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ScienceDirect
Aquaculture 274 (2008) 132–141
Aquaculture
www.elsevier.com/locate/aqua-online

Methionine intake affect hepatic sulphur metabolism in Atlantic salmon, *Salmo salar*

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Received 23 August 2007; received in revised form 24 October 2007; accepted 25 October 2007

Abstract

Atlantic salmon with body weight of 493 g were fed 6 graded levels of methionine in diets based on plant proteins for a period of 85 days with the aim to test whether methionine intake affected growth, nutrient accretion and hepatic sulphur metabolism. A negative control based on a mixture of plant proteins with low fish meal inclusion (5%) containing 1.64 g methionine 16 g^{-1} N was added five levels of DL-methionine resulting in dose levels from 1.64 to 2.98 g methionine 16 g^{-1} N. A control feed based on fish meal (26%) and plant proteins (44.9%) containing 2.30 g methionine 16 g^{-1} N was used as a control for growth performance. Feed intake and thus growth was generally lower in fish fed the plant protein based diets, while digestibility of amino acids was higher in fish fed the test diets as compared to those fed the fish meal based positive control diet. However, no significant differences in either feed intake or growth were present in fish fed either of the test diets containing graded levels of methionine. Neither carcass protein or lipid retention was affected by methionine intake as confirmed by the unaffected mRNA levels of growth hormone-insulin-like growth factor in hepatic and muscle tissues. Hepatic size as well as transsulfuration was significantly affected by methionine intake. Thus it is concluded that nutrient accretion was not the main effect of methionine intake (ranging from 35 to 90 mg $fish^{-1}$ day^{-1}). Rather methionine is essential to secure high synthesis of activated methyl groups for methylation reactions ensuring a healthy fish not developing increased liver size. Intakes exceeding 60 to 70 mg methionine daily in the fast growing seawater period results in increased transsulfuration analysed as increased hepatic taurine production keeping the hepatic free methionine constant at all intakes.

Keywords: Methionine intake; Plant proteins; Hepatic methylation; SAM; SAH; Homocystein; Taurine

1. Introduction

Aquaculture urgently needs to increase knowledge about the effect on growth and metabolism when the marine protein is substituted with sustainable plant proteins. By replacing the well balanced marine protein with plant protein sources, imbalances in IAA's easily arise, as soy the main alternative to marine protein, is low in methionine, but high in cysteine as compared to marine proteins. Additionally the plant proteins are low in the sulphur AA taurine. In fish both excess and restricted dietary methionine has been reported to affect feed intake, growth performance and composition of fish (Jackson and Capper, 1982; Rumsey et al., 1983; Mambrini et al., 1999; Sveier et al., 2001). The methionine requirement of Atlantic salmon has been reported to be in the range of 2.24 to 2.85 g 16 g^{-1} N (Scott, 1998; Sveier et al., 2001), while rainbow trout is reported to require 2.94 g methionine 16 g^{-1} N (Pack et al., 1995; Rodehutscord et al., 1997). The studies, however, did not include a positive control (Pack et al., 1995; Rodehutscord et al., 1997) or suffered from significantly ($P<0.05$) reduced growth in the test groups relative to the positive control (Sveier et al., 2001) which may arise uncertainty about the response used to determine the methionine requirement. Atlantic salmon frequently shows lower growth on

Abbreviations: SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; tHcy, total homocysteine; AA's, amino acids; IAA, indispensable AA's; DAA's, dispensable AA's; TSAA, total sulphuric AA's; IGF, insulin-like growth factor; IGFBP-1, IGF-binding protein-1; GH-R, growth hormone receptor.

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doi:10.1016/j.aquaculture.2007.10.051

purified/semi-purified diets, which can be related to problems with appetite or differences in either absorption or utilisation (Espe et al., 1993; Espe and Lied, 1994; Dabrowski and Guderley, 2002). To avoid this we previously designed a test diet to be used in AA studies resulting in growth not differing ($P>0.05$) from the positive control diet upon substitution of the AA under study in adequate amounts (Espe et al., 2006, 2007). This was achieved by using mixtures of plant proteins low in specific AA's supplemented with squid hydrolysate and fish solubles to improve acceptability and finally balanced with small amounts of the IAA's not under study, but added in low amounts as high levels of free AA's affect growth and protein accretion in Atlantic salmon (Espe and Lied, 1994).

Besides being used for protein synthesis, the main utilisation of methionine is as the donor of methyl groups. Sulphur metabolism mainly occurs in hepatic tissues and in the human liver it has been calculated that 85% of methylation reactions and 48% of methionine metabolism occur in this organ (Mato et al., 1997). The activated methionine, *S*-adenosyl methionine (SAM) is an important methyl donor, present in all living animals. Hepatic tissue besides being the central organ for the synthesis and degradation of SAM also plays a central role in its homeostasis (Friedel et al., 1989; Finkelstein, 1990). SAM is synthesised from methionine by transferring the adenosyl from adenosyl triphosphate (ATP) by the enzyme methionine adenosyltransferase (MAT), enabling SAM to transfer its methyl group to a large variety of substrates including nucleic acids, proteins, phospholipids, biogenic amines (Cantoni and Chiang, 1980; Cantoni, 1982; Mato et al., 1997). Upon donation of the methyl group, SAM is converted to *S*-adenosyl homocysteine (SAH). SAH is a potent competitive inhibitor of hepatic transmethylation reactions, as both an increase in SAH as well as a decrease in SAM or in the ratio of SAM/SAH inhibits the transmethylation reactions within hepatic tissue (Kerr, 1972; Cantoni and Chiang, 1980). Thus one should expect that dietary methionine affects hepatic sulphur metabolism both when present in excess as well as in limited amounts.

The present experiment was conducted to study the influence of methionine intake on growth, nutrient accretion and hepatic sulphur metabolism in Atlantic salmon growing from about 500 g to approximately 1 kg body weight when fed diets supporting growth close to a positive fish meal based control diet and being balanced in the dietary AA's except the methionine ranging from 1.64 to 2.98 g 16 g^{-1} N.

2. Materials and methods

2.1. The diets

Six diets were prepared in which one was without any addition of crystalline methionine (Diet 1, the negative control), while Diets 2 to 6 were added 0.12, 0.24, 0.36, 0.53 and 0.72 g crystalline DL-methionine 100 g^{-1} diet, respectively. The respective dietary methionine 16 g^{-1} N was 1.64, 1.84, 2.20, 2.34, 2.80 and 2.98 g. The positive control diet used to validate growth data was based on fish meal and mixed plant proteins and contained 2.30 g methionine 16 g^{-1} N. To all diets, 0.1 g yttrium oxide kg^{-1} was added to allow calculation of digestibility.

Seven mm dry extruded pellets containing 31 and 42 g lipids and protein 100 g^{-1} diet, respectively, were made. The compositions and chemical analyses of the experimental diets are given in Tables 1 and 2. Each experimental diet was fed to three (Diets 1, 3 and 6) or two (Diets 2, 4 and 5) replicate tanks. The positive control diet was fed to triplicate tanks.

2.2. The fish experiment

Fifty Atlantic salmon (*Salmo salar*) with a mean body weight of 493±19 g was used in each of 18 tanks. The experiment was conducted as earlier described (Espe et al., 2006). In short each tank was supplied by running seawater (salinity of 33 g L^{-1}, temperature 8–9 °C) and fish were reared under continuous light regime. Each tank was randomly assigned to the experimental diets, and the fish were fed to apparent satiation three times daily using an automatic feeding system. To all tanks feed collectors were connected to measure the actual feed intake. At the start of the experiment fish were bulk weighed and fish used for crude chemical composition were sampled. The fish were fed the experimental diets for a period of 85 days. At the end of the growth period, the fish were bulk weighed and pooled samples of faeces and fish used for analyses of crude composition were collected. No starvation was used prior to sampling of faeces, but prior to sampling of whole fish 2 days feed deprivation was adopted. The anaesthetic used prior to weighing was chlorobutanol (0.4 g L^{-1}). The experimental protocol was approved by the Norwegian Board of Experiments with Living Animals.

2.3. Sampling procedures

At the start of the experiment a pooled sample of 10 fish was analysed for its crude chemical composition, representing the chemical composition of all the fish, while at the end of the experiment 10 fish were pooled from each experimental tank. Pooled samples of faeces were collected from each tank by stripping. The remaining fish in each tank were fed their respective diets for another week to ensure tissue and blood samples of a normally metabolising

Table 1
Proximal composition (g kg^{-1}diet) of the experimental diets used

	Experimental diets	
	Control diet	Test diets ($n=6$)
Composition		
Fish meal	260	50
Fish soluble C.P.S.P	–	50
Wheat gluten	80	238
Corn gluten	140	–
Soybean meal	123	–
Soy protein concentrate	–	11
Squid hydrolysate	–	30
Amino acid mix [a]	–	68
Wheat grain	106.4	222.4–229.4
Yttrium oxide	0.1	0.1
Micronutrients [b]	18.5	43.5
DL-methionine	–	0–7
Fish oil	272	280
Chemical composition		Mean±SE
Dry matter	916	932±1.9
Crude protein	433	429±2.0
Crude fat	274	298±2.3
Ash	60	51±3.3
Carbohydrate	149	153±4.1
Energy (MJ/kg)	24.0	24.3±0.1

Wheat grain was exchanged with crystalline DL-methionine at 6 levels (0, 0.12, 0.24, 0.36, 0.53 and 0.72 g, see Materials and methods).

[a] AA mix (% of mixture): Biolysine60 25.74, L-Trp 1.76, L-Ile 6.47, L-His 5.88, L-Val 14.71, L-Leu 20.44, L-Arg 11.76, L-Thr 7.35, L-Phe 5.88. Carbohydrate calculated by the difference. Biolysine60 is composed of 47.3% Lys, 0.15% Trp, 0.5% Ile, 1.2% Val, 0.8% Leu and 1% Arg.

[b] Vitamins and minerals to fulfil the requirement for Atlantic salmon as given by NRC (1993).