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Dietary methionine supplementation alters the expression of genes involved in methionine metabolism in salmonids

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ABSTRACT

The objective of the present study was to investigate the effect of dietary methionine (Met) concentration and alternating feeding strategies in methionine delivery, on the mRNA transcript levels of genes involved in Met resynthesis (betaine-homocysteine methyltransferase, BHMT; S-adenosylhomocysteine hydrolase, SAHH) and net Met loss (taurine synthesis) (cystathionine beta-synthase, CBS) in Atlantic salmon (*Salmo salar*) liver. Salmon alevins (265 ± 3 mg) were distributed into 24 tanks (50 fish per tank; 3 replicates). The experimental diets were supplemented with L-methionine at 0, 1.9, 5.8, and 17.4 g/kg (M0, M1/3, M1, and M3, respectively). The M3 diet without glycine was prepared to examine Met toxicity (M3-G). These diets were provided via "mono-feeding strategy," meaning fish were fed a designated single diet. This experiment also included alternative feeding groups with "duo-feeding" strategy: AF1 (fish fed M0 for 2 days followed by M1 for 1 day), AF2 (fish fed M0 for 2 days followed by M3 for 1 day), and AF3 (fish fed two meals of M0 followed by one meal of M3). Salmon fed M0 diet had smaller weight compared to all other groups. There was no effect of alternative feeding on the growth except with the M0 group. The highest expression of CBS gene was found in the M0, M1/3, and AF-1 groups compared to the M3 and M3-G groups. The expression of CBS gene in the M1 group was lower compared to the AF-1 group. The expression of SAHH gene was the highest in the M3 group compared to the M1, M1/3, and AF-3 groups. The highest expression of BHMT gene was found in the M0, M1/3, and AF-1 groups, indicating enhanced re-methylation of homocysteine by betaine to Met. The lowest BHMT expression in M1, M3, M3-G, AF-2, and AF-3 groups compared to other treatments can be indicative of downregulation of remethylation in the liver. The alternate provision of methionine in a form of combination of low and high methionine containing diets seemed to support its efficient utilization in the salmon liver.

1. Introduction

Methionine is a source of sulfur required for the synthesis of cysteine and taurine as well as an essential methyl-donor in cellular metabolism. Changes in the metabolism of methionine can influence the production of nutrients essential for proper functioning of the skeletal, cardiovascular, and nervous systems (Miller and Kelly, 1997). A total of 48% of dietary methionine metabolism takes place in the liver (Corrales et al., 2002). The maintenance of methyl groups and homocysteine homeostasis in the hepatic tissue is dependent on the balance between S-adenosylhomocysteine (SAH) and its precursor, a powerful inhibitor of transmethylation reactions, S-adenosylmethionine (SAM). This SAM:SAH ratio can be affected by S-adenosylhomocysteine hydrolase (SAHH) activity, which is involved in the hydrolysis of SAH to homocysteine and adenosine. Homocysteine is a sulfur amino acid metabolite derived from methionine. The homeostasis of homocysteine is dependent on genetic factors and nutrient intake (i.e., folate, vitamin B_6, and vitamin B_{12}), and it can be regulated via conversion of homocysteine back to methionine (remethylation) or transition to cysteine and taurine (trans-sulfuration) in reactions requiring cystathionine β-synthase (CBS). Amino acids such as serine and glycine have been shown to alleviate methionine toxicity by enhancing formation of cystathionine/cysteine and increasing the activity of cystathionine β-synthase (CBS), ultimately removing an excess of homocysteine (Fukada et al., 2006; Kawakami et al., 2009). The remethylation of homocysteine in the liver in the process of Met regeneration can also be affected by dietary sulfur amino acid levels, such as cystine, through increase or decrease of betaine-homocysteine methyltransferase (BHMT).

The intake of certain nutrients, such as polyunsaturated fatty acids, can affect expression of genes involved in methionine/homocysteine

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metabolism (Huang et al., 2013). Ohuchi et al. (2009) suggested that dietary impact on the activity of enzymes, such as CBS and BHMT, are possibly regulated at the level of transcription since the enzyme activity is often paralleled with the increase/decrease in mRNA copy number (Yamamoto et al., 1996).

Therefore, the objective of the present experiment was to investigate the effect of dietary methionine concentration and alternating feeding strategies in methionine delivery, on the mRNA transcript levels of genes involved in methionine metabolism: S-adenosylhomocysteine hydrolase, betaine-homocysteine methyltransferase, and cystathionine beta-synthase in Atlantic salmon (*Salmo salar*) juveniles.

2. Materials and methods

2.1. Feeding trial

Salmon alevins (265 ± 3 mg) were randomly distributed into 24 tanks at a density of 50 fish per tank (3 replicates) and kept at 15 °C–19 °C in a semi-recirculation system. The optimum temperature range for Atlantic salmon at first feeding and for juvenile fish is 16 °C–20 °C (Dwyer and Piper, 1987; Peterson and Martin-Robichaud, 1989). The system had a refreshment rate of 3.3 L per min/440 L. The city water used was filtered through activated charcoal filters and additionally treated with sodium thiosulfate to keep chlorine level at < 0.1 mg/L. The outflow water returning to the system was additionally treated with filtration unit (Aquatic Life Support Filtration Unit, Aquanetics System Pak, San Diego, CA). Each fish tank received a flow rate of 0.3 L per min. The photoperiod was 13 h light: 11 h dark.

A casein-gelatin (CG)-based semi-purified diet was used as the basal diet and was formulated to contain 17.54% casein, 3.51% gelatin, and 30% amino acid mixture (refer to Table 1 for details). The experimental diets were supplemented with L-methionine at levels of 0, 1.9, 5.8, and 17.4 g/kg (designated as M0, M1/3, M1, and M3, respectively). The M3 diet was additionally prepared to examine Met toxicity without glycine (M3-G) (Table 2). These diets were provided to the fish following the "mono-feeding strategy," meaning fish were fed a designated single diet during the entire feeding experiment. This experiment also included three alternative feeding groups with "duo-feeding" strategy. That means that fish were fed two different diets alternatively on daily or hourly basis. Alternative feeding groups included: AF1 (fish that were sequentially fed M0 for 2 days followed by M1 for 1 day), AF2 (fish that were fed M0 for 2 days followed by M3 for 1 day), and AF3 (fish that were fed two meals of M0 followed by one meal of M3). Fish were fed three times a day and feeding rates were restricted to 4.8%–2.0% of biomass during the feeding experiment based on tri-weekly fish weight measurements (Lee, 2013; Storrebaken and Austreng, 1987).

After 9-week-long feeding experiment, liver was immediately collected eight hours after the last meal from three fish per tank, immediately frozen on dry ice, and stored at −80 °C. The weight of the liver taken during the sampling was used for the calculation of hepatosomatic index (HSI); 100 × liver weight/fish weight.

Table 1
Composition of the basal diet.

Ingredients	g/100 g
Casein (vitamin free)	17.54
Gelatin	3.51
Amino acid mixture[a]	30.00
CPSP[b]	4.00
Dextrin	15.56
α-Cellulose	1.79
CMC	2.00
Astaxanthin[c]	0.20
Stay-C 35[d]	0.06
Choline chloride	0.17
Vitamin mix[e]	2.00
Mineral mix[f]	3.00
Cod liver oil	6.06
Soy-lecithin	10.06
Palm oil[g]	4.06
Total	100.00

[a] Fifty-eight percent of protein was replaced from casein and gelatin to free amino acid mixture. The amino acid composition was presented in Table 2.
[b] CPSP , Fish Hydrolysate (SOPROPECHE, USA).
[c] NASX-1, Natural Astaxanthin (15,000 ppm) (JEHMCO Aquatic Breeder Supplies, USA).
[d] Sources were Rovimix series (Aquaculture Research Group, DSM Nutritional Products France, Animal Nutrition & Health Research, Saint-Louis, France).
[e] Vitamin mixture (mg/kg diet) sources were Rovimix series: retinyl acetate, 2.00; cholecalciferol, 0.10; DL-α-tocopheryl acetate, 125.00; menadione niacinamide bisulfite, 5.00; nicotinic acid, 25.00; riboflavin, 20.00; pyridoxine hydrochloride, 15.00; D-calcium pantothenate, 50.00; biotin, 1.00; folic acid, 5.00; cyanocobalamin, 0.05; *myo*-inositol, 500.00.
[f] Mineral mixture: Bernhart Tomarelli salt mixture (MP Biomedicals, Solon, OH, USA) mixed with 5 mg Se in the form of sodium selenite/kg.
[g] Palm oil: Red palm oil (Jungle Products Inc., NJ, USA).

Table 2
Free amino acid mixture (g/1000 g of complete diet).

Amino acids[a]	M0	M1/3	M1	M3	M3-G
Lysine	10.4	10.4	10.4	10.4	10.4
Arginine	8.7	8.7	8.7	8.7	8.7
Histidine	4.1	4.1	4.1	4.1	4.1
Threonine	8.0	8.0	8.0	8.0	8.0
Valine	7.0	7.0	7.0	7.0	7.0
Leucine	8.1	8.1	8.1	8.1	8.1
Isoleucine	5.2	5.2	5.2	5.2	5.2
Methionine	0.0	1.9	5.8	17.4	17.4
Phenylalanine	10.4	10.4	10.4	10.4	10.4
Tryptophan	4.0	4.0	4.0	4.0	4.0
Glycine	20.0	20.0	20.0	20.0	0.0
Aspartate	10.0	10.0	10.0	10.0	10.0
Glutamate	25.6	23.6	19.8	8.2	28.2
Serine	59.5	59.5	59.5	59.5	59.5
Proline	59.5	59.5	59.5	59.5	59.5
Alanine	59.5	59.5	59.5	59.5	59.5
Total	300.0	300.0	300.0	300.0	300.0

[a] All the amino acids are L-form (MP Biomedicals, LLC, Solon, OH).

2.2. Molecular biology analysis

2.2.1. Preparation of total RNA and cDNA synthesis

The nucleic acid analysis for BHMT, SAHH, and CBS were performed in the Department of Biotechnology and Molecular Sciences at University of Insubria, Italy. Total RNA was extracted from all samples of salmon liver using PureYield RNA Midiprep System (Promega, Italy), following the protocol described in Technical Manual #TM279 (average tissue weight 0.089 ± 0.03 g). The quantity of the extracted RNA was calculated by measuring the absorbance at 260 nm, whereas the integrity of RNA was assessed by agarose gel electrophoresis. The purity of RNA was assessed at 260/280 and averaged 2.10 ± 0.01. Crisp 18S and 28S bands, detected by ethidium bromide staining were indicator of the intact RNA.

After extraction, total RNA was reverse transcribed into cDNA in a mix containing oligo dT16 primer and dNTPs. This mix was heated, chilled on ice, and then reverse transcription buffer, DTT, RNaseOUT, and Moloney murine Leukaemia virus reverse transcriptase were added, as described in the M-MLV Reverse Transcriptase kit (Invitrogen).

2.2.2. Cloning and sequencing

To perform PCR, an aliquot of the resulting cDNA was amplified with GoTaq Polymerase (Promega) in a mix containing buffer, dNTPs, and each of the designed RT-PCR primer sets. PCR amplifications were performed for primer sets designed for each gene, using an automated Thermal Cycler (Mycycler, Biorad). The annealing temperatures depended on the melting temperatures of the primer set used. An