth index (%)	2.0±0.1	2.1±0.1	1.8±0.2	1.7±0.1	0.79	0.01	0.37
Feed intake (g kg ⁻¹ day ⁻¹)	16.8±0.1	16.5±0.6	16.6±0.5	16.0±0.3	0.11	0.16	0.59
Feed efficiency	0.97±0.03	1.0±0.03	0.92±0.06	0.92±0.03	0.58	0.03	0.41
Protein efficiency ratio	1.7±0.1	2.2±0.1	1.6±0.1	2.1±0.1	<10 ⁻⁴	0.02	0.27
N gain (mg kg ⁻¹ ABM day ⁻¹)	363±9	368±30	366±18	336±13	0.29	0.22	0.14
Fat gain (g kg ⁻¹ ABM day ⁻¹)	2.3±0.3	2.3±0.3	2.4±0.1	2.2±0.1	0.86	0.89	0.54
Protein retention (%)	24.5±0.5	33.2±2.6	25.1±0.9	31.2±1.7	<10 ⁻⁴	0.49	0.21
Lipid retention (%)	56.8±8.1	66.6±6.3	59.4±0.6	66.0±3.5	0.03	0.76	0.63

Data are means ± s.d. (N=3 tanks). Data were analysed by two-way ANOVA followed by a Student–Newman–Keuls multiple comparison test. Differences were considered statistically significant at $P<0.05$. ABM, mean biomass.

et al., 2010) adapted to trout tissues. PK enzyme activity was measured as described by Kirchner et al. (Kirchner et al., 2003). Enzyme reaction rates were determined by the increase or decrease in absorbance of NAD(P)H at 340 nm using a microplate spectrophotometer. Enzyme activity units (IU), defined as micromoles of substrate converted to product, per minute, at 37°C, were expressed per gram of liver. All of the measurements for each individual sample were performed in duplicate.

Statistical analysis

The results are presented as means ± s.d. The effects of diets, lines and the diet × line interaction on the different parameters were tested using StatView (version 5; SAS Institute, Cary, NC, USA) by means of a two-way ANOVA with diet and line as independent variables. *Post hoc* comparisons were made using a Student–Newman–Keuls multiple comparison test, and differences were considered statistically significant at $P<0.05$. When interactions were significant, means were compared using one-way ANOVA ($P<0.05$).

RESULTS

Growth performance, feed efficiency and nutrient retention

Feed intake was similar irrespective of line or diet (Table 2). At the end of the feeding trial, trout from the lean line (L) had a significantly higher body mass ($P=0.005$) than fish from the fat line (F), irrespective of diet. Feed efficiency was thus better in L line than in F line fish ($P=0.03$). Dietary treatments did not affect growth rate or final body mass. But as expected, the protein efficiency ratio was higher with the 20C diet ($P<0.0001$), because of the protein-

sparing effect of the carbohydrates in this diet. The L line exhibited better protein efficiency ($P=0.02$) than the F line. The 20C diet improved the protein ($P<0.0001$) and fat retention ($P=0.03$) in both the lines. Daily nitrogen and fat gains/retention were not significantly different between the two trout lines. No significant line × diet interaction was recorded for any of these parameters.

Whole-body and tissue composition

Dietary treatments did not modify the whole-body composition of fish from both lines, but an effect of genotype was found (Table 3). F line fish had higher whole-body lipid content ($P=0.046$) and reciprocally lower moisture content ($P=0.046$) than the L line fish. The protein content was similar in both lines. As expected, the target of genetic selection, the muscle lipid content was significantly higher in the F line fish than in the L line fish ($P=0.0004$), with no significant dietary influence. Both trout lines showed higher HSI when fed the 20C diet ($P<0.0001$). The measure of liver glycogen levels (Table 3) confirmed the increased storage of glucose as glycogen when fed the 20C diet ($P<0.0001$). The F line fish had higher liver glycogen levels ($P=0.03$) than the L line fish, irrespective of the diet. The glycogen levels in muscle remained unaffected by the diets or genetic selection. In perivisceral adipose tissue, glycogen content was negligible (data not shown).

Plasma metabolite levels

The results of post-prandial plasma metabolites measured at 2, 8 and 24 h after the meal are presented in Table 4. Plasma glucose levels were higher in fish of both lines fed the 20C diet at 2 and 8 h after the meal ($P<0.0001$ and $P=0.001$, respectively) compared

Table 3. Whole-body composition and tissue metabolites of rainbow trout from L and F lines fed 0C and 20C diets for 10 weeks

	L line		F line		P		
	0C	20C	0C	20C	Diet	Line	Diet × Line
Whole-body moisture content (%WM)	68.0±0.7	67.5±0.9	66.4±1.2	66.5±1.0	0.76	0.05	0.57
Whole-body protein content (%WM)	15.3±0.2	15.3±0.4	15.7±0.3	15.4±0.4	0.32	0.21	0.43
Whole-body lipid content (%WM)	14.5±1.1	14.6±1.1	15.7±0.7	15.7±0.5	0.95	0.05	0.91
Whole-body ash content (%WM)	2.0±0.1	2.1±0.03	2.1±0.1	2.1±0.1	0.34	0.63	0.11
Energy content (kJ g ⁻¹ WM)	9.2±0.3	9.3±0.3	9.7±0.4	9.7±0.4	0.97	0.04	0.75
Hepato-somatic index (%)	1.2±0.3	1.8±0.2	1.1±0.2	1.9±0.3	<10 ⁻⁴	0.39	0.13
Liver glycogen content (μmol glycosyl units g ⁻¹ WM)	153±31	625±132	261±75	700±101	<10 ⁻⁴	0.03	0.68
Muscle lipid content (% WM)	5.4±1.1	5.2±1.8	7.8±2.5	9.0±3.4	0.53	4 × 10 ⁻⁴	0.40
Muscle glycogen content (μmol glycosyl units g ⁻¹ WM)	5.5±3.1	6.6±2.0	4.9±2.8	3.5±2.6	0.90	0.11	0.28

Data are presented as means ± s.d. (N=3 pools for whole-body composition analysis; N=6 individuals for tissue glycogen estimations; N=9 individuals for hepato-somatic index and muscle lipid estimation) and were analysed by two-way ANOVA followed by a Student–Newman–Keuls multiple comparison test. Differences were considered statistically significant at $P<0.05$.

Table 4. Post-prandial plasma metabolites of rainbow trout from L and F lines fed 0C and 20C diets

Plasma metabolite	Post-prandial time	L line		F line		<i>P</i>		
		0C	20C	0C	20C	Diet	Line	Diet × Line
Glucose (mmol l ⁻¹)	2 h	4.3±0.6 ^b	5.9±1.1 ^b	4.4±0.4 ^b	6.3±1.7	<10 ⁻⁴	0.53	0.78
	8 h	5.4±1.2 ^a	7.6±2.0 ^a	5.6±1.1 ^a	6.9±1.5	0.001	0.68	0.43
	24 h	5.6±0.8 ^a	5.9±1.3 ^{a,b}	6.3±1.2 ^a	6.3±0.9	0.70	0.18	0.70
Triglycerides (mmol l ⁻¹)	2 h	5.6±2.2 ^b	6.2±1.7	9.3±3.6 ^b	9.0±4.3 ^b	0.92	0.004	0.66
	8 h	11.3±4.0 ^a	11.4±6.1	16.4±6.0 ^a	20.6±3.5 ^a	0.21	2×10 ⁻⁴	0.23
	24 h	5.5±2.7 ^b	7.8±5.4	9.6±3.1 ^b	7.5±1.7 ^b	0.01	0.02	0.42
Free fatty acids (mequiv. oleic acid l ⁻¹)	2 h	0.1±0.03 ^c	0.1±0.03 ^b	0.1±0.02 ^c	0.1±0.03 ^c	0.23	0.30	0.61
	8 h	0.22±0.05 ^a	0.19±0.05 ^a	0.2±0.04 ^a	0.18±0.03 ^a	0.10	0.33	0.77
	24 h	0.15±0.02 ^b	0.17±0.04 ^a	0.15±0.03 ^b	0.14±0.03 ^b	0.40	0.08	0.33
Free amino acids (mequiv. glycine ml ⁻¹)	2 h	0.47±0.07 ^b	0.51±0.05 ^a	0.5±0.08 ^b	0.48±0.06 ^b	0.71	0.88	0.24
	8 h	0.68±0.15 ^a	0.51±0.1 ^a	0.62±0.09 ^a	0.59±0.08 ^a	0.02	0.81	0.06
	24 h	0.45±0.08 ^b	0.39±0.06 ^b	0.47±0.05 ^b	0.47±0.05 ^b	0.19	0.03	0.24

Data are means ± s.d. (*N*=9 individuals). At each post-prandial time (row wise), the effect of diet, line and interaction were analysed by two-way ANOVA (*P*<0.05) followed by a Student–Newman–Keuls multiple comparison test. Within each dietary treatment (column wise), significant differences in post-prandial kinetics (2, 8 and 24 h after the last meal) are represented with different superscripts (one-way ANOVA, *P*<0.05).

with fish fed the 0C diet. However, the level of post-prandial hyperglycaemia induced by dietary carbohydrates was not very high (<8 mmol l⁻¹) in either trout line. Particularly in the F line fish fed the 20C diet, a statistically significant post-prandial hyperglycaemia was not observed but the plasma glucose levels were relatively higher at all time intervals. No significant effect of genetic selection was found for the plasma glucose level at any of the post-prandial times analysed. Plasma free fatty acid levels were not significantly different with respect to line or diet and all the treatments exhibited a similar post-prandial pattern with a peak at 8 h. The F line fish showed a significantly higher level of plasma triglycerides than the L line fish, irrespective of diet and time. The highest plasma triglyceride levels were recorded at 8 h after the meal, and were nearly twofold higher in the F line than in the L line (*P*=0.0002). Fish fed the 0C diet showed significantly higher plasma free amino acid levels than fish fed the 20C diet at 8 h, the point of post-prandial peak (*P*=0.02). The F line fish exhibited higher plasma free amino acids than the L line at 24 h (*P*=0.03).

mRNA levels of enzymes involved in intermediary metabolism

Data on the mRNA levels of the glycolytic enzymes analysed are illustrated in Fig. 1. In the liver, GK mRNA levels were induced several hundred-fold in fish in both the lines when fed the 20C diet (*P*<0.0001); this induction was also dependent on genotype, with the F line showing a higher expression than the L line, as indicated by the significant interaction between diet and line (*P*=0.04). Expression levels of 6PFK mRNA remained unchanged between the lines whatever the diet. PK mRNA levels were uncharacteristically reduced in fish fed the 20C diet compared with those fed the 0C diet (*P*<0.0001), and F line fish showed a higher PK expression than L line fish (*P*=0.04). In muscle and adipose tissue, the mRNA levels of the glycolytic genes analysed were similar between lines and remained unchanged with dietary treatment, with one exception in adipose tissue. An interaction between diet and genotype was observed for PK (*P*=0.01), where the L line exhibited decreased mRNA levels compared with the F line when fed the 20C diet. The mRNA levels of the hepatic gluconeogenic enzymes analysed were found to be differentially regulated by dietary carbohydrates (Fig. 2). The 20C diet upregulated the expression of G6Pase-2 (*P*=0.04), whereas it significantly downregulated the expression of FBPase (*P*<0.0001). Trout from the F line exhibited significantly higher expression levels of FBPase

and PEPCK mRNA than the L line (*P*<0.0001 for both). In the liver, mRNA expression levels of genes encoding enzymes involved in NADPH synthesis and lipogenesis (G6PD, ACLY and FAS) were significantly higher in the F line fish than in the L line fish (Fig. 3). The 20C diet enhanced the transcript levels of these lipogenic enzymes in the trout lines, particularly in the F line. A significant interaction between diet and line was observed for G6PD mRNA levels (*P*=0.04), with a strong induction in the F line when fed the 20C diet. No significant difference between the lines was observed for the transcription factor SREBP1c. Regardless of diet and line, no changes in relative transcript levels were observed for all the lipogenic enzymes examined in the adipose tissue except for the transcription factor SREBP1c, the expression of which was significantly higher in the F line than in the L line (*P*=0.01). Further concerning fatty acid bioconversion in the liver (Fig. 3), the F line demonstrated a significantly higher mRNA expression of D6D than the L line (*P*<0.0001) irrespective of diet. In the case of D9D, mRNA expression levels were considerably enhanced by the 20C diet in both lines (*P*=0.0003), but the level of induction varied significantly depending on the genotype, leading to a significant interaction (*P*=0.008). As shown in Fig. 4, dietary treatment induced no variation in the mRNA levels of candidate enzymes linked to fatty acid oxidation in all the three tissues analysed. But an effect of genetic selection was observed for the mRNA levels of the two hepatic β-oxidation enzymes investigated. The L line showed a significantly higher level of expression of both A and B isoforms of CPT1 than the F line (*P*=0.0002 and 0.0003, respectively), whereas an inverse line effect was observed for HOAD (*P*=0.0009).

Activities of enzymes involved in hepatic intermediary metabolism

To substantiate the results from gene expression analysis, the activities of two lipogenic enzymes, FAS and G6PD, were determined 24 h after the last meal (Fig. 5). The activity of FAS and G6PD were significantly enhanced by dietary carbohydrates in both fish lines (*P*=0.01 and *P*<0.0001, respectively), but no significant difference was observed between the lines. The PK activity was assessed because of its atypically decreased mRNA levels in both fish lines when fed the 20C diet in this study. In contrast to the mRNA levels, the activity of PK was not found to be different between the dietary treatments or the lines.

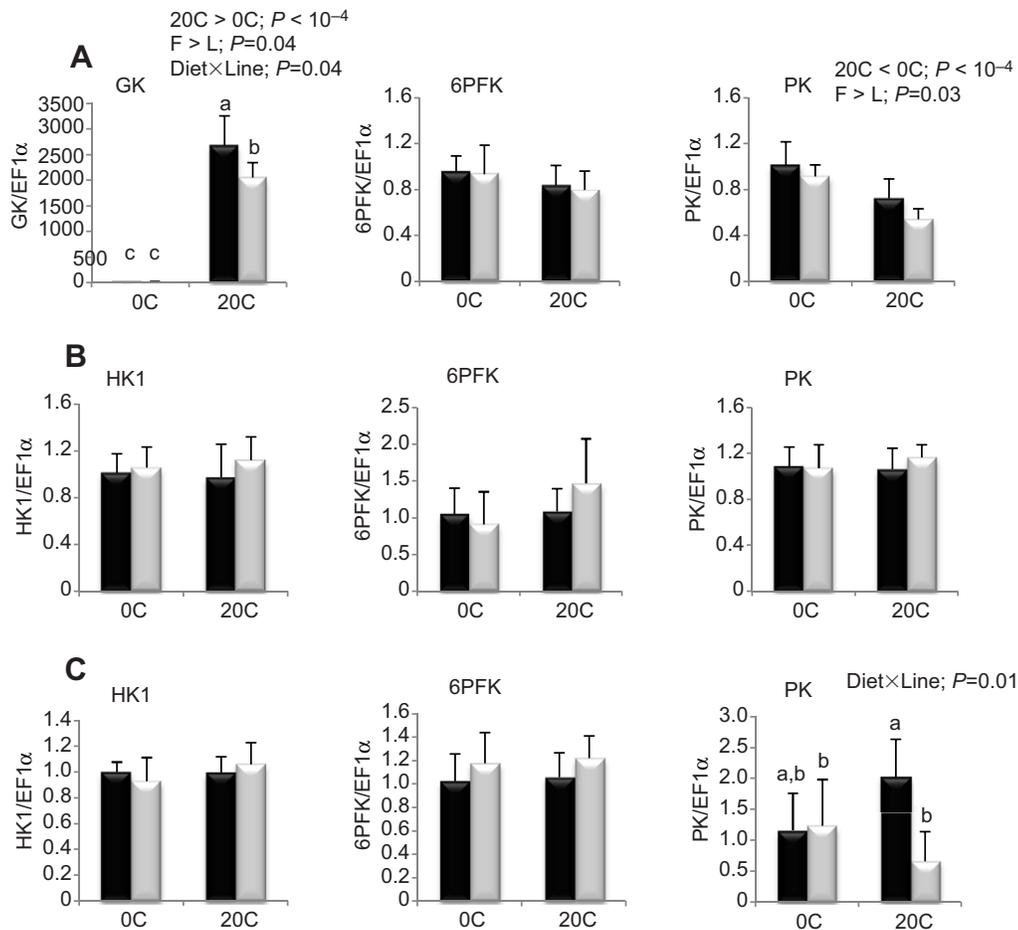


Fig. 1. Gene expression of selected glycolytic enzymes in the (A) liver, (B) muscle and (C) white adipose tissue of rainbow trout from a fat line (F; black bars) and a lean line (L; grey bars) fed a diet without (0C) or with (20C) carbohydrate, 8 h after the last meal. Glucokinase (GK), hexokinase 1 (HK1), 6-phosphofructo-1-kinase (6PFK) and pyruvate kinase (PK) mRNA levels were measured using real-time quantitative RT-PCR. Expression values are normalized by elongation factor-1 alpha (EF1 α)-expressed transcripts. Relative fold differences between treatments are presented as means \pm s.d. ($N=6$ individuals) and were analysed using two-way ANOVA ($P<0.05$), followed by a Student–Newman–Keuls multiple comparison test. Mean values not sharing a common lowercase letter are significantly different from each other (one-way ANOVA, $P<0.05$).

Hepatic Akt, S6 and AMPK phosphorylation status

To better understand the gene expression regulation in liver (in the two other tissues the gene expression was almost constant), we investigated the effect of diet with or without carbohydrates on the activation of different signalling molecules involved in the insulin pathway (Akt), the nutrient sensing pathway (TOR-S6) and cellular energy homeostasis (AMPK) in the two lines. Fig. 6 illustrates the results of western blot analyses of phosphorylated (P-) and total forms of Akt, S6 and AMPK obtained in F and L lines fed either the 0C or the 20C diet. Analysis of the AMPK ratio data indicated that the phosphorylation levels were significantly reduced in fish fed the 20C diet compared with fish fed the 0C diet in both lines ($P<0.0001$), but were not different between the lines. In contrast, S6 ribosomal protein ratio data showed that the 20C diet increased the activation levels compared with the 0C diet ($P<0.03$), with no effect of genetic selection. Akt protein phosphorylation was not influenced by diet; however, it was slightly modified by genetic selection ($P<0.04$).

DISCUSSION

The hypothesis of the present study was that the F line has a higher ability to metabolise dietary carbohydrates than the L line, as previously suggested by Kolditz et al. (Kolditz et al., 2008a) and Skiba-Cassy et al. (Skiba-Cassy et al., 2009), who worked with these trout lines fed diets containing constant level of carbohydrates (~10%). Our objective was thus to verify this hypothesis by analysing the metabolic response of the two trout lines to diets with or without carbohydrates. The effect of diet, genotype and their interaction are discussed separately below.

Effect of diets with (20C) or without (0C) carbohydrates

The diet with carbohydrate did not adversely impact feed intake, feed efficiency or growth of the trout lines because the inclusion level of gelatinised starch was within the acceptable limits and the energy from glucose was utilised as efficiently as that of protein (Kaushik et al., 1989; Kim and Kaushik, 1992; Wilson, 1994). The higher protein efficiency ratio and protein retention associated with dietary carbohydrate intake indicate a protein-sparing effect, as was expected (Kim and Kaushik, 1992; Hemre et al., 2002). Dietary carbohydrate intake resulted in larger liver and increased glycogen content, which is also well documented (Wilson, 1994; Hemre et al., 2002). Moreover, as expected post-prandial hyperglycaemia accompanied the intake of dietary carbohydrates, even though the level of induction was relatively moderate compared with previous studies using similar levels of carbohydrate intake (Panserat et al., 2000a; Bergot, 1979; Kaushik and Oliva Teles, 1985). In the F line, long-term carbohydrate intake resulted in an overall higher glycaemic profile that was not modulated post-prandially after a single meal. We studied the regulation of metabolism by dietary carbohydrates at the signalling, molecular and enzymatic levels in target tissues involved in glucose utilisation, i.e. liver, muscle and adipose tissue. However, we caution that in the present study it was not possible to reject completely any effect of dietary protein levels, as fish fed 20C diet ingested less protein. The absolute difference in carbohydrate intake (<1% versus 17.1%) between the two dietary groups, but not for protein (always higher than the requirement; 59% versus 45%), enables us to consider that the majority of the effects on intermediary metabolism are linked to the carbohydrate intake.

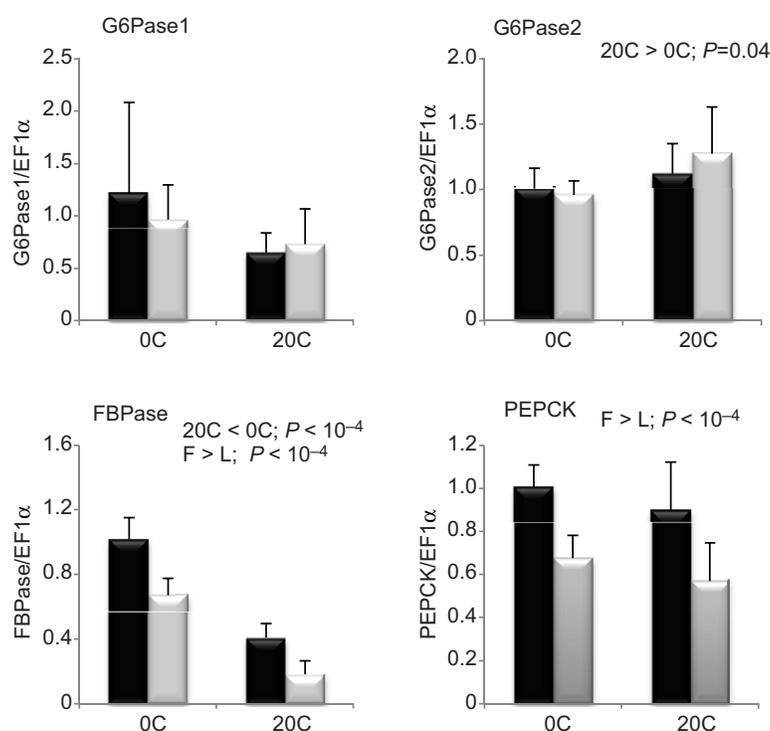


Fig. 2. Gene expression of selected gluconeogenic enzymes in the liver of rainbow trout from a fat line (F; black bars) and a lean line (L; grey bars) fed a diet without (0C) or with (20C) carbohydrate, 8 h after the last meal. Glucose-6-phosphatase isoform 1 (G6Pase1) and isoform 2 (G6Pase2), fructose 1,6-bisphosphatase (FBPase) and phosphoenolpyruvate carboxykinase (PEPCK) mRNA levels were measured using real-time quantitative RT-PCR. Expression values are normalised by elongation factor-1 alpha (EF1 α)-expressed transcripts. Relative fold differences between treatments are presented as means + s.d. ($N=6$ individuals) and were analysed using two-way ANOVA, followed by a Student–Newman–Keuls multiple comparison test. Differences were considered significant at $P<0.05$.

We analysed specific signalling pathways involved in nutrient (TOR-S6) and energy (AMPK) sensing in liver, the main tissue involved in nutrient homeostasis. We observed that carbohydrate intake decreases the phosphorylation of AMPK in rainbow trout just like in mammals (Assifi et al., 2005), which in turn enhances the activation of S6 mitochondrial protein (Wullschleger et al., 2006; Shaw, 2009). AMPK is a metabolic master switch by which cells sense and decode changes in cellular energy status, whereas S6 protein is a major indicator of the TOR signalling cascade involved in nutrient sensing and cell growth. In mammals, AMPK is known to exert a negative control over the TOR signalling (Rutter et al., 2003; Wullschleger et al., 2006; Shaw, 2009). Existence of these two proteins with a functional regulatory mechanism has already been reported in rainbow trout muscle and liver (Seiliez et al., 2008; Lansard et al., 2009; Polakof et al., 2011c). However, our results strongly suggest, for the first time in fish, that dietary carbohydrates contribute to a specific hepatic cell signalling response, as reflected by the decrease of AMPK phosphorylation as well as the increase of the S6 phosphorylation, when fish were fed with carbohydrates.

We analysed the glucose metabolism in liver, muscle and fat tissue. In liver, we reaffirm the recognised on–off regulation of GK in response to dietary carbohydrates at the transcriptional level (Panserat et al., 2000a; Panserat et al., 2009; Seiliez et al., 2011). The absence of molecular regulation of endogenous glucose production by dietary carbohydrates is also reiterated from the mRNA levels of the rate-limiting gluconeogenic enzymes, G6Pase and PEPCK (Panserat et al., 2000b; Panserat et al., 2001a; Kirchner et al., 2008). The synchronised higher expression of G6Pase 2 and GK suggests the possibility of a futile cycle following dietary carbohydrate intake; further investigations are required to validate it. Moreover, the existence of a paradoxical inhibition of hepatic PK by dietary carbohydrate at the mRNA level could also play a negative role in glucose utilisation in liver (Pilkis and Granner, 1992; Panserat et al., 2001b). This inhibition could result from an interaction between levels of dietary carbohydrates and

polyunsaturated fatty acids, as reported in rats (Yamada and Noguchi, 1999), but this hypothesis requires further confirmation. However, contrary to previous reports in trout (Panserat et al., 2001b; Kirchner et al., 2008), FBPase expression was downregulated by dietary carbohydrates, similar to mammals and a few other fish species (Pilkis and Granner, 1992; Panserat et al., 2002). Though the exact reason behind this downregulation remains elusive, this could explain the moderate hyperglycaemia observed, as the rate of glucose production from substrates entering the pathway at the triose phosphate level would be limited. In the white muscle, the absence of dietary effect in the expression of glycolytic enzymes confirms the low response of this tissue to a high influx of glucose, as shown previously (West et al., 1994; Hemre et al., 2002; Panserat et al., 2009). A similar absence of differences in the scarcely studied adipose tissue reinforces the theory that glucose utilisation is poorly regulated in the peripheral tissues of carnivorous fish (Moon, 2001).

There is a strong relationship between glucose and lipid metabolism in animals, which can impact glucose use (Randle, 1998). Indeed, excess carbohydrate intake can be converted to fatty acids through *de novo* lipogenesis (Towle et al., 1997; Dentin et al., 2006). Lipogenesis from dietary carbohydrate has been previously demonstrated in rainbow trout by elevated lipogenic enzyme activities and lipid retention ratios above 100% (Kaushik et al., 1989; Brauge et al., 1995; Alvarez et al., 2000). We found that dietary carbohydrate intake enhanced mRNA levels and activity of hepatic G6PD, representing a potential higher production of NADPH, the cytosolic reducing equivalent required for lipogenesis, as shown previously in different fish (Lin et al., 1977b; Hilton and Atkinson, 1982; Hemre et al., 2002; Panserat et al., 2009). Further, even though the transcript abundance of SREBP1c, a transcription factor involved in regulation of FAS gene expression (Eberle et al., 2004; Ferre and Foulfelle, 2007), did not increase with carbohydrate intake, we show evidence for increased hepatic lipogenic capacities through the enhanced activity of FAS and the elevated mRNA levels of ACLY, the main lipogenic enzyme diverting glycolytic carbon

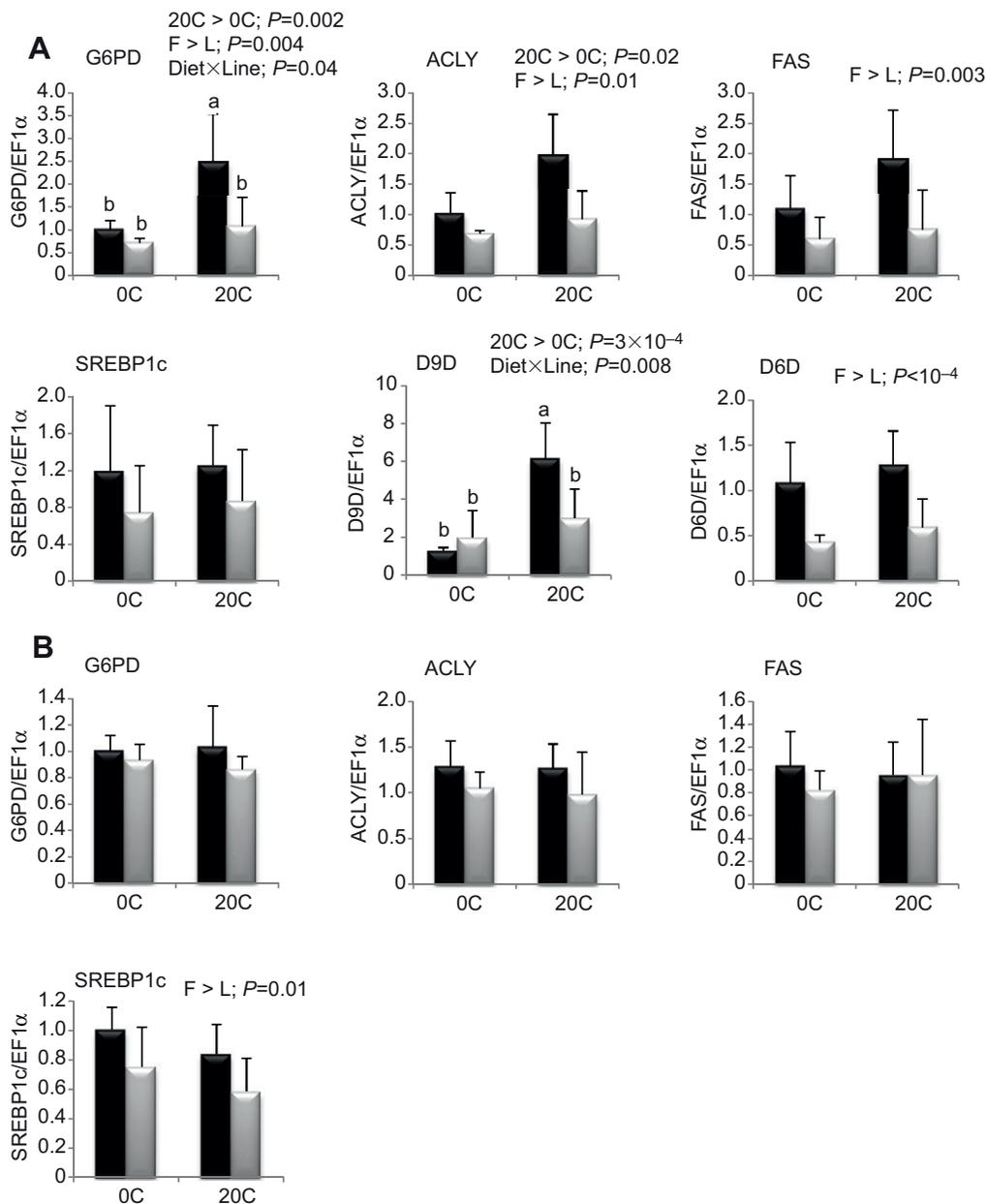


Fig. 3. Gene expression of selected enzymes and transcription factors involved in NADPH generation, lipogenesis and fatty acid bioconversion in the (A) liver and (B) white adipose tissue (excluding fatty acid bioconversion) of rainbow trout from a fat line (F; black bars) and a lean line (L; grey bars) fed a diet without (0C) or with (20C) carbohydrate, 8 h after the last meal. Glucose 6-phosphate dehydrogenase (G6PD), ATP citrate lyase (ACLY), fatty acid synthase (FAS), sterol regulatory element binding protein 1-like (SREBP-1c), $\Delta 9$ desaturase (D9D) and $\Delta 6$ desaturase (D6D) mRNA levels were measured using real-time quantitative RT-PCR. Expression values are normalised by elongation factor-1 alpha (EF1 α)-expressed transcripts. Relative fold differences between treatments are presented as means + s.d. ($N=6$ individuals) and were analysed using two-way ANOVA ($P < 0.05$), followed by a Student–Newman–Keuls multiple comparison test. Mean values not sharing a common lowercase letter are significantly different from each other (one-way ANOVA, $P < 0.05$).

flux into lipid biosynthesis (Lin et al., 1977a; Lin et al., 1977b). Another interesting observation is that D9D transcript levels are also elevated by feeding dietary carbohydrates, suggesting a higher synthesis of monounsaturated fatty acids following the carbohydrate-induced lipogenesis, as in mammals (Tocher et al., 1998; Ntambi et al., 2004). Looking further along the fatty acid bioconversion pathway, we did not find any effect of dietary carbohydrate on the expression of another desaturase, the D6D, contrary to Seiliez et al. (Seiliez et al., 2001). The high level of fish oil and fish meal in the diets probably supplied enough of the essential fatty acids (Vagner and Santigosa, 2010). In the adipose tissue, we found no stimulatory effect of dietary carbohydrates on the expression of lipogenic enzymes, as has been reported in catfish (Likimani and Wilson, 1982), confirming the lack of response in salmonids to dietary manipulations (Lin et al., 1977a; Lin et al., 1977b). There was also no dietary impact on regulation of fatty acid catabolism, as reflected by the constant levels of CPT1 and HOAD mRNAs. Overall, the enhanced hepatic lipogenic pathway may play a pivotal role in

storing excess glucose, ultimately leading to an improved glucose homeostasis in this glucose-intolerant species, as previously suggested using a specific anti-diabetic drug (Panserat et al., 2009; Polakof et al., 2011b).

Effect of genetic selection

We confirm the better growth potential of the L line compared with the F line, which has been reported from the third generation onwards, and it is not linked to a difference in the voluntary feed intake. Rather, it is associated with an improved feed efficiency and protein utilisation in the L line (Quillet et al., 2007; Kolditz et al., 2008a; Skiba-Cassy et al., 2009). The slightly higher whole-body lipid content in the F line can be attributed to the increased divergence in muscle lipid content with the progress of selection (Kolditz et al., 2008a; Skiba-Cassy et al., 2009). Moreover, the nutrient gain and retention estimates for protein and lipid do not reflect any preferential utilisation of energy substrates in the lines. Finally, and contrary to data from a previous study (Skiba-Cassy

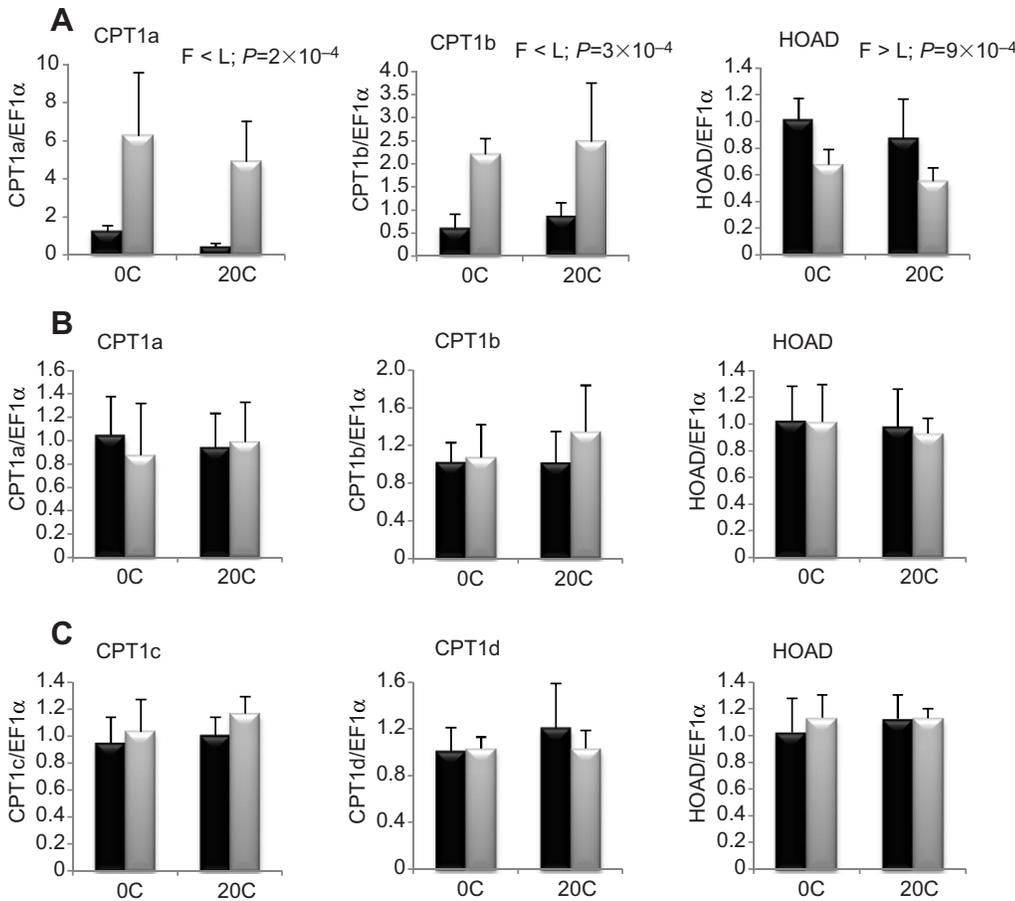


Fig. 4. Gene expression of selected fatty acid oxidation enzymes in the (A) liver, (B) muscle and (C) white adipose tissue of rainbow trout from a fat line (F; black bars) and a lean line (L; grey bars) fed a diet without (0C) or with (20C) carbohydrate, 8 h after the last meal. Carnitine palmitoyl transferase isoforms a (CPT1a), b (CPT1b), c (CPT1c) and d (CPT1d) and 3-hydroxyacyl-CoA dehydrogenase (HOAD) mRNA levels were measured using real-time quantitative RT-PCR. Expression values are normalised by elongation factor-1 alpha (EF1 α)-expressed transcripts. Relative fold differences between treatments are presented as means + s.d. ($N=6$ individuals) and were analysed using two-way ANOVA, followed by a Student–Newman–Keuls multiple comparison test. Differences were considered significant at $P<0.05$.

et al., 2009), the F and L lines did not show any difference in the post-prandial regulation of glycaemia, but the measures were not made in the same preceding conditions (fasted/refed). However, the analyses of the molecular actors in glucose and lipid metabolism clearly show significant differences between the two lines.

Studies at the transcriptional level suggest a higher glycolysis in the liver of F line as indicated by the elevated mRNA levels of the enzymes GK and PK (Kolditz et al., 2008a). The higher liver glycogen content in the F line suggests better glucose storage. However, we found that control over the hepatic endogenous glucose production was weaker in the F line than in the L line irrespective of the dietary carbohydrate levels, as indicated by the higher mRNA abundance of the gluconeogenic enzymes PEPCK and FBPase. This

indicates that the potential export of glucose synthesised *de novo* through unregulated gluconeogenesis may counter-balance the uptake of glucose from the bloodstream for glycolytic purposes in the F line, which may explain why we did not find any difference in the plasma glucose levels between the two lines. We must note here that the cell signalling analysis in liver cannot explain the differences in glucose metabolism between the two lines: there was a slightly lower activation of Akt phosphorylation in the liver of the F line (which show paradoxically higher insulin-dependent metabolic pathways—glycolysis and glycogen) and no difference in S6 and AMPK phosphorylation, in contrast to our previous study (Skiba-Cassy et al., 2009). In the muscle, we did not observe an enhanced glycolysis in the F line, which was suggested as a possible

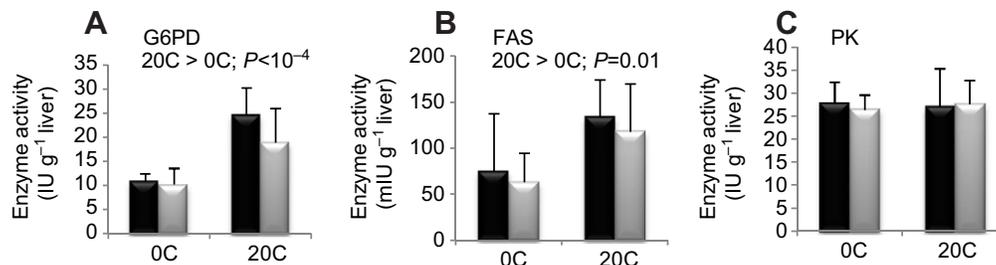


Fig. 5. Selected lipogenic and glycolytic enzyme activities in the liver of rainbow trout from a fat line (F; black bars) and a lean line (L; grey bars) fed a diet without (0C) or with (20C) carbohydrate, 24 h after the last meal. (A) Glucose-6-phosphate dehydrogenase (G6PD). (B) Fatty acid synthase (FAS). (C) Pyruvate kinase (PK). Results are means + s.d. ($N=6$ individuals) and were analysed using two-way ANOVA, followed by a Student–Newman–Keuls multiple comparison test. Differences were considered significant at $P<0.05$. Enzyme activity units (IU), defined as micromoles of substrate converted to product, per minute, at assay temperature, were expressed per gram liver tissue.

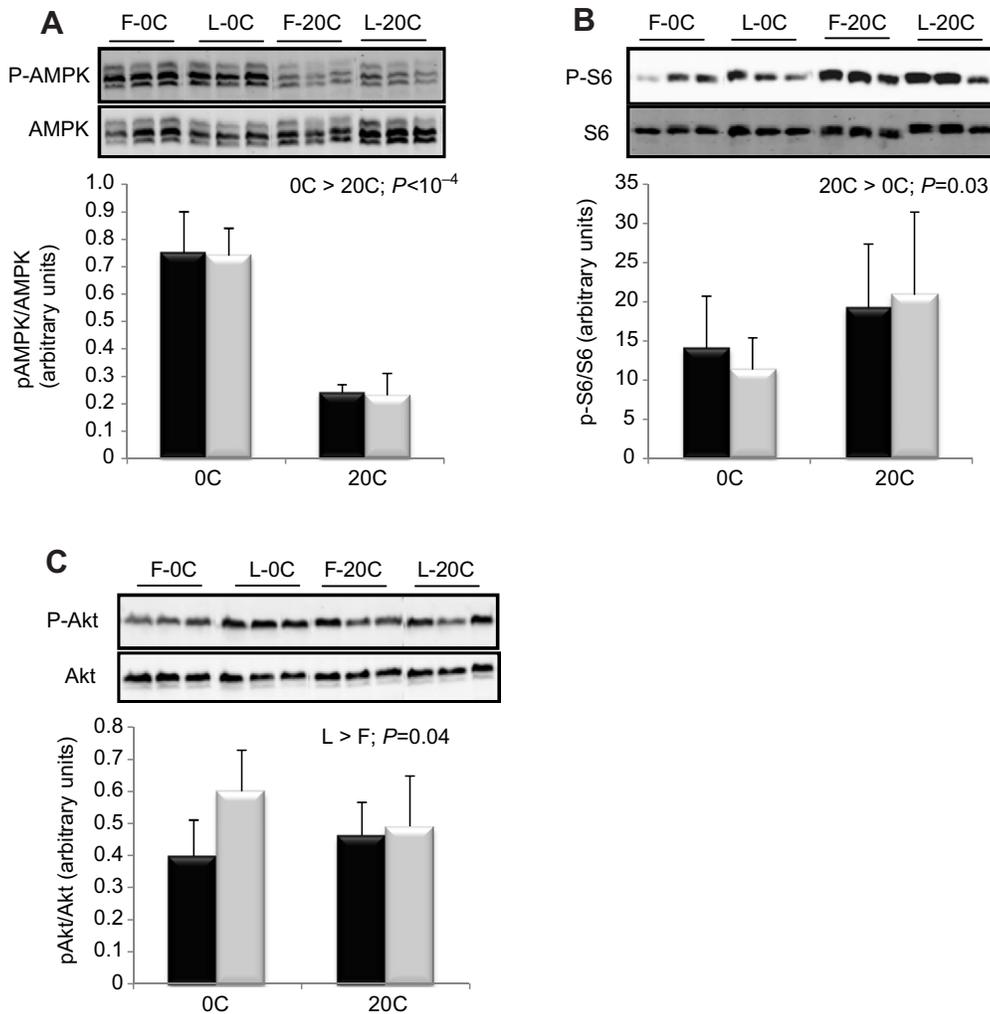


Fig. 6. Western blot analysis of (A) AMPK, (B) S6 and (C) Akt protein phosphorylation in the liver of rainbow trout from a fat line (F; black bars) and a lean line (L; grey bars) fed a diet without (0C) or with (20C) carbohydrate, 2 h after the last meal. Gels were loaded with 20 and 10 μ g of total protein per lane for AMPK and S6/Akt, respectively. Western blots were performed on six individual samples per treatment and a representative blot is shown here. Graphs represent the ratio between the phosphorylated protein and the total amount of the target protein. Data are means + s.d. ($N=6$) and were analysed using two-way ANOVA, followed by a Student–Newman–Keuls multiple comparison test. Differences were considered significant at $P<0.05$.

genetic predisposition by Kolditz et al. (Kolditz et al., 2008a). This inconsistency may be due to differences in the generation of selection investigated (fourth *versus* third), rearing temperature (17°C *versus* 11°C), diet composition or post-prandial time of analysis (8 h *versus* 24 h). Our hypothesis of a better utilisation of glucose in the peripheral tissues of the F line was therefore not validated.

Regarding the lipid metabolism, we confirm the higher hepatic lipogenic potential in the F line (FAS, ACLY and G6PD) as previously shown (Skiba-Cassy et al., 2009). This is also reflected by the elevated plasma triglyceride levels in the F line. In contrast, in Atlantic salmon similarly selected for muscle adiposity, the fat genotype exhibits lower plasma triglyceride levels reportedly because of an increased lipid uptake by peripheral tissues (Morais et al., 2011b), but the lipogenic capacity of the genotypes were in agreement with our results. In the adipose tissue, though we found an abundance of SREBP1c in the F line, it was not accompanied by higher expression of lipogenic enzymes. The trout adipose tissue was recently proved to have lipogenic ability with possible involvement in glucose homeostasis (Polakof et al., 2011d). Overall, we confirm that the F line is characterised by higher lipogenic capacities than the L line, suggesting a better ability to store excess glucose as triglycerides. Moreover, the lower abundance of the hepatic transcripts of CPT1 a and b isoforms (catalysing the first limiting step for lipid catabolism) in the F line points towards a lower utilisation of fatty acids for energy supply, confirming earlier

observations (Kolditz et al., 2008a). These data suggest a possibility for glucose to be used as energy source linked to a decrease in lipid oxidation in liver. Regarding fatty acid bioconversion, we found that the F line showed an enhanced ability through the higher D6D expression, although an opposite case of genotypic difference was reported in similarly selected Atlantic salmon fed a diet rich in vegetable oil (Morais et al., 2011a). The potentially decreased mitochondrial fatty acid oxidation coupled with increased lipogenesis and fatty acid bioconversion in the liver may define the fat muscle phenotype of the F line.

Interactions of diet and genotype

The F and L trout lines were recognised as unique biological material to study diet \times genotype interactions focusing on the metabolic utilisation of carbohydrates, based on previous results (Kolditz et al., 2008a; Skiba-Cassy et al., 2009). However, we found only limited interactions. Growth, feed efficiency and nutrient retention did not show any interaction between diet and line, as was previously observed in different rainbow trout families (Edwards et al., 1977; Austreng et al., 1977; Refstie and Austreng, 1981). But the possible genetic determinism of adaptability to dietary carbohydrates in salmonids has been suggested (Mazur et al., 1992; Blanc, 2002). Mazur et al. (Mazur et al., 1992) reported a reduced post-prandial hyperglycaemic response in a specific strain of chinook salmon fed high levels of carbohydrate, whereas we failed to find such a response in the F line, in contrast to Skiba-Cassy et al. (Skiba-Cassy

et al., 2009). In hepatic glucose metabolism, we show a diet \times line interaction for the glycolytic enzyme GK and the NADPH-producing enzyme G6PD. Dietary carbohydrates enhanced their expression more specifically in the F line. In an earlier study using the same genotypes, with diets varying in their lipid content, these two genes exhibited a diet \times line interaction (Kolditz et al., 2008b). This identifies them as potentially differentiated by genetic selection, responding also to dietary manipulations. Additionally, we report here a disordinal interaction for the hepatic D9D and adipose tissue PK expression, enhanced only in the F line when fed dietary carbohydrates. Though the few interactions endorse our hypothesis that the F line has a higher capacity for using high levels of dietary carbohydrates, they are insufficient to clearly establish this to be the case.

Conclusions

We combined genetic selection and nutritional strategies to investigate the genotype-specific metabolic response to dietary carbohydrates in trout. The enhanced hepatic lipogenic potential and the higher liver glycogen content in the F line illustrate its superior ability to store excess glucose compared with the L line. However, this does not result in an improved regulation of post-prandial glycaemia in the F line, possibly because of unregulated gluconeogenic enzyme transcripts. Because all our major results were obtained in the liver and not in the supposed major glucose utilisation tissues, i.e. muscle and white adipose tissue, our study evidences their lack of response to dietary manipulations with carbohydrates. The poor uptake and utilisation of glucose in these peripheral tissues may be one of the key factors underlying the impaired glucose homeostasis in rainbow trout. Moreover, the diet and genotype interactions that we observed in glucose metabolism (even being few) suggest that it is possible to obtain a trout genotype with an improved ability for lipid biosynthesis in the presence of dietary carbohydrates.

LIST OF ABBREVIATIONS

0C	diet without carbohydrates
20C	diet with carbohydrates
6PFK	6-phosphofructo-1-kinase
ACLY	adenosine triphosphate citrate lyase
Akt	protein kinase B
AMPK	5' adenosine monophosphate activated protein kinase
D6D	Δ 6 desaturase
D9D	Δ 9 desaturase
F	fat line
FAS	fatty acid synthase
FBPase	fructose 1,6-bisphosphatase
G6Pase	glucose 6-phosphatase
G6PD	glucose 6-phosphate dehydrogenase
GK	glucokinase
HK1	hexokinase 1
HSI	hepato-somatic index
L	lean line
PEPCK	phosphoenolpyruvate carboxykinase
PK	pyruvate kinase
SREBP1c	sterol regulatory element binding protein 1c
TOR	target of rapamycin

ACKNOWLEDGEMENTS

We thank M. J. Borthaire, E. Plagnes-Juan, C. Figueiredo-Silva, K. Dias, L. Larroquet, A. Surget and M. Larquier for technical assistance in the laboratory. We also acknowledge the technical staff of the INRA experimental fish farm at Donzacq (Y. Hontang, F. Sandres and F. Terrier) for conducting the feeding trial and the technical staff of the INRA experimental fish farm at Lees Athas (P. Maunas) for conserving the trout lines.

FUNDING

B.S.K.J. gratefully acknowledges the financial assistance provided by the Indian Council of Agricultural Research (ICAR) through the ICAR International Fellowship for his doctoral programme. This research was internally funded by the Nutrition, Metabolism and Aquaculture unit of the Institut National de la Recherche Agronomique (INRA), France.

REFERENCES

- Alvarez, M., Diez, A., Lopez-Bote, C., Gallego, M. and Bautista, J. (2000). Short-term modulation of lipogenesis by macronutrients in rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Br. J. Nutr.* **84**, 619-628.
- Assifi, M. M., Suchankova, G., Constant, S., Prentki, M., Saha, A. K. and Ruderman, N. B. (2005). AMP-activated protein kinase and coordination of hepatic fatty acid metabolism of starved/carbohydrate-refed rats. *Am. J. Physiol. Endocrinol. Metab.* **289**, E794-E800.
- Austreng, E., Risa, S., Edwards, D. and Hvidsten, H. (1977). Carbohydrate in rainbow trout diets. II. Influence of carbohydrate levels on chemical composition and feed utilization of fish from different families. *Aquaculture* **11**, 39-50.
- Bergot, F. (1979). Effects of dietary carbohydrates and of their mode of distribution on glycaemia in rainbow trout (*Salmo gairdneri* Richardson). *Comp. Biochem. Physiol.* **64A**, 543-547.
- Blanc, J. (2002). Interaction between diet and genetic aptitude for weight and growth in juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquac. Res.* **33**, 563-568.
- Brauge, C., Corraze, G. and Médale, F. (1995). Effects of dietary levels of carbohydrate and lipid on glucose oxidation and lipogenesis from glucose in rainbow trout, *Oncorhynchus mykiss*, reared in freshwater or in seawater. *Comp. Biochem. Physiol.* **111A**, 117-124.
- Cowey, C. B. and Walton, M. J. (1989). Intermediary metabolism. In *Fish Nutrition*, 2nd edn (ed. J. E. Halver), pp. 259-329. New York: Academic Press.
- Dentin, R., Denechaud, P. D., Benhamed, F., Girard, J. and Postic, C. (2006). Hepatic gene regulation by glucose and polyunsaturated fatty acids: a role for ChREBP. *J. Nutr.* **136**, 1145-1149.
- Eberle, D., Hegarty, B., Bossard, P., Ferre, P. and Foulle, F. (2004). SREBP transcription factors: master regulators of lipid homeostasis. *Biochimie* **86**, 839-848.
- Edwards, D., Austreng, E., Risa, S. and Gjedrem, T. (1977). Carbohydrate in rainbow trout diets. I. Growth of fish of different families fed diets containing different proportions of carbohydrate. *Aquaculture* **11**, 31-38.
- Enes, P., Panserat, S., Kaushik, S. and Oliva-Teles, A. (2009). Nutritional regulation of hepatic glucose metabolism in fish. *Fish Physiol. Biochem.* **35**, 519-539.
- Ferre, P. and Foulle, F. (2007). SREBP-1c transcription factor and lipid homeostasis: clinical perspective. *Horm. Res. Paediatr.* **68**, 72-82.
- Figueiredo-Silva, A., Corraze, G., Borges, P. and Valente, L. (2010). Dietary protein/lipid level and protein source effects on growth, tissue composition and lipid metabolism of blackspot seabream (*Pagellus bogaraveo*). *Aquac. Nutr.* **16**, 173-187.
- Folch, J., Lees, M. and Sloane-Stanley, G. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**, 497-509.
- Gjedrem, T. (2000). Genetic improvement of cold-water fish species. *Aquac. Res.* **31**, 25-33.
- Hemre, G. I. and Kahrs, F. (1997). 14C-glucose injection in Atlantic cod, *Gadus morhua*, metabolic responses and excretion via the gill membrane. *Aquac. Nutr.* **3**, 3-8.
- Hemre, G. I., Mommsen, T. P. and Kroghdahl, A. (2002). Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. *Aquac. Nutr.* **8**, 175-194.
- Henryon, M., Jokumsen, A., Berg, P., Lund, I., Pedersen, P. B., Olesen, N. J. and Slierendrecht, W. J. (2002). Genetic variation for growth rate, feed conversion efficiency, and disease resistance exists within a farmed population of rainbow trout. *Aquaculture* **209**, 59-76.
- Hilton, J. W. and Atkinson, J. L. (1982). Response of rainbow trout (*Salmo gairdneri*) to increased levels of available carbohydrate in practical trout diets. *Br. J. Nutr.* **47**, 597-607.
- Hilton, J. W., Atkinson, J. L. and Slinger, S. J. (1987). Evaluation of the net energy value of glucose (cerelose) and maize starch in diets for rainbow trout (*Salmo gairdneri*). *Br. J. Nutr.* **58**, 453-461.
- Kaushik, S., Médale, F., Fauconneau, B. and Blanc, D. (1989). Effect of digestible carbohydrates on protein/energy utilization and on glucose metabolism in rainbow trout (*Salmo gairdneri* R.). *Aquaculture* **79**, 63-74.
- Kaushik, S. J. and de Oliva Teles, A. (1985). Effect of digestible energy on nitrogen and energy balance in rainbow trout. *Aquaculture* **50**, 89-101.
- Keppeler, D. and Decker, K. (1974). Glycogen determination with amyloglucosidase. In *Methods of Enzymatic Analysis* (ed. H. U. Bergmeyer), pp. 1127-1131. New York: Academic Press.
- Kim, J. and Kaushik, S. (1992). Contribution of digestible energy from carbohydrates and estimation of protein/energy requirements for growth of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **106**, 161-169.
- Kirchner, S., Kaushik, S. and Panserat, S. (2003). Effect of partial substitution of dietary protein by a single gluconeogenic dispensable amino acid on hepatic glucose metabolism in rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol.* **134A**, 337-347.
- Kirchner, S., Panserat, S., Lim, P., Kaushik, S. and Ferraris, R. (2008). The role of hepatic, renal and intestinal gluconeogenic enzymes in glucose homeostasis of juvenile rainbow trout. *J. Comp. Physiol.* **178B**, 429-438.
- Kolditz, C. I., Borthaire, M., Richard, N., Corraze, G., Panserat, S., Vachot, C., Lefèvre, F. and Médale, F. (2008a). Liver and muscle metabolic changes induced by dietary energy content and genetic selection in rainbow trout (*Oncorhynchus mykiss*). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **294**, R1154-R1164.
- Kolditz, C. I., Pabouef, G., Borthaire, M., Esquerré, D., SanCristobal, M., Lefèvre, F. and Médale, F. (2008b). 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**, 506-521.
- Lansard, M., Panserat, S., Seilliez, I., Polakof, S., Plagnes-Juan, E., Geurden, I., Médale, F., Kaushik, S., Corraze, G. and Skiba-Cassy, S. (2009). Hepatic protein kinase B (Akt)-target of rapamycin (TOR)-signalling pathways and intermediary metabolism in rainbow trout (*Oncorhynchus mykiss*) are not significantly affected by feeding plant-based diets. *Br. J. Nutr.* **102**, 1564-1573.
- Likimani, T. A. and Wilson, R. P. (1982). Effects of diet on lipogenic enzyme activities in channel catfish hepatic and adipose tissue. *J. Nutr.* **112**, 112-117.
- Lin, H., Romsos, D. R., Tack, P. I. and Leveille, G. A. (1977a). Influence of diet on *in vitro* and *in vivo* rates of fatty acid synthesis in coho salmon (*Oncorhynchus kisutch* (Walbaum)). *J. Nutr.* **107**, 1677-1682.
- Lin, H., Romsos, D. R., Tack, P. I. and Leveille, G. A. (1977b). Influence of dietary lipid on lipogenic enzyme activities in coho salmon, *Oncorhynchus kisutch* (Walbaum). *J. Nutr.* **107**, 846-854.
- Mazur, C. N., Higgs, D., Plisetskaya, E. and March, B. (1992). Utilization of dietary starch and glucose tolerance in juvenile chinook salmon (*Oncorhynchus tshawytscha*) of different strains in seawater. *Fish Physiol. Biochem.* **10**, 303-313.
- Mommsen, T. and Plisetskaya, E. (1991). Insulin in fishes and agnathans: history, structure, and metabolic regulation. *Rev. Aquat. Sci.* **4**, 225-259.
- Moon, T. W. (2001). Glucose intolerance in teleost fish: fact or fiction? *Comp. Biochem. Physiol.* **129B**, 243-249.
- Moore, S. (1968). Amino acid analysis: aqueous dimethyl sulfoxide as solvent for the ninhydrin reaction. *J. Biol. Chem.* **243**, 6281-6283.
- Morais, S., Pratoomyot, J., Taggart, J., Bron, J., Guy, D., Bell, J. and Tocher, D. (2011a). Genotype-specific responses in Atlantic salmon (*Salmo salar*) subject to dietary fish oil replacement by vegetable oil: a liver transcriptomic analysis. *BMC Genomics* **12**, 255-271.
- Morais, S., Pratoomyot, J., Torstensen, B. E., Taggart, J. B., Guy, D. R., Gordon Bell, J. and Tocher, D. R. (2011b). Diet × genotype interactions in hepatic cholesterol and lipoprotein metabolism in Atlantic salmon (*Salmo salar*) in response to replacement of dietary fish oil with vegetable oil. *Br. J. Nutr.* **106**, 1457-1469.
- Navarro, I., Leibush, B., Moon, T., Plisetskaya, E., Banos, N., Mendez, E., Planas, J. and Gutierrez, J. (1999). Insulin, insulin-like growth factor-I (IGF-I) and glucagon: the evolution of their receptors. *Comp. Biochem. Physiol.* **122B**, 137-153.
- Naylor, R. L., Goldberg, R. J., Primavera, J. H., Kautsky, N., Beveridge, M. C. M., Clay, J., Folke, C., Lubchenco, J., Mooney, H. and Troell, M. (2000). Effect of aquaculture on world fish supplies. *Nature* **405**, 1017-1024.
- Ntambi, J. M., Miyazaki, M. and Dobrzyn, A. (2004). Regulation of stearoyl-CoA desaturase expression. *Lipids* **39**, 1061-1065.
- Olsvik, P., Lie, K., Jordal, A. E., Nilsen, T. and Hordvik, I. (2005). Evaluation of potential reference genes in real-time RT-PCR studies of Atlantic salmon. *BMC Mol. Biol.* **6**, 21-29.
- Panserat, S., Médale, F., Blin, C., Brèque, J., Vachot, C., Plagnes-Juan, E., Gomes, E., Krishnamoorthy, R. and Kaushik, S. (2000a). Hepatic glucokinase is induced by dietary carbohydrates in rainbow trout, gilthead seabream, and common carp. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **278**, R1164-R1170.
- Panserat, S., Médale, F., Brèque, J., Plagnes-Juan, E. and Kaushik, S. (2000b). Lack of significant long-term effect of dietary carbohydrates on hepatic glucose-6-phosphatase expression in rainbow trout (*Oncorhynchus mykiss*). *J. Nutr. Biochem.* **11**, 22-29.
- Panserat, S., Plagnes-Juan, E., Brèque, J. and Kaushik, S. (2001a). Hepatic phosphoenolpyruvate carboxykinase gene expression is not repressed by dietary carbohydrates in rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* **204**, 359-365.
- Panserat, S., Plagnes-Juan, E. and Kaushik, S. (2001b). Nutritional regulation and tissue specificity of gene expression for proteins involved in hepatic glucose metabolism in rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* **204**, 2351-2360.
- Panserat, S., Plagnes-Juan, E. and Kaushik, S. (2002). Gluconeogenic enzyme gene expression is decreased by dietary carbohydrates in common carp (*Cyprinus carpio*) and gilthead seabream (*Sparus aurata*). *Biochim. Biophys. Acta* **1579**, 35-42.
- Panserat, S., Skiba-Cassy, S., Seilliez, I., Lansard, M., Plagnes-Juan, E., Vachot, C., Aguirre, P., Larroquet, L., Chavernac, G., Médale, F. et al. (2009). Metformin improves postprandial glucose homeostasis in rainbow trout fed dietary carbohydrates: a link with the induction of hepatic lipogenic capacities? *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **297**, R707-R715.
- Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* **29**, e45.
- Pilkis, S. and Granner, D. (1992). Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annu. Rev. Physiol.* **54**, 885-909.
- Planas, J. V., Capilla, E. and Gutiérrez, J. (2000). Molecular identification of a glucose transporter from fish muscle1. *FEBS Lett.* **481**, 266-270.
- Polakof, S., Skiba-Cassy, S. and Panserat, S. (2009). Glucose homeostasis is impaired by a paradoxical interaction between metformin and insulin in carnivorous rainbow trout. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **297**, R1769-R1776.
- Polakof, S., Mommsen, T. P. and Soengas, J. L. (2011a). Glucosensing and glucose homeostasis: from fish to mammals. *Comp. Biochem. Physiol.* **160B**, 123-149.
- Polakof, S., Médale, F., Larroquet, L., Vachot, C., Corraze, G. and Panserat, S. (2011b). Regulation of *de novo* hepatic lipogenesis by insulin infusion in rainbow trout fed a high-carbohydrate diet. *J. Anim. Sci.* **89**, 3079-3088.
- Polakof, S., Panserat, S., Craig, P. M., Martyres, D. J., Plagnes-Juan, E., Savari, S., Aris-Brosou, S. and Moon, T. W. (2011c). The metabolic consequences of hepatic AMP-kinase phosphorylation in rainbow trout. *PLoS ONE* **6**, e20228.
- Polakof, S., Médale, F., Larroquet, L., Vachot, C., Corraze, G. and Panserat, S. (2011d). Insulin stimulates lipogenesis and attenuates beta-oxidation in white adipose tissue of fed rainbow trout. *Lipids* **46**, 189-199.
- Quillet, E., Le Guillou, S., Aubin, J. and Fauconneau, B. (2005). Two-way selection for muscle lipid content in pan-size rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **245**, 49-61.
- Quillet, E., Le Guillou, S., Aubin, J., Labbé, L., Fauconneau, B. and Médale, F. (2007). Response of a lean muscle and a fat muscle rainbow trout (*Oncorhynchus mykiss*) line on growth, nutrient utilization, body composition and carcass traits when fed two different diets. *Aquaculture* **269**, 220-231.
- Randle, P. J. (1998). Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. *Diabetes Metab. Rev.* **14**, 263-283.
- Refstie, T. and Austreng, E. (1981). Carbohydrate in rainbow trout diets. III. Growth and chemical composition of fish from different families fed four levels of carbohydrate in the diet. *Aquaculture* **25**, 35-49.
- Rutter, G. A., Xavier, G. D. S. and Leclerc, I. (2003). Roles of 5'-AMP-activated protein kinase (AMPK) in mammalian glucose homeostasis. *Biochem. J.* **375**, 1-16.
- Seilliez, I., Panserat, S., Kaushik, S. and Bergot, P. (2001). Cloning, tissue distribution and nutritional regulation of a $\Delta 6$ -desaturase-like enzyme in rainbow trout. *Comp. Biochem. Physiol.* **130B**, 83-93.
- Seilliez, I., Gabillard, J. C., Skiba-Cassy, S., Garcia-Serrana, D., Gutiérrez, J., Kaushik, S., Panserat, S. and Tesseraud, S. (2008). An *in vivo* and *in vitro* assessment of TOR signaling cascade in rainbow trout (*Oncorhynchus mykiss*). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **295**, R329-R335.
- Seilliez, I., Panserat, S., Lansard, M., Polakof, S., Plagnes-Juan, E., Surget, A., Dias, K., Larquier, M., Kaushik, S. and Skiba-Cassy, S. (2011). 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**, 733-743.
- Shaw, R. J. (2009). LKB1 and AMP-activated protein kinase control of mTOR signalling and growth. *Acta Physiol.* **196**, 65-80.
- Skiba-Cassy, S., Lansard, M., Panserat, S. and Médale, F. (2009). Rainbow trout genetically selected for greater muscle fat content display increased activation of liver TOR signaling and lipogenic gene expression. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **297**, R1421-R1429.
- Stone, D. A. J. (2003). Dietary carbohydrate utilization by fish. *Rev. Fish. Sci.* **11**, 337-370.
- Tacon, A. G. J. and Metian, M. (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. *Aquaculture* **285**, 146-158.
- Tocher, D., Leaver, M. and Hodgson, P. (1998). Recent advances in the biochemistry and molecular biology of fatty acyl desaturases. *Prog. Lipid Res.* **37**, 73-117.
- Towle, H. C., Kaytor, E. N. and Shih, H. M. (1997). Regulation of the expression of lipogenic enzyme genes by carbohydrate. *Annu. Rev. Nutr.* **17**, 405-433.
- Vagner, M. and Santigosa, E. (2010). Characterization and modulation of gene expression and enzymatic activity of delta-6 desaturase in teleosts: a review. *Aquaculture* **315**, 131-143.
- West, T., Schulte, P. and Hochachka, P. (1994). Implications of hyperglycemia for post-exercise resynthesis of glycogen in trout skeletal muscle. *J. Exp. Biol.* **189**, 69-84.
- Wilson, R. (1994). Utilization of dietary carbohydrate by fish. *Aquaculture* **124**, 67-80.
- Wullschlegel, S., Loewith, R. and Hall, M. N. (2006). TOR signaling in growth and metabolism. *Cell* **124**, 471-484.
- Yamada, K. and Noguchi, T. (1999). Nutrient and hormonal regulation of pyruvate kinase gene expression. *Biochem. J.* **337**, 1-11.