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Dietary supplementation of glutamate and arginine to Atlantic salmon (*Salmo salar* L.) increases growth during the first autumn in sea

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ABSTRACT

During the sea water phase, Atlantic salmon is exposed to shifting environmental conditions affecting the physiology and metabolism of the fish. Strategic dietary supplements may enable the fish to sustain a higher growth rate during particular periods. In this experiment, a combination of two amino acids (L-arginine and L-glutamate, with supplementation levels 1.1% and 0.75%, respectively) was used as dietary supplements since earlier observations have indicated that these amino acids have versatile functions, influencing growth, reproduction and the immune response in animals. In the case of arginine, the control diet was set at assumed requirement level, while the test diet was supplemented further. The effects of this supplementation on feed intake and growth parameters were studied, as well as selected organ weight, biochemical, molecular and hormonal indicators. During the first experimental period (May–July), few significant effects were found. However, in the second period of the experiment (July–September), a higher specific feeding rate (SFR), thermal growth coefficient (TGC) and specific growth rate (SGR) ($p<0.05$) occurred in fish fed the supplemented diet, together with a trend ($p<0.1$) for increased final body weight. The measured gene expression levels of Insulin-like growth factor (IGF) and growth hormone receptor gene did not correlate with the increased growth observed during the second period in fish fed the supplemented diet. Furthermore, no difference in total plasma IGF-I concentrations was found after the first period. After the second period, fish fed the supplemented diet had in fact a significantly lower plasma IGF-I level. Additionally, we found a higher relative weight of gastrointestinal sections and elevated levels of plasma arginine, urea and ornithine after the second period in fish fed the supplemented diet. We conclude that dietary supplementation of L-arginine and L-glutamate significantly increased feeding rate and growth during the second feeding period in autumn. This response appeared to occur when the two conditions of rapidly decreasing day lengths and high feed intake, were met.

1. Introduction

The seawater phase is the most value-adding phase during grow-out of farmed Atlantic salmon. However, during this phase, the fish in sea is exposed to shifting environmental conditions during the seasons. Especially temperature and photoperiod vary considerably, as experienced along the Norwegian coast. These are dramatic changes affecting the physiology and metabolism of the fish. Effects of seasonal variation on growth rate, feed utilization and product quality have been documented (Thorpe et al., 1989). In particular, the period after sea transfer of 1+ smolt in spring, and the first spring following sea transfer for 0+ smolt in the autumn, are periods that should be focussed on. These periods are characterized by decreased feed intake, low energy retention, decrease in body energy level and condition factor (Alne et al., 2010); often this situation is complicated by disease outbreaks (Alne et al., 2009).

Strategic dietary supplements may improve the fish's performance during these periods (Burrells et al., 2001a,b; Rørvik et al., 2007; Alne et al., 2009). In this context, specific amino acids are some of several candidate supplements. There is growing evidence that in addition to being building blocks, amino acids have specific functions, acting as metabolic regulators necessary for maintenance, growth, reproduction and immune response (Meijer, 2003; Li et al. 2009). Some of the amino acids with the most versatile functions are the glutamate family, in

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particular arginine, glutamate, and glutamine. Arginine is a precursor of compounds such as nitric oxide, polyamines, proline, ornithine, creatine and agmatine which are important in cell proliferation, signalling, and growth regulation (Wu and Morris, 1998; Flynn et al, 2002; Ball et al., 2007; Morris, 2007). In fish, the release of growth hormone, Insulin-like growth factor-I (IGF-I), and other hormones can be stimulated by arginine (Plisetskaya et al., 1991; Baños et al, 1999; Mommsen, 2001). Unlike arginine, glutamate and glutamine are thought to be dietary dispensable in fish. Glutamate and glutamine have central metabolic roles in mammals and are interconvertible (Neu et al., 1996; Young and Ajami, 2001; Tapiero et al., 2002). In fish, this interconversion is critical for ammonia homeostasis (Anderson, 2001; Terjesen, 2008). Glutamate is also a precursor for gamma amino butyric acid (GABA) and for purine and pyrimidine nucleotides (Neu et al., 1996; Tapiero et al., 2002; Li et al., 2009). Furthermore, glutamate and glutamine are important energy sources in the intestine (Neu et al., 1996). Regarding fish, Yan and Qiu-Zhou (2006) tested the effect of dietary glutamine supplementation on growth performance, intestinal structure and function in juvenile Jian carp. Weight gain, feed efficiency, intestinal development and enzyme activities were found to be enhanced up to a supplementation level of 1.2% in the diet. The authors (Yan and Qiu-Zhou, 2006) suggested that glutamine is responsible for these positive effects by serving as a precursor in nucleotide biosynthesis in rapidly replicating cells and as an important energy substrate in enterocytes. To our knowledge these potential beneficial effects of dietary glutamine supplementation have not been studied in Atlantic salmon.

Given these vital functions of arginine and glutamate, a feeding trial was done using the amino acids as supplements for Atlantic salmon during the first period in sea cages. The main objective of the experiment was to test the effects on feed intake, growth, feed conversion ratio (FCR) and nutrient retention of a combined arginine/glutamate supplement, added in a commercial diet and to a level above assumed dietary arginine requirements (16–22 g/kg DM: Lall et al., 1994; Berge et al., 1998; Rollin et al., 2003). The trial was performed with Atlantic salmon smolts in a 6 month period after sea transfer, to be as close as possible to industry-realistic environmental conditions, including natural photoperiod. Glutamate was chosen as a supplement instead of glutamine due to the thermal instability of the latter (e.g. Sowden et al., 2002). In addition, the experiment included several other parameters to improve the understanding of any observed effects in the production parameters. Relative gut weight measurements were done to assess if salmon fed this diet respond similar to the situation in carp, where supplemented glutamine increased gut weight and proliferation (Yan and Qiu-Zhou, 2006). Measurements of growth hormone (GH)-receptor expression and IGF-I were used to study if the test diet resulted in GH-IGF axis changes as has been reported before for arginine injections (Plisetskaya et al., 1991; Baños et al, 1999; Mommsen, 2001). Furthermore, we included insulin-like growth factor binding proteins (IGFBPs), since they may modulate the action of IGF by controlling the availability of free IGF to its receptors in the target tissues (Duan, 2002). Finally, we wanted to study how the arginine and glutamate supplement affected their plasma free pools, and two points in their catabolic pathways by measuring plasma free amino acids, and liver and muscle expression of arginase and glutamate dehydrogenase (GDH).

2. Material and methods

2.1. Fish and facilities

The experiment was done at the Nofima Marin sea water research station at Averøy, in North-Western Norway. Atlantic salmon hatched at the Nofima Marin research station at Sunndalsøra, Norway, 1 year earlier were used. The trial lasted from transfer to sea in May 2007 until September 2007. The fish were stocked in 6 net pens (5×5×5 m, 125 m^3 volume each) with 400 fish per pen, and the dietary treatments were randomly assigned to triplicate cages each. The mean body weight of the fish at the start of the experiment was 106±3.3 g (mean±SD, $n=6$; six cages).

2.2. Diets and feeding

Two extruded diets, of which diet E was supplemented with L-glutamate (0.75%) and L-arginine (1.1%) (Degussa, Germany), were produced by Biomar AS (Table 1). The diets C and E were changed between the periods, since the period 2 diets were better optimized for large salmon following the first period after stocking (Einen and Roem, 1997). The amino acids were added in the feed mix prior to the pellet extrusion process. The diets were not designed to be isonitrogenous, due to the low amounts of supplemented amino acids, and as a matter of fact, the differences in N content being within analytical error (Table 1). Furthermore, we avoided using a replacement nitrogen compound in the control feed in order not to introduce a confounding factor. The fish were fed by automatic feeders to 10–20% overfeeding, four times per day. Uneaten feed was collected immediately after each meal and pumped up into wire mesh strainers as described by Einen et al. (1999). Each diet was tested for recovery of dry matter under the environmental conditions present during the experiments as described by Helland et al. (1996), and the weight of uneaten feed recorded was corrected for dry matter losses during feeding and collection.

2.3. Samplings and recordings

The fish were counted and bulk weighted at the beginning of the experiment (mid May 2007, called sampling point E1), after 9 weeks on experimental diets (mid July 2007, sampling point E2), and at the final sampling 19 weeks after the start of the experiment (end of September 2007, E3). To ensure safe sampling, the E2 sampling was done 9 weeks after starting the experiment based on historical data on sea temperatures.

Blood and tissue samples were collected from 9 fish per cage on each sampling point, from fish representing the cage mean with regard to body weight (e.g. Rørvik et al, 2007; Alne et al, 2009). The fish were anesthetized by MS 222 (metacaine, 0.1 g L^{-1}; Alpharma Animal Health Ltd), and blood samples were collected from the caudal blood vessel using EDTA vacutainers. The fish were subsequently killed by a blow to the head. The blood samples were centrifuged at 4 °C and 630 ×g for 10 min, and equal volumes of plasma from three fish were pooled in each of the replicate cryotube, flash-frozen in liquid nitrogen and then stored at −80 °C. Samples of muscle and liver tissues were prepared and flash-frozen in liquid nitrogen and stored at −80 °C for later RNA extraction.

To analyze proximal chemical composition, additional three pooled samples per cage, each from five fish, were prepared by dissecting the fish into carcass, liver and viscera samples. Individual body weight and length were recorded for all sampled fish. Hepatosomatic index (HSI) was calculated from pooled liver samples as: ((pooled liver weight/5)/average body weight) × 100. At sampling point E3, three fish from each cage were dissected and weights of the gastrointestinal tract (GIT) were taken after removing the intestinal contents and fat tissue. The GITs were separated into sections: stomach, pyloric intestine, mid intestine and distal intestine and the weights of these segments were recorded and related to the body weight of the same fish.

2.4. Quantitative PCR

Total RNA from muscle and liver tissue samples was extracted from 12 fish per dietary treatment at sampling points E2 and E3 (total of 48 fish). Extraction was done by using TRIZOL Reagent (Invitrogen)