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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.

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

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(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

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, 5000 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.