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ScienceDirect
Aquaculture
Aquaculture 263 (2007) 168–178
www.elsevier.com/locate/aqua-online

Assessment of lysine requirement for maximal protein accretion in Atlantic salmon using plant protein diets

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Received 6 September 2006; received in revised form 16 October 2006; accepted 16 October 2006

Abstract

Two trials (I and II) were conducted to finally determine the Lys requirement in the Atlantic salmon during the fast growing period in seawater. First three test diets with well balanced AA's as well as a IAA:DAA ratio close to 1, but differing in amount and source of an attractant, were developed allowing for both a reduction of dietary Lys and a reasonable performance when supplementing Lys. The diets were based on our previous study in which all fish meal was replaced by plant proteins, of which resulted in both inferior body weight gain and lipid deposition ([Espe, M., Lemme, A., Petri, A., El-Mowafi, A. 2006. Can Atlantic salmon grow on diets devoid of fish meal? Aquaculture 255, 255–262]). Basically, the amino acid profiles of all test diets did not differ ($p>0.05$) from the control fish meal based diet although 90% of the fish meal was replaced by plant proteins. The diets were fed to Atlantic salmon of BW 327 g for a period of 85 days. The test diet supporting both growth and deposition of protein and lipid not differing ($p>0.05$) from the fish meal control finally consisted of plant proteins supplemented with 5% fish meal, 5% fish soluble and 3% squid hydrolysate. This diet then was used in a dose response experiment aiming to determine Lys requirement in the fast growing salmon. Atlantic salmon with BW of 642 g were fed graded amounts of Lys (2.85 to 9.19 g Lys/16 g N) for a period of 85 days. Dietary Lys did not affect growth ($p>0.05$), but the protein accretion suggested an optimum dietary Lys supply of 5.04 g /16 g N (corresponding to 0.12 g Lys/fish/day). This response was accompanied with reduced fat accretion. To obtain weight gain to the level present in the fish meal control diet 3.17 g Lys/16 g N was sufficient. Thus lean growth rather than body weight gain should be the response parameter to determine Lys requirement in salmon.

Keywords: Lysine requirement; Amino acids; Test diet development; Atlantic salmon; Plant proteins; Protein accretion

1. Introduction

Dose response experiments generally are used to determine AA requirement in fish (Robbins et al., 1979; Cowey, 1992; Pack et al., 1995; Rodehutscord et al., 1997). Ideally the only ingredient to vary within such experiments should be the AA being investigated, while all other substances should be kept constant. Further, the test diet used should allow for similar growth compared to a traditional control diet, when the tested AA is added in adequate amounts. As a consequence such experimental diets should allow for normal feed intake because this will of course affect fish performance.

Atlantic salmon (*Salmo salar*, L.) being a strict carnivore fish naturally grows on fish meal based diets

Abbreviations: AA's, amino acids; IAA's, indispensable amino acids; DAA's, dispensable amino acids; BW, body weight.

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doi:10.1016/j.aquaculture.2006.10.018

which are sufficient in all IAA's as well as DAA's (Njaa, 1990) and which are well accepted by the salmon resulting in good growth performance. As fish meal diets cannot be made sufficient low in the IAA's, fish meal cannot be used as the main protein source in experiments aiming to determine AA requirements or to study the AA metabolism. One strategy to avoid this is to produce diets low in fish meal thus deficient in the AA to be tested which is then gradually supplemented with crystalline AA's. Often also other AA's have to be supplemented. Main drawback of this strategy is that feed intake and thus overall performances might be adversely affected by using diets with high amounts of crystalline AA's. It also has been postulated that due to different absorption peaks of crystalline AA's compared to those of protein bound AA utilisation of dietary AA for anabolic purposes is impaired (Plakas et al., 1980; Murai et al., 1982; Cowey and Walton, 1988; Espe et al., 1993, 1999; Berge et al., 1994). Another strategy is to choose protein sources sufficiently low in the AA to be studied enabling to make diets from 2 times below to 2 times above the anticipated requirement. This strategy was applied by Berge et al. (1997, 1998), in studying the requirement of Lys and Arg in Atlantic salmon but survival, feed intake and growth were severely affected as compared to the control fish meal diet. Another methodology is to coat the AA, as used by De la Higuera et al. (1998) which used corn gluten that is low in Lys and studied the effect of adding crystalline or coated Lys in carp diets. Although coating Lys improved the growth it still was lower than in the reference diet.

Generally, reduced growth might be due to anti-nutritional components in the protein sources in the test diets, low digestibility of such proteins, or bad palatability resulting in lowered feed intake. Previously, we fed Atlantic salmon with diets consisting of plant proteins added 5% fish hydrolysate and either stick water (5%) or squid hydrolysate (3%) but no fishmeal. Diets were balanced in AA's as well as using a ratio of IAA's to DAA's close to 1, by adding low amounts of crystalline AA's to simulate the AA profile of the fish meal control diet not to jeopardise utilisation of AA's (Espe et al., 2006). The salmon accepted the feed, but feed intake and subsequently weight gain were still lower ($p<0.05$) as compared to those fed the control fish meal based diet. Even though the protein gain was equal to the control fed fish ($p>0.05$), the lipid gain was less ($p<0.05$). Therefore, no diet to be used in requirement studies in Atlantic salmon as well as in studies of the AA-metabolism allowing for equal feed intakes and equal protein and lipid gain as in a fish meal reference diet exist. The present study thus aimed to develop a test diet based on our previous obtained results (Espe et al., 2006) that can be made low in IAA's and that allows for acceptable feed intakes and growth when the AA under study is added to the experimental diet. The ability of the developed test diets to support growth was then tested in a dose response experiment with salmon of higher BW to determine the requirement of Lys in the fast growing seawater period.

2. Material and methods

2.1. Trial I: development of the test diet

In a previous experiment diets devoid of fish meal resulted in impaired feed intake (13%) and, consequently, growth (10%) compared to a fish meal control in Atlantic salmon (Espe et al., 2006). Therefore the present diets were supplemented with 5% fish meal plus 5% fish solubles. Apart from this the diets were formulated similarly as previously described by Espe et al. (2006): Diet 1: control based on fish meal, Diet 2: based on plant protein sources with only 5% fish meal, Diet 3: as diet 2, but supplemented with 5% stick water and Diet 4: as diet 2, but supplemented with 3% squid hydrolysate (Table 1) as we previously found both the stick water and the squid hydrolysate to improve feed intakes. Calculated total dietary Lys from raw materials without supplemented Lys in diets 1 through 4 was 3.08, 1.10, 1.33, 1.24 g/100 g diet. Test diets (Diets 2–3) were supplemented with crystalline IAA's to the level present in the control fish meal based diet, but low levels of crystalline AA were added not to jeopardise utilisation of the AA's. To all diets, 0.1 g/kg yttrium oxide as inert indicator was added to allow for calculation of digestibility. All dry ingredients were ground, mixed and extruded using Wenger X-85 extruder. The extruded feed was dried and the oil was added in a vacuum coater. Due to the high gluten content, feed did not expand enough to absorb all the added oil. This explains the lower lipid and higher protein analysed values (Tables 1 and 3). Analysed dietary AA compositions in the test diets and the control diet are given in Table 2. Each experimental diet was fed to three replicate tanks each containing 40 fish with mean BW 330±6 g. Tanks were equipped with feed collectors to measure the actual feed intake as previously described (Espe et al., 2006). The experimental protocol as described in Espe et al. (2006) was adopted. In short cylindrical fibreglass tanks with water volume of 0.5 m^3 supplied with running seawater (30 g/L, temperature 8±1 °C and flow rate of 0.8 L/kg body mass/min) was used. Fish were fed three times daily and a continuous light regime was used. At the start of the experiment fish was bulk weighed, before ten fish were used for chemical composition of the