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Fillet quality and health of Atlantic salmon (*Salmo salar* L.) fed a diet supplemented with glutamate

Thomas Larsson [a,b,*], Erling Olaf Koppang [c], Marit Espe [d], Bendik Fyhn Terjesen [a], Aleksei Krasnov [a], Helena Maria Moreno [e], Kjell-Arne Rørvik [a,b], Magny Thomassen [a,b], Turid Mørkøre [a,b]

[a] Nofima, N-1431 Ås, Norway
[b] Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, N-1432 Ås, Norway
[c] Norwegian School of Veterinary Science, N-0033 Oslo, Norway
[d] National Institute of Nutrition and Seafood Research, N-5004 Bergen, Norway
[e] Products Department, Institute of Food Science Technology and Nutrition, ICTAN, CSIC, 28040 Madrid, Spain

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ABSTRACT

Atlantic salmon were fed a standard extruded dry feed (Control) or the same feed supplemented with 1.5% L-glutamate (Glu) (triplicate net pens per diet) from May 2009 (body weight = 105 g) to May 2010 (body weight = 3.1 kg). No significant differences were observed in growth (TGC = 3.1) or feed conversion ratio (1.0) between the dietary treatments. Instrumental texture analyses showed that Glu supplementation resulted in significantly ($P \leq 0.05$) firmer fillets after ice storage (10.1 vs. 9.1 N) and after frozen storage (8.7 vs. 6.3 N). Additionally the Glu group had less organ adhesions (score = 0.5 vs. 1.1), lower hepato-somatic index (0.91 vs. 0.99%) and less fat accumulated in the livers (1.8 vs. 2.1 g). The condition factor, carcass and fillet yield, and cardio- and spleen-somatic indices were unaffected by dietary treatment. Hepatocellular vacuolization, intestinal inflammation and muscle degeneration were observed in the Control (50, 13, and 5%, respectively) and the Glu group (40, 7, and 25%, respectively). No abnormal observations were found in the spleen or kidney. Plasma analyses revealed significantly lower activity of creatine kinase (3.3 vs. 5.8 U/mL) and alanine aminotransferase (5.8 vs. 7.5 U/L) in the Glu fed group. Muscle pH was significantly higher in the Glu group (6.22 vs. 6.19), but the fat-, protein-, amino acid-, and collagen contents were similar. The Glu group had significantly higher concentrations of saturated fatty acids and docosapentaenoic acid (1.9 vs. 1.7 and 17.4 vs. 17.1% of fatty acids, respectively), and $n-3$ fatty acids and $n-3/n-6$ ratio in the muscle tended to be higher ($P < 0.09$). Collagen properties determined as degree of glycation, solubility, thermal behaviour, pyridinoline bonds and structure were similar for both dietary groups. Compared with the Control, the skeletal muscle of salmon fed the Glu supplemented diet showed up-regulation of genes involved in stress response, mitochondrial functions, and amino acid and lipid metabolism, whereas several genes involved in cytoskeletal structure were down-regulated. Glu supplementation resulted in firmer fillets, coinciding with altered energy metabolism and improved health related parameters. It is suggested that optimal dietary amino acid levels for growth may differ from optimal levels for good fish health and flesh quality.

1. Introduction

Fillet firmness of farmed Atlantic salmon is an important quality trait for consumer acceptance and soft fillets are downgraded by the processing industry (Ando, 1999; Michie, 2001; Veland and Torrissen, 1999). Texture is influenced by both ante- and post-mortem factors (Hyldig and Nielsen, 2001). Ante-mortem factors affecting fillet texture include genetic background (Gjøen and Bentsen, 1997; Larsson et al., 2012a), feed and feeding (Einen and Thomassen, 1998), environmental factors (Johnston, 2008) and health status (Larsson et al., 2012b; Lerfall et al., 2012). The present study focuses on the impact of dietary composition with special emphasis on supplementation of the amino acid glutamate (Glu).

Amino acids are important for maintenance, growth, reproduction and immune response (Li et al., 2009). Furthermore, catabolism of amino acids is more important for energy dissipation in fish than in terrestrial animals (Panserat and Kaushik, 2010). Protein and amino acid

* Corresponding author at: Nofima, PO Box 210, 1431 Ås, Norway. Tel.: +47 64970100.
E-mail addresses: thomas.larsson@nofima.no (T. Larsson), erling.o.koppang@nvh.no (E.O. Koppang), marit.espe@nifes.no (M. Espe), bendik.terjesen@nofima.no (B.F. Terjesen), aleksei.krasnov@nofima.no (A. Krasnov), hmoreno@ictan.csic.es (H.M. Moreno), kjell-arne.rorvik@nofima.no (K.-A. Rørvik), magny.thomassen@umb.no (M. Thomassen), turid.morkore@nofima.no (T. Mørkøre).

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requirements for maximum growth in salmon have been studied previously (NRC, 2011; Wilson, 2002), but there is limited knowledge on the effect of dietary amino acid levels on flesh quality. Glu is a non-essential metabolically versatile amino acid (Brosnan, 2000; Neu et al., 1996) that affects the oxidation of cytoplasmically produced nicotinamide adenine dinucleotide (NADH) in many cells (Minarik et al., 2002), has an anaplerotic function in the tricarboxylic acid (TCA) cycle (Brosnan, 2000; Gibala, 2001), and serves as a signalling agent between the immune and nervous systems (Boldyrev et al., 2005). Additionally, Glu is a key transamination partner and is required for the synthesis of glutathione, an important component in the defence against oxidative stress (Amores-Sanchez and Medina, 1999; Johnson et al., 2003). Furthermore, Glu is a vital substrate for the ammonia detoxification mechanism in nervous tissues in salmon (Kolarevic et al., 2012). However, few studies have investigated the effect of Glu supplementation in salmon diets, although during the period of decreasing day length and high feed intake, Oehme et al. (2010) observed increased feeding rate, growth, and gut weight, of salmon fed a diet supplemented with a combination of arginine and Glu.

A recent transcriptome study of salmon with known pedigrees revealed novel strong positive correlations between firmness of salmon fillets and both the expression of genes coding for proteins involved in aerobic energy metabolism and expression of immune genes (Larsson et al., 2012a). Hence, a holistic approach is required to determine underlying causes of deviating firmness of salmon fillets.

The focus of the present study was to investigate the dietary effect of supplementing salmon feed with Glu on fillet firmness and parameters related to fish health and energy metabolism. Analyses included mechanical measurements of texture, composition of liver and muscle, histology of different tissues, transcriptome profiling of muscle and biomarkers commonly used as health indicators. Additionally, biometric parameters were recorded.

2. Materials and methods

2.1. Fish and sampling

Atlantic salmon (*Salmo salar* L.) smolts (105 g) from Salmar ASA, Norway were transferred to seawater cages (125 m^3) in April 2009 at Nofima research station at Averøy, Norway (500 fish per cage). The fish had been vaccinated in March of the same year (MINOVA 6 Vet., Intervet Norbio, Bergen, Norway). The fish were fed a basis dry feed as control diet, or the same feed supplemented with L-glutamate (Glu), from May 2009 to May 2010. Diets were fed to randomly distributed cages, in triplicate. Sea temperature was logged daily and was 8 °C in May of both years, with the highest and lowest temperatures registered in August 2009 (16 °C) and February 2010 (3 °C), respectively (average temperature was 8.7 °C).

At harvest in May 2010 (average body weight = 3.1 kg), the fish were anaesthetized (MS 222 metacaine, ALPHARMA, Animal Health Ltd., Hampshire, UK, 0.1 g/L) in batches of 10 fish in a 1000 litre tank with fresh seawater (8 °C) before blood sampling. Thereafter, they were bled to death for 20 min in another tank with seawater after sectioning the left gill arches. The fish sampled for analyses from each net pen (n = 10) were selected randomly among fish representing the average body weight. The salmon were weighed (whole body and gutted weight) and fork length was registered. Organ adhesions were classified according to a standardized scoring system by using a scale from 0 to 6, where 0 equaled no adhesions and 6 the highest possible degree of adhesions (Midtlyng et al., 1996). The weight of the liver, heart, and spleen was recorded for calculation of organ indices (% of body weight). Liver samples were stored at −20 °C and −80 °C for later analyses of fat and free amino acids, respectively. Gaping was scored immediately after filleting. Various organs and white muscle were sampled for histological examination. White muscle was sampled for analyses of free amino acids, fat, and gene expression. The left fillets were weighed, packed in Styrofoam boxes with ice and transported to Nofima (Ås, Norway) and analyzed five days post-mortem for texture, gaping and pH. At day five, a cutlet from each fillet anterior to the dorsal fin was sampled, stored in a sealed plastic bag on ice for one week, and then analyzed for texture on day 12. Both at day zero and day five, the Norwegian quality cut (NS 9401, 1994) was frozen at −20 °C and used for analysis of collagen properties, as well as texture after six weeks of frozen storage (day five). See Fig. 1 for an overview of sampling locations and time of sampling for the various analyses performed.

2.2. Diets and feeding

The experiment was carried out using two diets, where the basis feed was a standard dry feed (Skretting AS, Norway), formulated to meet known nutritional requirements of salmonid fish (NRC, 1993). The feed was first coated with 3.6% distilled water (Control), in which step the experimental diet (Glutamate) was added with 1.5% L-glutamate (Glu, Meihua Holding Group Co., Ltd., Hebei, China) dissolved in the water (70 °C, 0.43 kg/L). Both feeds were then top-dressed with rapeseed oil (20 mL/kg) to eliminate possible taste differences. The Glutamate diet had 22% higher Glu content compared with the Control (Table 2). Pellet size was adjusted according to size of fish and was 9 mm during the last five months of the trial.

The fish were fed in excess of recorded feed intake four times per day with automatic feeders. Uneaten feed was collected immediately after each meal as described by Einen et al. (1999). Each feed was tested for recovery of dry matter under the environmental conditions present during the experiment (Helland et al., 1996), and the weight of uneaten feed registered was corrected for dry matter losses during feeding and collection. Protein, crude fat (Folch et al., 1957), total amino acids, dry matter, ash, starch and energy were analyzed as described by Oehme et al. (2010). The formulation and composition of the control feed given to the fish from December 2009 to May 2010 (Optiline V 2500-20A 9) are shown in Tables 1 and 2.

2.3. Texture, gaping and pH

Texture analyses were performed instrumentally (TA-XT2, Stable Micro Systems Ltd., Surrey, England) by pressing a flat-ended cylinder (12.5 mm diameter, type P/0.5) into the fillet perpendicular to the muscle fibres at 1 mm/s (Fig. 1). The instrument was equipped with a 30 kg load cell and the trigger force was 9 g. The force (Newton, N) required to puncture the fillet surface was registered from the resulting time–force curve.

Fillet gaping was assessed using a scale from 0 to 5, where 0 indicated no gaping and 5 extreme gaping (Andersen et al., 1994).

Muscle pH was analyzed using a pH meter [330i, Wissenchaftlich-Techniche Werkstätten GmbH (WTW), Weilheim, Germany] connected to an electrode (BlueLine 21, Schott Instruments Electrode, SI Analytics GmbH, Mainz, Germany) and a temperature probe (TFK 325, WTW).

2.4. Chemical composition and analyses of collagen

Protein was analyzed in white skeletal muscle using the Kjeldahl total nitrogen method (Commission dir. 93/28/EEC), and free amino acids were analyzed in the white muscle and liver (Espe et al., 2006). Total lipids were extracted from the white muscle and liver (Hara and Radin, 1978) and determined gravimetrically. Fatty acids (FAs, % of total) were determined from these extractions by gas chromatography (Trattner et al., 2011).

Isolation of connective tissue and analyses of collagen content, solubility, amino acid composition, glycation, thermal behaviour (differential scanning calorimetry) and molecular bonds (Fourier transform infrared spectroscopy) were performed as described by Moreno et al. (2012).