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Parental and early-feeding effects of dietary methionine in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

We studied the effect of changes in dietary methionine (Met) supply in broodstock and first-feeding rainbow trout fry (offspring). Three plant-based diets differing in Met level (deficient, adequate or in excess of the established requirement) were fed to the broodstock (male and female) for 6 months prior to spawning (diets BD, BA and BE, respectively). The offspring from the parental Met-groups was then challenged in turn with the different Met fry-diets (FD, FA and FE, respectively) for 3 weeks from first-feeding. At spawning, females fed diet BD had significantly higher plasma total and LDL-cholesterol and slightly lower plasma triacylglycerol. Diet BD reduced female (but not male) growth, weight of spawn and egg size, but had no effect on relative fecundity. The free amino acid profile of oocytes was modified, with levels of Met and Cys correlating positively with the Met-levels of broodstock diets. SAM and SAH levels in oocytes followed the same pattern, as opposed to SAM/SAH ratio. At the swim-up stage, no significant effect of parental diet on fry weight was noted, whereas survival was the highest in fry from BE-broodstock. The subsequent 21-day fry feeding with different Met levels highly affected the daily growth index with a significant interaction between the parental-diet and fry-diet effects. The expression of a number of genes regulating sulfur amino acid metabolism was modified either directly by the dietary Met supply in both broodstock liver and in whole fry (e.g. BHMT1, GR, GSTπ, MsrA1) or indirectly by the parental Met intakes as seen in the swim-up fry (e.g. BHMT1, MTR, GSTπ, MsrA1). Importantly, long-lasting parental effects linked to broodstock Met-intake were seen in the fry, 21-days after first-feeding and irrespective of the fry diet (CTH, MsrA1, MsrB2, SOD2). Similarly, parental effects were noted on the gene expression of both NPY and POMC feeding peptides in fry prior to exogenous feeding which persisted for POMC in the 21-day fry. Parental effects were also demonstrated on the key myogenic gene Myog, on fMHC and GDH

in swim-up fry, which persisted for GDH in 21-day fry. In summary, our results demonstrate that dietary Met levels of rainbow trout broodstock affect various traits in the offspring, some of which persisted during the first weeks of exogenous feeding. Further studies need to evaluate the long-term persistence of the parental effects over time and to elucidate the mechanisms, whether epigenetic or not.

Keywords

methionine metabolism; broodstock; fry; reproduction; programming; rainbow trout

Highlights of the manuscript

- Dietary methionine deficiency not only reduces rainbow trout female and fry growth but also egg size.
- Parental dietary methionine levels affect the free methionine, cysteine, S-adenosyl-methionine and S-adenosyl-homocysteine levels in rainbow trout oocytes.
- Dietary methionine levels affect the expression of various genes involved in sulfur amino acid metabolism, feed intake and muscle growth in rainbow trout offspring.
- Some effects of parental methionine intakes persist during the first weeks of exogenous feeding irrespective of the dietary methionine level fed to the offspring.

Statement of relevance

Determining the multiple effects of dietary methionine levels on reproductive, growth performance and metabolism in offspring will help improve formulations of low fish meal feeds for rainbow trout at sensitive life cycle stages.

1. Introduction

Broodstock nutrition influences reproductive performance and larval quality of fish (Izquierdo et al., 2001). During ovarian development, dietary and maternal reserves are mobilized and transported into the oocytes where they are expected to fulfill the nutritional requirements for embryonic development and growth of the yolk sac larvae until the start of exogenous feeding. This input is particularly important in rainbow trout, a species with large eggs and long pre-hatch developmental periods, in which yolk constituents are likely to play an important role in determining offspring fitness (Palace and Werner, 2006). Hence, a better understanding of nutritional requirements of broodstock could lead to improved larval quality and hatchery production. Dietary components as diverse as fatty acids, vitamins, pigments and proteins have all been shown to affect egg and embryo survival (Brooks et al., 1997; Fontagné-Dicharry et al., 2010; Izquierdo et al., 2001; Palace and Werner, 2006).

There is very little information on the effects of dietary proteins and amino acids on broodstock performance (Brooks et al., 1997). In the general context of the need for reducing our reliance on fish meal (FAO, 2016), one point is to look at the effects of plant protein-rich feeds especially with regard to their essential amino acid profile.

In addition to its role as a precursor in protein synthesis, the sulfur-containing essential amino acid methionine (Met) participates in a wide range of other metabolic reactions including the production of S-adenosyl-methionine (SAM), homocysteine, cysteine, glutathione, taurine, phosphatidylcholine and other phospholipids (NRC, 2011). SAM serves as a methyl-donor in many methyltransferase reactions and is converted to S-adenosyl-homocysteine (SAH). Met deficiency has been shown to lead to reduced methylation of DNA and histones that can cause changes in gene expression and development (Roberts and Selker, 1995; Sadhu et al., 2013). Moreover, consequences of nutritional deficiency may persist in

offspring, even in the absence of the nutritional deficiency, since histone methylation patterns have the potential to be inherited epigenetically across cellular generations (Feil, 2006). On the other hand, maternal epigenetic programming seen early in life has been shown to be reversible in adult life through methyl donor supplementation (Weaver et al., 2005), underlining the possible interacting effects between parental and early-life nutritional events.

The objective of the study was to assess the effect of changes in dietary Met supply on rainbow trout broodstock performance. In addition, rainbow trout fry from the different parental Met-groups were in turn challenged to feed with different dietary Met levels in order to evaluate possible parental nutritional history on the offspring's dietary Met-responses. The present study, part of a broader survey, focusses on parental and early fry dietary effects on survival, growth and sulfur amino acid metabolism in the progeny.

2. Materials and methods

2.1. Experimental broodstock and fry diets

Diets were based on plant-derived proteins and fish oil (Table 1). In each feeding trial, the three diets used had similar levels of crude protein (44% in broodstock trial and 48% in fry trial) and total lipid (16% in broodstock trial and 14% in fry trial) and differed in Met content. The Met level was set at 0.5, 1 or 2% of the diet in deficient (BD and FD), adequate (BA and FA) and excess (BE and FE) diets, respectively in both feeding trials according to NRC (2011). Met was supplied as crystalline DL-Met at the expense of glutamic acid, a non-essential amino acid (Table 2). Diets were manufactured using a twin-screw extruder (BC 45, Clextral, France) at the INRA experimental facilities in Donzacq (Landes, France).

2.2. Experimental fish and dietary trial conditions for broodstock trial

Four- and three-year-old rainbow trout (*Oncorhynchus mykiss*) females and males from the same genetic group from the INRA experimental fish farm of Lées-Athas (Pyrénées-Atlantiques, France) were used as broodstock. Rainbow trout broodstock were randomly allocated to three circular 8-m diameter tanks supplied with flow-through spring water at 8 ± 1 °C with 25 females (initial mean body weight: 1.52 ± 0.24 kg, second reproduction season) and 10 males (0.28 ± 0.06 kg) per tank. Fish were hand-fed twice a day to apparent satiation from April to October over a 6-month period prior to spawning under natural photoperiod. Each fish was individually weighed and tagged with passive integrated transponder (PIT) tags (12 × 2 mm, ISO 11784/11785, IER, Suresnes, France) injected in the dorsal muscle. During reproductive season, all females were checked for ovulation two times a week.

At spawning, fish were anesthetized with benzocaine (30 mg/L) and oocytes from 8 females per dietary group were collected and fertilized synchronously with a common pool of milt from males from the same dietary group, on a single day. Blood samples were collected from the caudal vein into heparinized syringes and fish were killed subsequently by a sharp blow to the head. Livers were dissected, weighed for calculating hepato-somatic index (HSI, percentage of weight of liver out of weight of fish) and immediately frozen in liquid nitrogen and stored at -80 °C. Plasma was recovered from centrifuged (3000 × g for 5 min) blood samples, immediately frozen and stored at -80 °C before analysis. Two pools of 50 oocytes from each female were weighed and measured to calculate the average weight and diameter of oocytes. The weights of whole fish, spawn (total eggs obtained from each female after stripping and removal of coelomic fluid) and eviscerated fish were recorded to calculate absolute and relative fecundity (number of eggs/female and number of eggs/kg female,

respectively), gonado-somatic index (GSI, percentage of weight of spawn out of weight of female) and viscero-somatic index (VSI, percentage of weight of viscera out of weight of fish).

Eggs from each female were incubated in small trays supplied with flow-through spring water at 8 ± 1 °C to obtain individual hatching data. The fertilization rates (total number of developing eggs/total number of eggs) of each batch of eggs were assessed one day post-fertilization (dpf). Dead eggs or fry were removed every two days and survival rates were calculated at the eyed stage (32 dpf), hatching (44 dpf) and swim-up stage (66 dpf).

All experimental procedures complied with the EU Directive 2010/63/EU for the protection of animals used for scientific purposes and the French Decree no. 2013-118 for animal experimentation.

2.3. Experimental fish and dietary trial conditions for fry trial

At the swim-up stage, which corresponds to the beginning of exogenous feeding, a representative sample of swim-up fry from all eight of spawned females (only six for BD group, due to low fertilization rates of two females) was randomly collected and pooled for each dietary broodstock group and transferred to the INRA experimental fish farm in Donzacq (Landes, France). Rainbow trout fry from each of the three broodstock groups were randomly distributed into nine tanks (400 fish per 50-L fiberglass tank) supplied with flow through spring water at 17 ± 1 °C and hand-fed four or six times per day to apparent satiation under natural photoperiod. Each of the three fry diets was tested in triplicate for each of the three broodstock treatments during three weeks (n = 27 tanks). The survival was calculated from daily mortality and from the final number of surviving fish recorded in each tank. Fish were randomly sampled at the start (n = 10 swim-up fry per broodstock treatment) during tank

allocation before first-feeding and at the end of the experiment after a 16-h food-deprivation (n = 9 fish per treatment or 3 fish per tank). The sampled fry were euthanized by an overdose of benzocaine, weighed, frozen in liquid nitrogen and stored at -80 °C prior to analysis.

2.4. Chemical analyses of diets and oocytes

Proximate composition of diets was determined according to the following procedures: dry matter after drying at 105 °C for 24 h, protein (N × 6.25) by the Kjeldahl method after acid digestion, ash by incineration at 550 °C for 16 h and gross energy in an adiabatic bomb calorimeter. Total lipid was extracted and measured gravimetrically according to Folch et al. (1957) using dichloromethane instead of chloroform.

The total amino acid composition of the diets was determined after acid hydrolysis (6 M HCl at 116º C for 24 h in nitrogen-flushed glass vials). Protein-bound amino acid analysis of oocytes was made after protein precipitation (6% trichloroacetic acid at 4 ºC over 24 h, trichloroacetic acid was then discarded) followed by acid hydrolysis. Free amino acid, SAM and SAH analysis of oocytes was performed after homogeneization in 0.1 M HCl on ice, centrifugation at 1500 × g at 4 ºC for 15 min and deproteinization of the supernatant by centrifugal ultrafiltration (10 kDa cut-off, 2500 × g at 4 ºC for 20 min). For total amino acid analysis of diets, protein-bound and free amino acid analysis of oocytes, samples were pre-column derivatized with Waters AccQ Fluor Reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) using the AccQ Tag method (Waters, Milford, MA). Samples for SAM and SAH analysis were not derivatized. All analyses were performed by ultra-high-performance liquid chromatography (UPLC) on a Waters Reversed-Phase Amino Acid Analysis System, using norvaline as an internal standard. Amino acids were identified by retention times of standard mixtures (Waters) and pure standards (Sigma, Madrid, Spain).

Cysteine was determined as cysteic acid and the cysteine results therefore represent the sum of cysteine and cystine in the sample. Instrument control, data acquisition and processing were achieved by the use of Waters Empower software.

2.5. Metabolite analyses in broodstock plasma

Levels of glucose, triacylglycerol, total cholesterol and high-density lipoprotein (HDL) cholesterol were determined on plasma samples of broodstock females using enzymatic colorimetric kits in 96-well microtiter plates (bioMérieux, Marcy l'Etoile, France for glucose and HDL-cholesterol and Sobioda, Montbonnot-Saint-Martin, France for triacylglycerol and total cholesterol). Low-density lipoprotein (LDL) cholesterol was calculated based on total cholesterol, HDL-cholesterol and triacylglycerol measurements according to the Friedewald formula (Friedewald et al., 1972). Total amino acid concentrations were assessed on plasma samples as ninhydrin positive substances by colorimetric analysis (Moore, 1968) with glycine as a standard.

2.7. Gene expression analysis in broodstock liver and whole fry

Total RNA was extracted from broodstock female livers and whole offspring using TRIzol® reagent (Invitrogen, Cergy-Pontoise, France). For quantitative RT-PCR, cDNA was generated from 1 mg total RNA using SuperScript III RT (Invitrogen) and a mix of oligo(dT)15 and random primers (Promega, Charbonnières, France). For each sample, RT was performed in duplicate and quantitative PCR analyses were performed in LightCycler® 480 Instrument II (Roche Diagnostics, Meylan, France) using LightCycler® 480 SYBR Green I Master mix (Roche Diagnostics). Total reaction volume was 6 µL, with 2 µL of the diluted

RT reaction mixture (dilution 40) and 4 µL of master mix added with 0.4 mM of each primer (Table 3). The PCR protocol was initiated at 95 °C for 10 min for initial denaturation of the cDNA and hot-start Taq-polymerase activation, followed by 45 cycles of a three-step amplification program (15 sec at 95 °C, 10 sec at the melting temperature of 60 °C, 4.8 sec at 72 °C). Melting curves were systematically monitored (temperature gradient ranging from 65 to 97 °C) at the end of the last amplification cycle to confirm the specificity of the amplification reaction. Relative quantification of target gene transcripts were performed using Elongation Factor 1α (EF1α) as the reference gene and BA or BA-FA as the reference group using the ΔΔ Ct method (Pfaffl, 2001).

2.8. Statistical analyses

Results are given as means ± standard deviation (SD) or standard error of the mean (SEM). Differences between dietary groups were analyzed using one-way or two-way ANOVA to test the effect of broodstock and fry nutrition and their interaction. The Newman-Keuls multiple range test was used to compare means when a significant difference was found. Percentage, weight and gene expression data were arc-sin, log- or rank-transformed before analysis when necessary. Statistical analyses were performed with the computing program StatBox 6 (Grimmer Logiciels, Paris, France), SigmaStat 3 (SPSS, Chicago, IL, USA) or R software (R Development Core Team, 2008) and differences were considered significant when P values were < 0.05.

3. Results

3.1. Growth and spawning performance of rainbow trout broodstock

All diets were readily accepted by rainbow trout broodstock. Feed intake decreased in the last two months of the feeding trial for the 3 broodstock groups and was associated with a decrease in growth in females fed diet BD before spawning (Fig. 1). Final mean body weight of broodstock females fed diet BD was significantly lower than final mean body weight of females fed diets BA and BE (Table 4). Growth performance of males was not significantly different between broodstock groups (Fig. 1 and Table 4). HSI and VSI were not significantly different between groups for both females and males. Two females died during the feeding trial before spawning in groups BA and BE, two males in group BD and three males in group BE. The number of females and males that were ready to spawn at the end of October was not significantly different among dietary groups (32 and 63% for BD, 61 and 60% for BA and 52 and 57% for BE, respectively). However, due to the low number of males in each dietary group, plasma metabolites and hepatic gene expression were analyzed only in the females. The weight of spawn was lower in females fed BD than in females fed BE, probably in relation with the final size of fish as GSI and relative fecundity were not significantly affected by dietary Met supply (Table 4). Mean egg weight and diameter were significantly affected by the level of Met in the broodstock diets with smaller eggs produced by females fed diet BD. As for egg size, at the swim-up stage, the lowest mean wet weight was recorded in the BD-group but it was not significantly different from the ones of the two other groups. A significant decline in survival from the eyed stage onwards was noticeable in the BD- and BA-groups fed the lowest Met levels. The best final survival from fertilization to swim-up stage was recorded in group BE (77 vs. 45% for the two other groups).

3.2. Metabolite composition of broodstock female plasma

The levels of circulating glucose, HDL-cholesterol and total free amino acids in the broodstock female plasma, sampled at least 16 h after the last meal, were not significantly modified by dietary Met supplementation (Fig. 2). On the other hand, triacylglycerol levels were significantly reduced in females fed the deficient diet BD compared to females fed the control diet BA whereas total cholesterol and LDL-cholesterol concentrations were significantly higher in females fed diet BD.

3.4. Amino acid composition of oocytes

In oocytes from the three dietary groups, the main protein-bound amino acids were glutamic acid for non-essential amino acids and leucine for essential amino acids that represented 13 and 9% of total protein-bound amino acids, respectively (Table 5). Met supplementation of broodstock diet led to moderate changes in protein-bound amino acid composition of oocytes. Arginine, threonine and valine were slightly decreased in BA-oocytes whereas lysine was decreased in BE-oocytes compared to the two other dietary groups.

The free amino acid composition of oocytes was more affected by Met levels of broodstock diet than protein-bound amino acid profile. In the three dietary groups, the main free amino acids were arginine for essential amino acids and glutamic acid for non-essential amino acids that represented 18 and 12% of total free amino acids, respectively (Table 6). Free Met and cysteine concentrations were significantly higher in BE-oocytes and were significantly lower in BD-oocytes. Levels of arginine, leucine, lysine, alanine and ornithine were significantly lower in BD-oocytes compared to the two other dietary groups. Free histidine, phenylalanine, threonine, β-alanine, aspartic acid, glycine and taurine contents were significantly higher in BA-oocytes compared to the two other dietary treatments. On the

contrary, free serine and γ-aminobutyric acid levels were higher in BD-oocytes than in BE-oocytes (4.99 and 1.04 vs. 4.76 and 1.00 mg/g, respectively).

SAM and SAH concentrations were significantly higher in BE-oocytes and lower in BD-oocytes compared to BA-oocytes (Fig. 3). The SAM/SAH ratio was significantly higher in BD-oocytes than in BA- and BE-oocytes.

3.5. Growth performance of rainbow trout fry from the start of exogenous feeding onwards

Survival and growth of the 21-day fry was affected by the broodstock nutritional history, with the lowest results observed in BD-offspring (Table 7). The best survival from the swim-up stage onwards was noticed in BE-offspring whereas the best growth was recorded in BA-offspring. Growth, but not survival of the fry, was clearly affected by the Met level of the fry diet, being the lowest in rainbow trout fry fed the lowest dietary Met level (diet FD). Fry fed diet FE exhibited the highest growth. As for mean body weight, daily growth index, which is independent of initial weight, was affected both by broodstock and fry Met level, but with a significant interaction. The difference of daily growth index between FA and FE was recorded only within BD-group and the difference between dietary broodstock groups was recorded only within FD- and FA groups (Fig. 4).

3.6. Gene expression levels for Met metabolism in broodstock liver and offspring

The expression of genes involved in Met metabolism was assessed in broodstock female livers (Table 8) and whole-body of offspring at the swim-up stage (before first-feeding) as well as at the 21-day post first-feeding stage (Fig. 5). The expression of BHMT1b gene was reduced in liver of females fed the deficient diet BD (Table 8) and in whole fry fed

the deficient diet FD (Fig. 5C). Also, the expression of BHMT1a gene was significantly reduced in BD- and BE-offspring at the swim-up fry stage (Fig. 5A) and in whole fry fed the diets FD and FE (Fig. 5C). On the contrary, the expression of BHMT1a gene was increased in BD group at the 3-week post first-feeding stage (Fig. 5B) and the expression of MTR gene, also involved in the remethylation of homocysteine to Met, was increased both in BD group compared to BE at the swim-up fry stage (Fig. 5A) and in FD group compared to FA and FE groups at the 3-week post first-feeding stage (Fig. 5C). However, this effect of Met deficiency in broodstock diets on the expression of MTR gene was no more significant in 3-week post first-feeding fry (Fig. 5B).

The expression of GR, GSTπ and MsrA1 genes was higher both in liver of females fed the deficient diet BD compared to diet BE (Table 8) and in 3-week fry fed the deficient diet FD compared to diet FA (Fig. 5C). GSTπ and MsrA1 mRNA levels were also increased in BD group compared to BE group at the swim-up fry stage (Fig. 5A) and this effect was still significant for MsrA1 gene at the 3-week post first-feeding stage (Fig. 5B). The expression of CTH gene was reduced in 3-week fry from the BE-group compared to BA group (Fig. 5B) and in 3-week fry fed the diets FE and FA compared to the deficient diet FD (Fig. 5C). The expression of MsrB2 gene was reduced in 3-week fry from the BD-group compared to BA group (Fig. 5B) and in 3-week fry fed the diet FD compared to FA (Fig. 5C). The expression of other considered genes was only affected by fry nutrition (Fig. 5C). Gclc and MsrB1 mRNA levels were increased in 3-week fry fed the deficient diet FD compared to diets FA and FE. Fry fed the diet FD had also a higher expression of CBS with a significant interaction since the dietary fry effect was only seen within the BD-group.

3.7. Gene expression levels linked with feed intake, growth and quality in offspring

The expression of genes involved in appetite regulation and muscle growth was assessed in whole-body offspring at the swim-up stage and at 21 days post first-feeding (Fig. 6). The swim-up fry from the Met-deficient broodstock group (BD) had a higher expression of POMC along with a lower expression of the NPY feeding peptide (Fig. 6A). This parental effect remained significant in the 21-day fed fry for POMC, but disappeared for NPY (Fig. 6B). Fry fed the Met-deficient FD diet had a higher expression of POMC (Fig. 6C) with a significant interaction since the broodstock effect was seen only within the FD-group and since the dietary fry effect was only seen within the BD-group. The FD fry diet enhanced NPY expression (Fig. 6C), yet only in fry of the BD and BA broodstock group whereas it decreased NPY expression in the BE broodstock group (interaction). Dietary Met deficiency in broodstock enhanced the expression of Myog and decreased the expression of fMHC in fry at the swim-up stage (Fig. 6A). The parental effects on Myog were no longer significant 21 days later (Fig. 6B). Feeding the FD-diet up-regulated the expression of Myog in 21-day post first-feeding fry but had no effect on fMHC expression at this stage (Fig. 6C). The expression of GDH was up-regulated in BD-offspring at both swim-up stage and 21 days post first-feeding (Fig. 6A and B). Fry fed diet FD, as well as those fed diet FE, had a higher GDH expression than those fed the adequate diet FA (Fig. 6C). The expression of SOD2 gene was lower in BE-offspring in both the swim-up and the 21-day post first-feeding fry (Fig. 6A and B). SOD2 mRNA levels were not significantly affected by fry nutrition (Fig. 6C). Broodstock and fry dietary Met deficiency led to an up-regulation of the expression of LDLR gene at both swim-up stage and 21 days post first-feeding (Fig. 6). A significant interaction for the expression of this gene was noticed at 21 days post first-feeding as dietary broodstock effect was recorded only within the FD-group and as the dietary fry effect was recorded only within the BD-group.

4. Discussion

We studied the effect of changes in dietary Met supply in broodstock and first-feeding rainbow trout fry (offspring). The first part of the discussion outlines the direct dietary Met effects on growth and Met metabolism at both life stages. The second part highlights the effects of parental Met intake on progeny performance, providing highly novel data in the field of fish nutrition.

4.1. Impact of dietary Met levels on growth and Met metabolism

Little is known on the impact of changes in dietary Met supply in fish at both studied life stages: broodstock and first-feeding. Feed intake, growth and reproductive performance recorded in the broodstock trial were in line with our previous data using rainbow trout broodstock reared under similar conditions of temperature and photoperiod (Fontagné-Dicharry et al., 2010). The decreased feed intakes seen over the last two months of the broodstock trial also agree with previous observations during late vitellogenesis in rainbow trout (Washburn et al., 1990). The early growth performances of the first-feeding fry fed plant-based diets were intermediate in between those obtained with semi-purified (Daprà et al., 2011) and with practical (Geurden et al., 2007) diets under similar rearing conditions.

Feeding the low Met diet to the first-feeding trout resulted in poor growth in accordance with findings in juvenile fish (Belghit et al., 2014; Figueiredo-Silva et al., 2015; NRC, 2011). Reduced growth, though to a lesser degree, was also noticed in female broodstock fed the low Met content, mainly due to the weight loss seen in the period preceding spawning which may reflect an inferior resistance to starvation. On the contrary, no difference in growth was seen in rainbow trout male broodstock highlighting thus the sex or

life stage specificity concerning the impact of dietary Met level on growth performance. Feeding high levels of methionine (two times the established requirement) did not negatively impact growth of the broodstock nor of the fry in agreement with reports in fish juveniles (Kaushik and Seiliez, 2010; NRC, 2011).

In fish, as in mammals, changes in dietary Met supply have been shown to modify levels of circulating plasma lipids. In our study, broodstock females fed the low Met diet had slightly reduced plasma triacylglycerol and increased levels of total and LDL-cholesterol, in line with reports in juvenile fish for triacylglycerol (Elmada et al., 2016; Luo et al. 2005; Skiba-Cassy et al., 2016), total cholesterol (Zhou et al., 2011), or in rodents for LDL-cholesterol levels (Elshorbagy et al., 2011; Hidiroglou et al., 2004; Velez-Carrasco et al., 2008). In contrast, lower dietary Met supply has been found to decrease plasma cholesterol in a previous study with juvenile rainbow trout (Craig and Moon, 2013). Some inconsistencies regarding the response of total and HDL-cholesterol levels to changes in dietary Met intake also exists in the aforementioned studies with rodents. Such discrepancy may be species or life-stage specific, e.g. linked to the involvement of cholesterol in steroid metabolism and reproduction. The dietary fat level or the presence of other methyl donors such as choline and betaine might also interfere. Life-stage specific effects of dietary Met on lipid metabolism has also been suggested by Espe et al. (2016a) regarding fat accumulation in liver, as reflected by changes in the HSI index. In our study, trout broodstock had similar HSI, irrespective of the diet.

Dietary Met deficiency altered expression of genes involved in Met metabolism with enhanced expression of MTR, CBS, CTH, Gclc, GR, GSTπ, MsrA1 and MsrB1 genes and reduced expression of BHMT and MsrB2 in whole fry at 21 days post-first feeding. Similar effects of dietary Met deficiency on GR, GSTπ, MsrA1 and BHMT gene expression were found in liver of broodstock females.

The increase in gene expression of CBS, which catalyzes the first step of the transsulfuration pathway (from homocysteine to cystathionine), by dietary Met deficiency has also been noticed in salmon alevins though associated with increased BHMT mRNA (Kwasek et al., 2014) in contrast to the decreased BHMT response seen here in both whole fry and broodstock liver. BHMT, like MTR, is an enzyme involved in the remethylation of homocysteine to Met. BHMT utilizes betaine, a product of choline oxidation, whereas MTR is 5-methyltetrahydrofolate and vitamin B12-dependent. Betaine has been shown to increase BHMT gene expression (Sparks et al., 2006), but to repress MTR gene expression (Kacprzak et al., 2003). As such, the lower BHMT and higher MTR mRNA levels seen in the Met-restricted trout might indicate that betaine was limiting despite the dietary supplementation of choline, its precursor. Indeed, fish appear able to synthesize choline if adequate methyl donors such as Met are present in the diet (NRC, 2011). In case of dietary Met deficiency, synthesis of choline may thus be reduced and so betaine levels. However, the Met-deficient broodstock diet did not result in a higher HSI, indicative of increased liver lipid, a common symptom linked to Met and choline deficiency in fish (NRC, 2011) and mammals (Henning et al., 1989). In order to ascertain this, the concentrations of metabolites including betaine and homocysteine should be assessed in broodstock liver and whole fry for a better understanding on the effects of dietary Met deficiency on the expression of genes involved in the remethylation process of Met.

The increase in glutathione metabolism-related gene expression (CBS, CTH, Gclc, GR and GSTπ) including the transsulfuration pathway with dietary Met restriction is in line with findings in yeast and mammals (Bella et al., 1999; Castellano et al., 2015; Kwon and Stipanuk, 2001; Tsai et al., 2010; Wheeler et al., 2003; Wolf et al., 2010). However, gene expression data do not necessarily correlate with changes in protein or enzyme levels, as illustrated in mice fed a Met-deficient diet which displayed slightly increased CBS mRNA

associated however with decreased CBS protein and activity (Tang et al., 2010). The present transcriptional data should therefore be confirmed by quantifying resulting enzymes and metabolites.

Dietary Met deficiency also reduced expression of MsrB2 gene while it increased that of MsrA1 and MsrB1 genes. The enhanced expression of MsrA and MsrB1 in our study possibly relates with observations in mice where both enzymes were found to play a role in supplying Met for growth when Met is limited (Zhao et al., 2012). Both free and protein-bound Met can be oxidized by reactive oxygen and nitrogen species and MsrA and MsrB can reverse the Met oxidation in order to both replenish the Met pool and contribute to antioxidant defense mechanism (Kim, 2013). The higher expression of MsrA and MsrB associated with the higher expression of Gclc, GR and GSTπ in rainbow trout fed the Met-deficient diets would be indicative of oxidative stress, in agreement with the antioxidant properties of Met in fish (Elmada et al., 2016). In order to substantiate this, the antioxidant status of the fish should be assessed.

Exposing the fry during 21 days to the Met-deficient diet modified the gene expression of neuropeptides playing a role in appetite regulation. Fish as most animals reduce feed intake of a diet showing an essential amino acid deficiency (Figueiredo-Silva et al., 2015; Rolland et al., 2015; Saravanan et al., 2013). Due to their small size, food intake in fry could not be monitored here. Yet, the higher expression of the anorexigenic feeding peptide POMC in fry fed the Met-deficient diet suggests reduced appetite in that group. At the same time, the expression of the NPY neuropeptide, known as being orexigenic, was also superior in the Met-deficient group. This might be due to the post-prandial sampling as the response of NPY in fish brain to changes in amino acid profile was found to occur just before the meal, i.e. pre-prandially (Nguyen et al., 2013).

The Met level of the fry diet had no effect on the expression of fMHC, in line with previous findings in juvenile Atlantic salmon fed low dietary Met (Espe et al., 2016b), but acted on other muscle growth-related genes. The Met-deficient diet at first-feeding up-regulated Myog expression in trout fry, different from reports in broilers (Wen et al., 2014). Both sampling age and Met level might be involved in this discrepancy, as myogenin expression in cultured broiler satellite cells is dependent on both factors (Harthan et al., 2014). It could also be due to differences in muscle hyperplastic intensity after hatching. As the growth potential of fish following first-feeding is linked to skeletal muscle hyperplasia (Alami-Durante et al., 1997), a higher level of Myog, involved in early differentiation/fusion, was expected in the faster growing fry, which was not the case. The higher expression of Myog in Met-deficient fry might be linked to a decrease in methylation level of Myog promoter, based on results obtained in cells treated with a DNA methylation inhibitor (Hupkes et al., 2011) or in sole larvae reared at different temperatures (Campos et al., 2013). Dedicated studies on epigenetic mechanisms involved are needed to verify this hypothesis. The increased mRNA level of GDH in whole fry when dietary Met is both limited or in excess, suggests that the process of amino acid deamination is very sensitive to dietary Met imbalance at this stage of development. In juvenile trout, hepatic GDH mRNA was upregulated by Met-excess but not by Met-deficiency (Skiba-Cassy et al., 2016). As Met is a rate-limiting amino acid for protein synthesis, complementary studies are needed to assess the long-term phenotypic consequences of fry dietary Met imbalance.

4.2. Impact of the Met level in broodstock diet on offspring performance

The present results are the first to provide evidence of the role of dietary Met in reproductive performance in fish. Dietary Met deficiency reduced the size of rainbow trout

eggs similar to those observed in Nile tilapia fed diets low in taurine (Al-Feky et al., 2016) or in rainbow trout fed diets without fish meal (Lazzarotto et al., 2015). The reduced egg size was associated with increased plasma total and LDL-cholesterol in Met-deficient broodstock, which may indicate a disruption of lipid metabolism and reproductive function, as observed in diabetic rat (McLean et al., 1995). The possible role of dietary Met on the regulation of cholesterol metabolism and reproductive performances deserves further studies. Moreover, we found some indication that parental Met levels affected cholesterol metabolism in progeny, as suggested by the altered expression of LDL-receptor in the trout fry. Also in mammals, changes in methylation potential (e.g. betaine) have been shown to affect LDL-receptor gene expression in progeny (Cai et al., 2014).

In our study, the parental Met deficiency reduced egg size and decreased the later growth performance of offspring. However, the body weight of the offspring at the swim-up stage was unaffected, probably due to the high mortality in this group at eyed stage which may have selected the more robust individuals. This decreased growth of offspring from broodstock fed the Met-deficient diet was in line with data obtained in rainbow trout fed high levels of plant protein ingredients (Lazzarotto et al., 2015; 2016). Reduced growth was also noticed in grand-offspring of duck fed Met-deficient diets but only during the first four weeks of life (Brun et al., 2015) and in gilthead seabream progeny from broodstock fed vegetable oils but only in early stages when fed a commercial diet (Izquierdo et al., 2015). Of interest, duck grand-offspring then exhibited higher weight gain during late force-feeding (Brun et al., 2015) and gilthead seabream offspring showed a higher growth and feed utilization when challenged with a plant-based diet at the 4-month-old juvenile stage (Izquierdo et al., 2015). Unlike our results, parental dietary Met deficiency did not affect birth weight of sheep, with adult offspring being instead heavier (Sinclair et al., 2007). It is therefore important to follow

growth performance of rainbow trout offspring over the long term under different challenging nutritional conditions to rule out the likely relation with egg size and larval quality.

In our study, the parental Met excess also decreased growth performance of offspring compared to the adequate parental Met level, despite its beneficial effect on offspring survival. This parental effect of Met excess is clearly different from the fry diet effect of Met excess which improved growth performance of the fry compared to the adequate Met fry diet, suggesting different roles for excess Met according to the life stage.

Met levels in broodstock diets affected the free amino acid concentrations of rainbow trout oocytes, especially those of Met and cysteine, which levels correlated with those of SAM and SAH. Similar effects due to changes in the dietary Met supply have been seen in muscle but not in liver of juvenile Atlantic salmon (Espe et al., 2016b). During vitellogenesis, essential nutrients such as amino acids are passed to the oocyte from the liver through the blood. As such, the essential amino acid profile of oocytes would be expected to reflect more that of liver than that of muscle, as seen in striped catfish (Kabir et al., 2015). Successful embryonic development in fish has been related with the balance of amino acids present in the egg (Brooks et al., 1997; Srivastasa et al., 1995). So the improved survival of swim-up fry from the Met-excess fed parents possibly relates with the free amino acid profile of the oocyte. However, total free amino acid concentrations including Met may change during early ontogenesis of rainbow trout due to metabolic activity (Suzuki et al., 1991; Zeitoun et al., 1977). So the free amino acid profile, as well as levels of SAM and SAH, observed in the oocyte at spawning is likely to be different in first-feeding fry. Further studies should investigate the effects of changes in SAM-related methylation potential during early ontogenesis of rainbow trout. In this regard, the high SAM/SAH ratio in the Met-deficient oocytes, often used as an index of cellular methylation, is intriguing and suggests a peculiar

SAH metabolism compared to mammals where Met-deficient diets do not seem to reduce SAH levels (Henning et al., 1989).

Met levels in broodstock diets altered the gene expression of some enzymes involved in Met metabolism in swim-up fry and even in 21-day post first-feeding fry. Contrary to the direct dietary effects noticed in broodstock or fry, the impact of parental Met-excess was more prominent than that of parental Met-deficiency on the progeny's gene expression of MTR, CTH, GSTπ, MsrA1 and SOD2. Maternal micronutrient imbalance (excess or deficient folic acid and vitamin B12) has been shown to reduce expression of MTR and increase expression of CBS in the rat (Khot et al., 2014), and to adversely affect antioxidant defense mechanisms with reduced SOD and increased lipid peroxidation in rat offspring (Roy et al., 2014). This suggests that the Met-excess parental diet was an imbalanced diet for MTR expression and antioxidant defense mechanisms in the offspring. On the other hand, a temporary imbalanced amino acid diet (with Met-deficiency) resulted in prolonged up-regulation of MsrA and GST genes in juvenile pigs (Schwerin et al., 2008), in line with the present results and which suggests that the Met-excess broodstock diet should be considered not as an imbalanced but as the adequate diet for oxidative stress response gene expression (MsrA and SOD2) transmitted to the offspring. Ongoing studies on the antioxidant status of rainbow trout offspring will throw more light on this point.

We know practically nothing on the possible parental dietary effects on the ontogeny and expression of appetite-regulating brain peptides in offspring. Transcripts of both POMC and NPY central feeding peptides have been found in Atlantic salmon during embryonic development, with POMC being provided as maternal transcript, and prior to exogenous feeding (Moen et al., 2010). We also found that both peptides were expressed in the pre-feeding swim-up fry. Moreover, their expression level was highly dependent on the parental broodstock diet, as that of POMC was increased by 60% while that of NPY was reduced by

more than 50% in the Met-deficient versus Met-adequate offspring. In terms of central feeding regulation, this may suggest that the swim-up fry from the Met-deficient fed parents are 'programmed' for a more anorectic feeding behavior. Of interest, whereas the parental effects on NPY had disappeared following the 21-day feeding period, POMC expression in offspring remained affected by the parental Met-deficiency, which may hint at an epigenetic effect governed by changes in methyl donor capacity. In studies on mammalian models, maternal nutrition is well recognized to alter POMC promotor methylation in offspring, as reported for example in sheep (Stevens et al., 2010) or in rat (Coupé et al., 2010).

Met level of broodstock feeds affected the expression of markers of early muscle differentiation/fusion (Myog) and fast muscle differentiation/hypertrophy (fMHC) in first-feeding fry but not 21 days later, indicating an early pre-feeding response of myogenesis to parental Met deficiency. To our knowledge, these data are the first to demonstrate a regulation of the expression of Myog by manipulations of parental nutrition in fish. The findings that broodstock dietary Met limitation up-regulated Myog and down-regulated fMHC mRNA in first-feeding trout fry, suggest that parental Met limitation led to a delay in muscle differentiation at first-feeding. The changes that had occurred in first-feeding rainbow trout from the Met-deficient broodstock might have potential long-term consequences. Indeed, as differentiation and proliferation are exclusive in skeletal muscle formation (Lassar et al., 1994), the increased Myog expression in first-feeding fry from dietary Met-deficient broodstock might have a negative impact on the proliferation of muscle progenitors. The consequences of a decrease in proliferation will be a decreased number of muscle precursors available for muscle growth (Harthan et al., 2014). The down-regulation of fMHC (a main component of sarcomeres) expression together with the up-regulation of GDH (amino acid catabolism) expression in fry from the Met-deficient broodstock suggests that a parental Met-deficiency may also limit the hypertrophic growth of muscle fibres. Our further studies will

determine the effect of Met supply on other markers of muscle precursor fate (determination, proliferation) and of muscle growth processes (hyperplasia and hypertrophy), and assess the long-term consequences on growth of a parental change in Met availability.

4.3. Is the impact of Met level in fry diet affected by the broodstock nutrition?

Above data show that changes in broodstock Met supply impact on a large variety of traits in the offspring. In addition, we tested whether i) the pre-natal Met supply can influence how offspring deals with changes in Met supply after hatching, i.e. during the first 3 weeks of feeding, and vice versa, whether ii) the Met supply to the fry affected the further consistency of the parental effects. Such interaction effect was evident on growth rate for which feeding Met in excess (diet FE) appeared to have washed out the parental effects while these persisted in fry fed diet FD. In the same line, fry from the Met-deficient parents (BD) benefited from receiving diet FE whereas no such benefits were seen in the other fry. In contrast, feeding the parents a Met-deficient diet clearly did not positively impact the fry's response to the FD diet. This would have implied some adaptive adjustments to reduced Met in the offspring, as seen in studies where offspring performed better when they experienced the same conditions before and after birth (Izquierdo et al., 2015; Sinclair et al., 2007). Instead, the dual Met-deficiency induced other interaction effects as illustrated at the level of POMC or LDL-R expression, for instance. For both genes the effect of feeding parents the Met-deficient diet was significant only in the FD group, and vice versa, the effect of the fry diet Met deficiency was only detected in fry originating from Met-deficient broodstock.

5. Conclusion

In summary, our results demonstrate that dietary Met levels of rainbow trout broodstock affect various traits in the offspring, some of which persisted during the first weeks of exogenous feeding. Further studies are warranted to evaluate the further persistence of the parental effects over time and to elucidate the mechanisms, whether epigenetic or not.

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Table 1

Formulation and composition of the experimental diets.

Diet	BD	BA	BE	FD	FA	FE
Ingredients (%)						
Fish soluble protein concentrate [a]	4.0	4.0	4.0	5.0	5.0	5.0
Fish oil [a]	13.6	13.6	13.6	7.0	7.0	7.0
Soybean protein concentrate [a]	27.0	27.0	27.0	24.0	24.0	24.0
Faba bean protein concentrate [b]	19.0	19.0	19.0	27.5	27.5	27.5
White lupin meal [c]	11.0	11.0	11.0	18.0	18.0	18.0
Dehulled pea meal [b]	6.0	6.0	6.0	7.0	7.0	7.0
Wheat gluten [d]	4.0	4.0	4.0	-	-	-
Whole wheat [e]	8.0	8.0	8.0	-	-	-
Soybean lecithin [f]	-	-	-	3.0	3.0	3.0
Carophyll® pink [g]	0.03	0.03	0.03	-	-	-
$CaHPO_4 \cdot 2H_2O$ [e]	3.55	3.55	3.55	2.7	2.7	2.7
Vitamin premix [h]	1.0	1.0	1.0	2.0	2.0	2.0
Mineral premix [i]	1.0	1.0	1.0	2.0	2.0	2.0
L-lysine [j]	0.32	0.32	0.32	0.3	0.3	0.3
L-glutamic acid [k]	1.5	1.0	0.0	1.5	1.0	0.0
DL-methionine [l]	0.0	0.5	1.5	0.0	0.5	1.5
Analytical composition						
Dry matter (DM, %)	95.6	95.9	96.4	95.9	95.8	96.2
Crude protein (% DM)	43.9	43.8	43.9	48.2	48.2	48.2
Total lipid (% DM)	16.7	16.0	16.6	14.7	13.4	13.7

Gross energy (kJ/g DM)	22.4	22.2	22.4	23.0	23.0	23.1
Ash (% DM)	7.9	8.0	7.8	8.0	8.1	8.1

[a] CPSP Special G, crude fish oil and Estrilvo from Sopropêche (Wimille, France).

[b] Fabaqua 55 and Primatex from Sotexpro (Berméricourt, France).

[c] Farilup 500 from Terrena (Martigné-Ferchaud, France).

[d] Roquette (Lestrem, France).

[e] Sud-Ouest Aliment (Haut-Mauco, France)

[f] Louis François (Croissy-Beaubourg, France).

[g] DSM (Basel, Switzerland), contained 8% astaxanthine.

[h] Vitamin premix (IU or g/kg premix): retinyl acetate, 500,000 IU; cholecalciferol, 250,000 IU; DL-α-tocopheryl acetate, 5,000 IU; sodium menadione bisulfate, 1 g; thiamin-HCl, 0.1 g; riboflavin, 0.4 g; niacin, 1 g; D-calcium pantothenate, 2 g; pyridoxine-HCl, 0.3 g; D-biotin, 20 mg; folic acid, 0.1 g; cyanocobalamin, 1 mg; L-ascorbyl-2-polyphosphate, 5 g; *myo*-inositol, 30 g; choline, 100 g. All ingredients were diluted with α-cellulose.

[i] Mineral mixture (g/kg premix): $CaHPO_4 \cdot 2H_2O$, 500; $CaCO_3$, 215; $Mg(OH)_2$, 124; KCl, 90; NaCl, 40; $FeSO_4 \cdot 7H_2O$, 20; $ZnSO_4 \cdot 7H_2O$, 4; $MnSO_4 \cdot H_2O$, 3; $CuSO_4 \cdot 5H_2O$, 3; NaF, 10; KI, 0.04; Na_2SeO_3, 0.03; $CoCl_2 \cdot 6H_2O$, 0.02. All ingredients were diluted with α-cellulose.

[j] Ajinomoto-Eurolysine (Paris, France).

[k] Acros (Geel, Belgium).

[l] Evonik (Essen, Germany).

Table 2

Analyzed amino acid composition of the diets as g/100 g dry feed.

Diet	BD	BA	BE	FD	FA	FE
Essential amino acids [a]						
Arginine	3.14	3.23	3.94	4.66	4.84	4.57
Histidine	0.98	1.00	1.00	1.15	1.18	1.14
Isoleucine	1.66	1.67	1.72	2.22	2.22	2.21
Leucine	2.97	3.04	3.01	3.59	3.58	3.57
Lysine	2.53	2.60	2.55	2.78	2.69	2.81
Methionine	0.51	1.04	2.04	0.57	1.18	2.15
Phenylalanine	1.88	1.95	1.92	2.36	2.46	2.35
Threonine	1.54	1.58	1.55	1.67	1.71	1.65
Valine	1.77	1.77	1.84	2.44	2.45	2.45
Non-essential amino acids						
Alanine	1.67	1.73	1.70	1.71	1.67	1.71
Aspartic acid + Asparagine	4.02	4.12	4.05	3.85	3.69	3.82
Cysteine	0.60	0.59	0.60	0.55	0.47	0.57
Glutamic acid + Glutamine	9.09	8.86	7.75	7.39	6.77	6.10
Glycine	1.76	1.81	1.79	2.15	2.18	2.09
Proline	2.14	2.21	2.19	2.00	2.01	1.97
Serine	2.07	2.15	2.06	2.34	2.32	2.27
Tyrosine [b]	-	-	-	1.93	1.94	1.90

[a] Tryptophan was not analyzed.

[b] Tyrosine was not determined in broodstock diets.

Table 3

Oligonucleotide forward (F) and reverse (R) primers used to assay gene expression by real-time quantitative RT-PCR.

Gene	Accession no. (GenBank or INRA-SIGENAE)	Primer sequence (5' → 3')	Amplicon size (bp)
EF1α	AF498320.1	F: TCCTCTTGGTCGTTTCGCTG	159
		R: ACCCGAGGGACATCCTGTG	
BHMT1a	FR908041.1	F: CAGAGAAGCACGGTAACTGG	188
		R: TTCTTTGTGCTGCATCAGGT	
BHMT1b	FR905322.1	F: GCTGAGGAGCTAGCCACAGA	140
		R: GGCTTCAGCTTCTCCCAGTA	
MTR	FYV3OTN01A0UO6.s.om.10	F: AATGCAGGTCTGCCCAATAC	137
		R: CTGATGTGTGCAGGAGTCGT	
CBS	FR904728.1	F: CAAGGCTCTCAGCACATCCA	132
		R: ACCATCATCGAGCCCACCT	
CTH	FR904293.1	F: TGGCTTGAGACTCCCACCAA	132
		R: GCGCTGGAAGTAGGCTGACA	
Gclc	CDQ64687.1	F: AGGCCAGAGTATGGCAGCTA	176
		R: CAGCCTAACCTTGGGAATGA	
GR	HF969248.1	F: CTAAGCGCAGCGTCATAGTG	108
		R: ACACCCCTGTCTGACGACAT	
GSTπ	BX302932.3	F: TCGCTGACTGGACGAAAGGA	196
		R: CGAAGGTCCTCAACGCCATC	
MsrA	BT073399.1	F: GATGGCTATGTTTGGGATGG	136
		R: ACCTGTGCAGGTCTCTTCGT	

MsrB1	BX313019.3	F: CTGGCCTGCCTTCACTGAGA	87
		R: TTCCCACATCGGACCTTGAA	
MsrB2	CA354807.1	F: AGGGGACAGAGATGCCCTTC	149
		R: CCCATGAGCCTCTTTGAACG	
POMCa	NM_001124718.1	F: CTCGCTGTCAAGACCTCAACTCT	117
		R: GAGTTGGGTTGGAGATGGACCTC	
NPY	NM_001124266.1	F: CTCGTCTGGACCTTTATATGC	246
		R: GTTCATCATATCTGGACTGTG	
Myog	Z46912	F: CAGTACATTGAGAGGCTGCAGGCA	132
		R: CTCACTCGACGACGACACCCTG	
fMHC	Z48794	F: AGAAACTGGAGTCCAGGGTG	215
		R: TGAGCTCCTTGACTCTGCGC	
GDH	AJ419571.1	F: ATCAAGCCCTGCAACCACGTCCT	140
		R: TCTTCACTGTAACGGATCCCCCCTTT	
SOD2	CA352127.1	F: TCCCTGACCTGACCTACGAC	201
		R: GGCCTCCTCCATTAAACCTC	
LDLR	AF542091.1	F: AACTGCGGTCACAGGTCAAA	186
		R: ACGGGGTTGTCAAAGTGGAT	

F, forward primer; R, reverse primer; EF1α, elongation factor 1α; BHMT1, betaine-homocysteine methyltransferase 1; MTR, 5-methyltetrahydrofolate-homocysteine methyltransferase or methionine synthase; CBS, cystathionine-β-synthase; CTH, cystathionine γ-lyase; Gclc, glutamate-cysteine ligase catalytic subunit; GR, glutathione reductase; GSTπ, glutathione-S-transferase π; MsrA, methionine-S-sulfoxide reductase A; MsrB, methionine-R-sulfoxide reductase B; POMCa, proopiomelanocortin A; NPY,

neuropeptide Y; Myog, myogenin; fMHC, fast myosin heavy chain; GDH, glutamate dehydrogenase; SOD2, superoxide dismutase 2; LDLR, low-density lipoprotein receptor.

Table 4

Growth, spawning parameters and offspring performance of rainbow trout broodstock fed different Met levels over 6 months at a constant water temperature of 8 ± 1 °C.

Diet	BD	BA	BE
Male performance			
Initial mean body weight (kg, n = 10)	0.28 ± 0.07	0.29 ± 0.05	0.27 ± 0.08
Final mean body weight (kg, n = 7-10)	0.58 ± 0.18	0.51 ± 0.10	0.57 ± 0.18
HSI (%, n = 4-6)	0.96 ± 0.18	0.94 ± 0.11	1.05 ± 0.23
VSI (%, n = 4-6)	10.4 ± 2.9	11.6 ± 1.0	11.4 ± 2.8
Female performance			
Initial mean body weight (kg, n = 25)	1.45 ± 0.26	1.51 ± 0.19	1.60 ± 0.26
Final mean body weight (kg, n = 23-25)	1.92 ± 0.36[b]	2.38 ± 0.37[a]	2.31 ± 0.41[a]
HSI (%) [1]	1.20 ± 0.18	1.03 ± 0.25	1.20 ± 0.19
VSI (%) [1]	11.9 ± 2.2	11.1 ± 1.6	12.2 ± 1.6
GSI (%) [1]	9.2 ± 3.6	10.9 ± 3.3	12.3 ± 2.6
Spawn weight (g) [1]	189 ± 73[b]	262 ± 81[ab]	295 ± 65[a]
Absolute fecundity [1]	5148 ± 1629	4889 ± 1286	5693 ± 1288
Relative fecundity [1]	2465 ± 553	2016 ± 413	2355 ± 405
Offspring performance			
Egg diameter (mm)	3.9 ± 0.5[b]	4.4 ± 0.3[a]	4.4 ± 0.4[a]
Egg mean weight (mg)	42 ± 13[b]	61 ± 10[a]	60 ± 14[a]
Fertilization rate (%)	73 ± 45	98 ± 2	99 ± 0
Survival at eyed stage (%)	38 ± 37[c]	65 ± 25[b]	83 ± 10[a]

Hatching rate (%)	36 ± 36[b]	59 ± 22[b]	80 ± 12[a]
Survival at swim-up stage (%)	35 ± 35[b]	56 ± 21[b]	77 ± 13[a]
Mean weight of swim-up fry (mg)	71 ± 8	83 ± 16	79 ± 18
Daily growth index [1]	0.07 ± 0.02	0.06 ± 0.02	0.05 ± 0.01

Values are means ± SD (n = 8, unless otherwise stated). Within rows, means not sharing a common superscript letter are significantly different ($P < 0.05$) according to one-way ANOVA followed by a Newman-Keuls test.

[1] HSI, hepato-somatic index = (wet liver weight, g/weight of fish, g) × 100; VSI, viscero-somatic index = (wet viscera weight, g/weight of fish, g) × 100; GSI, gonado-somatic index = (wet spawn weight, g/weight of female, g) × 100; spawn weight = total egg weight per female after stripping and removal of coelomic fluid; absolute fecundity = egg number per female; relative fecundity = egg number/weight of female, kg; daily growth index = 100 × [(fry mean weight at swim-up)$^{1/3}$ – (egg mean weight at spawning)$^{1/3}$]/duration (68 days).

Table 5

Protein-bound amino acid composition of oocytes from rainbow trout females fed different Met levels (mg/g dry oocyte).

Diet	BD	BA	BE
Essential amino acids [1]			
Arginine	29.1 ± 1.4[a]	26.6 ± 1.6[b]	29.0 ± 0.4[a]
Histidine	12.7 ± 0.9	12.8 ± 0.7	12.3 ± 0.2
Isoleucine	20.2 ± 0.8	20.3 ± 0.8	20.2 ± 0.6
Leucine	39.6 ± 2.4	40.2 ± 1.5	38.3 ± 1.1
Lysine	37.0 ± 4.1[a]	39.6 ± 3.0[a]	32.6 ± 1.1[b]
Methionine	21.3 ± 1.7	21.2 ± 2.1	21.5 ± 1.2
Phenylalanine	20.0 ± 1.7	21.1 ± 2.1	20.9 ± 0.9
Threonine	26.8 ± 1.8[a]	24.4 ± 1.0[b]	26.0 ± 0.8[ab]
Valine	24.2 ± 1.6[a]	21.5 ± 1.2[b]	25.2 ± 1.3[a]
Non-essential amino acids			
Alanine	28.9 ± 1.9	28.6 ± 2.2	28.3 ± 0.5
Aspartic acid	51.7 ± 2.4	51.9 ± 1.5	53.5 ± 1.9
Cysteine	5.3 ± 0.4	5.2 ± 0.6	5.3 ± 0.2
Glutamic acid	60.7 ± 3.2	57.7 ± 1.2	59.8 ± 2.2
Glycine	24.5 ± 1.1	25.3 ± 1.0	25.2 ± 0.8
Proline	20.2 ± 1.1	19.3 ± 0.7	20.1 ± 0.5
Serine	19.3 ± 1.1	19.6 ± 0.9	19.4 ± 0.4
Tyrosine	19.2 ± 0.8	19.1 ± 0.9	19.1 ± 0.3

Values are means ± SD. Within rows, means not sharing a common superscript letter are significantly different ($P < 0.05$) according to one-way ANOVA followed by a Newman-Keuls test.

[1] Tryptophan was not analyzed.

Table 6

Free amino acid composition of oocytes from rainbow trout females fed different Met levels (mg/g dry oocyte).

Diet	BD	BA	BE
Essential amino acids	65.3 ± 1.7[b]	69.0 ± 1.1[a]	68.0 ± 1.1[a]
Arginine	23.0 ± 0.5[b]	23.9 ± 0.4[a]	23.6 ± 0.3[a]
Histidine	3.0 ± 0.2[b]	3.2 ± 0.1[a]	3.0 ± 0.2[b]
Isoleucine	4.0 ± 0.2	4.1 ± 0.1	4.1 ± 0.1
Leucine	7.4 ± 0.1[b]	7.7 ± 0.2[a]	7.6 ± 0.1[a]
Lysine	8.5 ± 0.4[b]	9.4 ± 0.1[a]	9.2 ± 0.3[a]
Methionine	3.2 ± 0.2[c]	4.1 ± 0.1[b]	4.5 ± 0.1[a]
Phenylalanine	3.4 ± 0.1[b]	3.8 ± 0.1[a]	3.5 ± 0.1[b]
Threonine	3.7 ± 0.2[b]	3.9 ± 0.1[a]	3.7 ± 0.2[b]
Tryptophane	1.8 ± 0.0	1.8 ± 0.0	1.8 ± 0.0
Valine	7.2 ± 0.3	7.1 ± 0.1	7.0 ± 0.2
Non-essential amino acids	65.5 ± 2.7	67.4 ± 1.2	65.3 ± 1.0
Alanine	4.9 ± .2[b]	5.2 ± 0.1[a]	5.1 ± 0.1[a]
Asparagine	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0
Aspartic acid	13.3 ± 0.5[b]	13.9 ± 0.3[a]	13.4 ± 0.1[b]
β-alanine	0.2 ± 0.0[b]	0.2 ± 0.0[a]	0.2 ± 0.0[b]
Cysteine	1.4 ± 0.0[c]	1.5 ± 0.0[b]	1.6 ± 0.0[a]
γ-aminobutyric acid	1.0 ± 0.0[a]	1.0 ± 0.0[b]	1.0 ± 0.0[b]
Glutamine	4.6 ± 0.3	4.6 ± 0.1	4.6 ± 0.1
Glutamic acid	15.9 ± 0.7	15.9 ± 0.3	15.4 ± 0.5

Glycine	3.6 ± 0.1[b]	3.8 ± 0.1[a]	3.7 ± 0.1[b]
Hydroxyproline	4.4 ± 0.2	4.4 ± 0.0	4.3 ± 0.1
Ornithine	0.4 ± 0.0[b]	0.4 ± 0.0[a]	0.4 ± 0.0[a]
Proline	1.6 ± 0.1	1.6 ± 0.0	1.6 ± 0.0
Serine	5.0 ± 0.3[a]	5.1 ± 0.2[a]	4.8 ± 0.0[b]
Taurine	4.5 ± 0.3[b]	4.8 ± 0.1[a]	4.4 ± 0.1[b]
Tyrosine	3.8 ± 0.2	3.9 ± 0.1	3.8 ± 0.1
Total free amino acids	130.8 ± 4.4[b]	136.4 ± 2.2[a]	133.2 ± 2.1[a]

Values are means ± SD. Within rows, means not sharing a common superscript letter are significantly different ($P < 0.05$) according to one-way ANOVA followed by a Newman-Keuls test.

Table 7

Effects of rainbow trout broodstock and fry nutrition on growth performance of offspring fed different Met levels at a constant water temperature of 17 ± 1 °C for 3 weeks.

	Dietary broodstock group			Dietary fry group			*P* value		
	BD	BA	BE	FD	FA	FE	B	F	B × F
Survival (%)	95.9±0.4^{b}	95.3±0.8^{b}	98.4±0.3^{a}	96.3±0.5	96.6±1.0	96.6±0.6	<0.001	0.493	0.087
Mean body weight (mg)	186±17^{c}	239±15^{a}	219±16^{b}	152±9^{x}	238±9^{y}	254±7^{z}	<0.001	<0.001	0.348
Daily growth index [1]	0.73±0.08^{b}	0.85±0.06^{a}	0.76±0.07^{b}	0.50±0.03^{x}	0.89±0.02^{y}	0.95±0.02^{z}	<0.001	<0.001	**0.022**
Theoretical biomass (g) [1]	17.9±1.7^{c}	22.8±1.4^{a}	21.5±1.6^{b}	14.7±0.9^{x}	23.0±0.9^{y}	24.5±0.6^{z}	<0.001	<0.001	0.122

Values are means ± SEM of nine rearing tanks (three broodstock or fry diets). Within rows and for each diet-related effect: broodstock (B) or fry (F) diet, means not sharing a common superscript letter are significantly different according to two-way ANOVA. Significant broodstock diet × fry diet interactions (B × F) are highlighted in bold type.

[1] Daily growth index was calculated as 100 × [(final mean body weight)$^{1/3}$ – (initial mean body weight at swim-up)$^{1/3}$]/duration (21 days). Theoretical biomass was calculated as the product of survival by the mean weight of 100 larvae.

Table 8

Hepatic gene expression in rainbow trout females fed different Met levels.

Dietary group	BD	BA	BE
BHMT1a	0.5 ± 0.1	1.2 ± 0.3	1.5 ± 0.3
BHMT1b	0.5 ± 0.1^{b}	1.4 ± 0.4^{a}	1.6 ± 0.3^{a}
MTR	1.3 ± 0.2	1.0 ± 0.1	1.0 ± 0.0
CBS	1.2 ± 0.2	1.0 ± 0.1	1.1 ± 0.1
CTH	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
Gclc	1.2 ± 0.1	1.0 ± 0.1	1.0 ± 0.0
GR	1.4 ± 0.1^{a}	1.0 ± 0.1^{b}	0.9 ± 0.1^{b}
GSTπ	3.9 ± 0.3^{a}	1.0 ± 0.1^{b}	1.1 ± 0.3^{b}
MsrA1	1.4 ± 0.1^{a}	1.1 ± 0.1^{ab}	1.0 ± 0.2^{b}
MsrB1	0.9 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
MsrB2	0.9 ± 0.1	1.1 ± 0.1	0.9 ± 0.0

Transcript expression was normalized to EF1α RNA and values are expressed as a ratio of the BA group. Values are means of eight livers ± SEM. Within rows, means not sharing a common superscript letter are significantly different ($P < 0.05$) according to one-way ANOVA followed by a Newman-Keuls test.

Figure captions

Fig. 1. Growth of rainbow trout males (A) and females (B) fed different Met levels over 6 months at a constant water temperature of 8 ± 1 °C. Each point represents means ± SEM (n = 25 females or 10 males). Means not sharing a common superscript letter are significantly different ($P < 0.05$) according to one-way ANOVA followed by a Newman-Keuls test.

Fig. 2. Plasma composition of rainbow trout females fed different Met levels over 6 months at a constant water temperature of 8 ± 1 °C. Bars represent means ± SD (n = 8). Means not sharing a common superscript letter are significantly different ($P < 0.05$) according to one-way ANOVA followed by a Newman-Keuls test.

Fig. 3. SAM and SAH contents of oocytes from rainbow trout broodstock fed different Met levels over 6 months at a constant water temperature of 8 ± 1 °C. Bars represent means ± SD (n = 8). Means not sharing a common superscript letter are significantly different ($P < 0.05$) according to one-way ANOVA followed by a Newman-Keuls test.

Fig. 4. Interacting effects of rainbow trout broodstock and fry nutrition on daily growth index of offspring fed different Met levels at a constant water temperature of 17 ± 1 °C for 3 weeks. Bars represent means ± SD (n = 3). Means not sharing a common superscript letter are significantly different ($P < 0.05$) according to two-way ANOVA followed by a Newman-Keuls test.

Fig. 5. Effects of rainbow trout broodstock (A and B) and fry (C) nutrition on whole-body gene expression related to Met metabolism of offspring at swim-up (A) and fed different Met

levels at a constant water temperature of 17 ± 1 °C for 3 weeks (B and C). Bars represent means ± SEM (n = 10 individuals for the swim-up fry and 27 individuals (nine rearing tanks representing three broodstock or fry diets) for the 3-week fed fry) and are normalized to EF1α RNA and expressed as fold-changes of mRNA abundance compared with the control group BA-FA. Within each stage and each diet-related effect (broodstock or fry diet), means not sharing a common superscript letter are significantly different ($P < 0.05$) according to two-way ANOVA followed by a Newman-Keuls test. Unless otherwise stated in the text, the interaction between broodstock and fry nutrition was not significant ($P > 0.05$).

Fig. 6. Effects of rainbow trout broodstock (A and B) and fry (C) nutrition on whole-body gene expression related to appetite, growth and quality of offspring at swim-up (A) and fed different Met levels at a constant water temperature of 17 ± 1 °C for 3 weeks (B and C). Bars represent means ± SEM (n = 10 individuals for the swim-up fry and 27 individuals (nine rearing tanks representing three broodstock or fry diets) for the 3-week fed fry) and are normalized to EF1α RNA and expressed as fold-changes of mRNA abundance compared with the control group BA-FA. Within each stage and each diet-related effect (broodstock or fry diet), means not sharing a common superscript letter are significantly different ($P < 0.05$) according to two-way ANOVA followed by a Newman-Keuls test. Unless otherwise stated in the text, the interaction between broodstock and fry nutrition was not significant ($P > 0.05$).

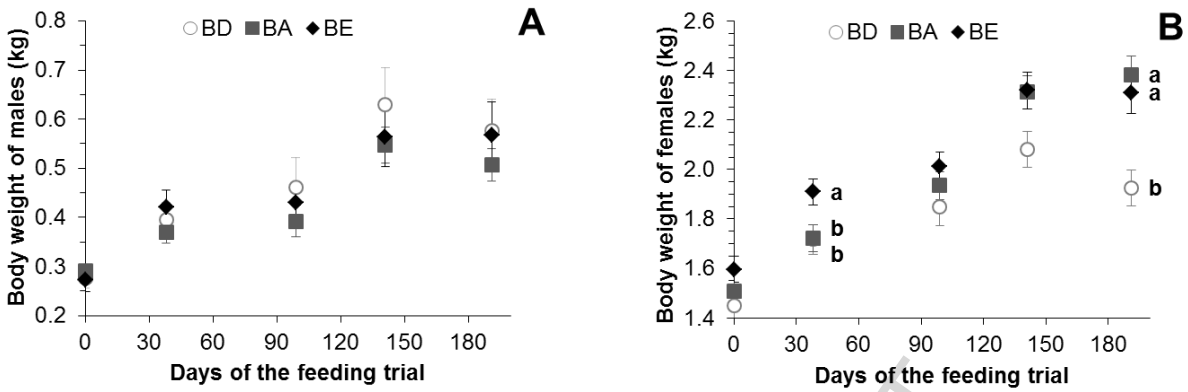

Fig. 1

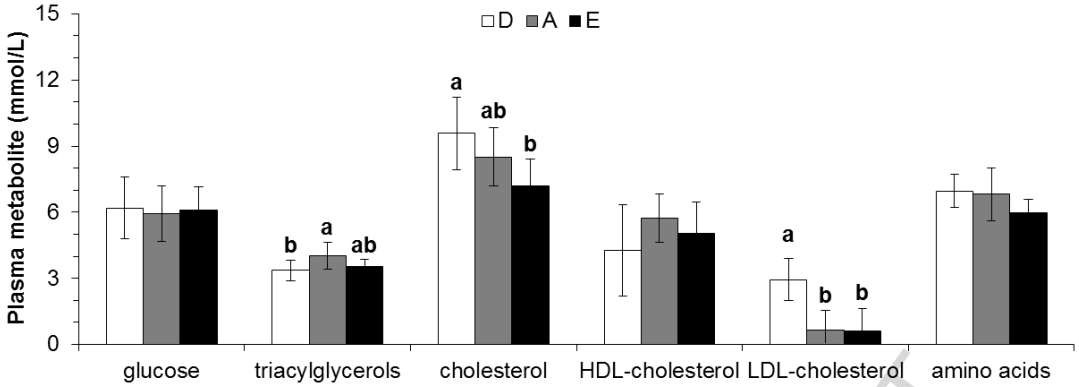

Fig. 2.

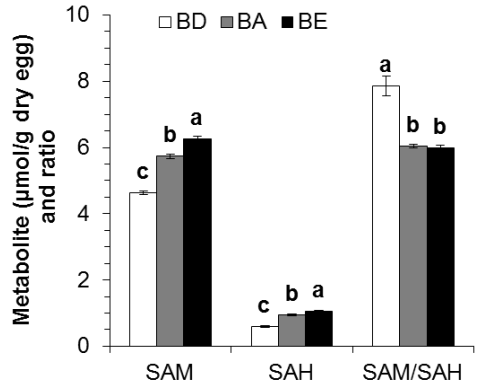

Fig. 3.

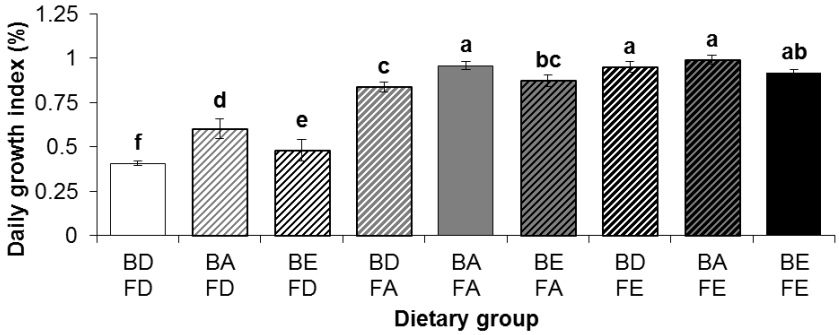

Fig. 4.

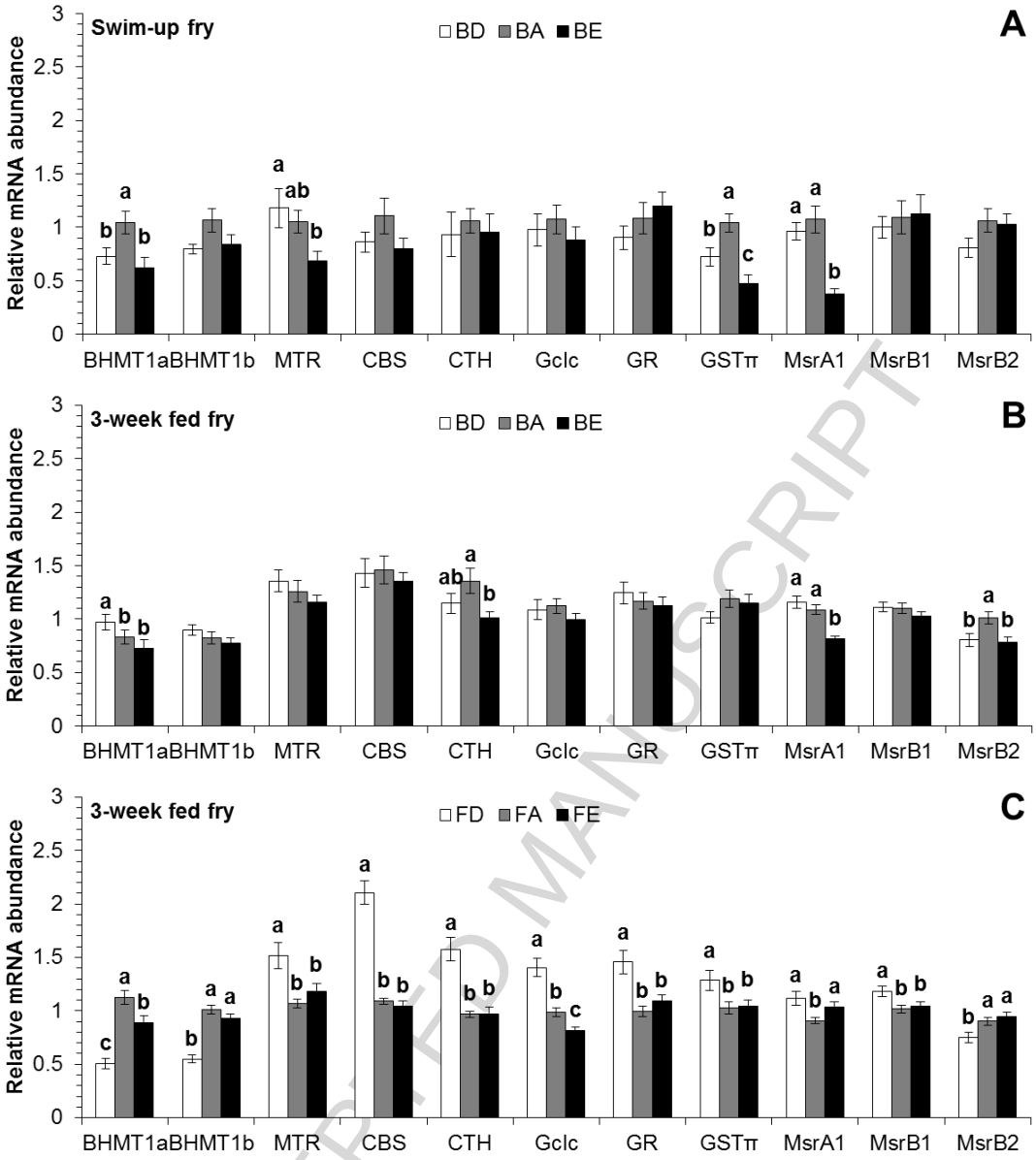

Fig. 5.

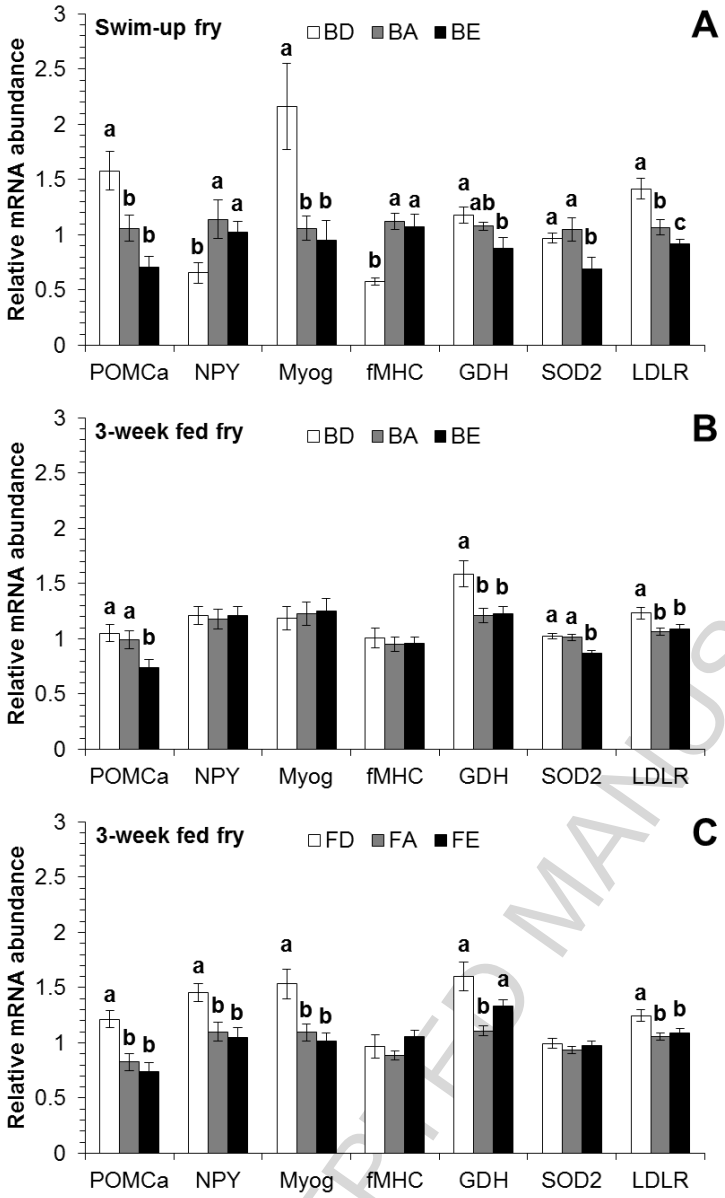

Fig. 6.