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# Growth and quality of Atlantic cod (*Gadus morhua*) fed with high and low fat diets supplemented with glutamate

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ABSTRACT

This study aimed to investigate how supplementation of glutamate to high and low energy Atlantic cod (*Gadus morhua*) diets, affects fish performance, muscle quality and lipid metabolism. This was investigated by feeding Atlantic cod extra dietary glutamate (0.2%) supplementation at two different fat levels (13 and 24% total fat) for a period of 94 days (weight increased from 200 g to 425 g). Overall, only marginal effects of glutamate supplementation could be observed at both fat levels. Differences between groups were mainly explained by fat level in the diet and not by glutamate. Higher fat level reduced feed conversion rate and increased hepatosomatic index (HSI) and hepatic fat content. However, a quality parameter like fillet lightness was significantly increased by glutamate in the low fat group whereas fillet firmness was increased by glutamate in the high fat group. Metabolic studies in isolated liver cells revealed a tendency for increased β-oxidation and lower deposition of lipids in the liver cells from Atlantic cod fed with extra glutamate. PPARα was also upregulated in hepatocytes from glutamate fed Atlantic cod. QPCR analysis of transcripts for key metabolic genes in tissues like intestine, liver or muscle revealed almost no gene regulatory effects of dietary lipid or glutamate levels. Supplementation of Atlantic cod diets with extra glutamate may therefore have the potential to improve the quality parameters like color and firmness without compromising growth and health of the animals.



## 1. Introduction

Interest for farming of Atlantic cod (*Gadus morhua*) increased during the course of the 2000s because of low catch quotas of wild Atlantic cod and high prices of the product. Profitability in Atlantic cod farming has been low, mainly because of high production cost. Feed is the largest cost component and comprises between 50–60% of the production cost (Hemre et al., 2003). Attempts have been made to reduce feed cost by replacing expensive fish meal and fish oil with plant ingredients (Hansen et al., 2007; Tibbetts, 2012) or by optimizing nutrient and energy content in order to promote fast growth rate and high utilization of the feed (Grisdale-Helland et al., 2007; Hatlen et al., 2007; Hemre et al., 2003; Karlsen et al., 2006; Lekva et al., 2010; Walker et al., 2010). Earlier studies have suggested an optimal main nutrient composition of ~50% protein, ~20% fat and ~15% carbohydrates (Dossantos et al., 1993; Grisdale-Helland et al., 2008; Lie et al., 1988; Lied and Braaten, 1984; Morais et al., 2001; Rosenlund et al., 2004). Atlantic cod is known to deposit large quantities of the dietary fat in the liver when fed to satiety (Dossantos et al., 1993; Lie et al., 1988). More than 80% of the fat content of the Atlantic cod can be found in the liver (Albrektsen et al., 2006), whereas skeletal muscle contains less than 2% in farmed Atlantic cod (Morkore et al., 2007). The deposition of fat in the liver is positively correlated to dietary fat level and feed intake (Hansen et al., 2007; Jobling et al., 1991; Lie et al., 1988). As much as 69% of the variation in HSI is explained by protein:energy ratio (Grisdale-Helland et al., 2008). A change in distribution of the ingested energy away from liver storage to muscle protein synthesis would be beneficial in Atlantic cod aquaculture. Large HSI contributes to a high production cost, because the liver is a by-product with lower price compared to the carcass. A strategy to reduce production cost could thus be to develop strategies to prevent excessive deposition of liver fat and rather to use the energy for production of edible product.

The concept of functional feeds, i.e. feed with additives aiming to enhance growth and health performance with the use of feed additives, is well established in feeding of aquatic species (Bridle et al., 2005; Gatesoupe, 2008; Ringo et al., 2007; Tacchi et al., 2011). With respect to Atlantic cod, there is not much information published on the use of feed additives. Based on growth or nitrogen balance, amino acids (AAs) have generally been classified as essential (EAAs) or nonessential

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(NEAAs). The main element of this is that the requirement for NEAA can be met by endogenous synthesis to provide building blocks in protein synthesis. However, more recent studies have demonstrated that some of the NEAAs (mainly glutamine, glutamate and arginine) have important regulatory roles in metabolism, gene expression and immunity (Brasse-Lagnel et al., 2010; Cetinbas et al., 2010; Newsholme et al., 2003). These developments have introduced the term functional amino acids (FAAs), which are AAs that regulate metabolic pathways to improve growth, health and survival (Wu, 2010). L-glutamate is one of the AAs that has received special attention (Brosnan and Brosnan, 2013). This AA plays a central role as a signaling molecule in neurotransmission (as glutamate and its metabolite GABA) (Gecz, 2010), insulin secretion (Treberg et al., 2010) and activation of umami taste receptors (Chaudhari and Roper, 1998). In addition, glutamate serves as a precursor of the amino acid glutamine and the intracellular antioxidant glutathione, an important player in the cellular redox system (Burrin and Stoll, 2009). This knowledge has motivated the food industry to investigate the effects of dietary supplementation of FAAs on growth, quality and stress resistance in production animals like poultry and pigs (Dai et al., 2009, 2012; Wu et al., 1996). In some of these studies a positive effect of FAAs was found on animal health and production parameters. Glutamate is extensively used in prepared foods as a flavor enhancer and is generally recognized as a safe food additive. Dietary glutamate is a major oxidative fuel in the gut, and metabolic products are also an important source of energy for the liver (Newsholme et al., 2003; Windmueller and Spaeth, 1975). This increased understanding of the role of various AA in animal and human nutrition has also led to the development of functional aquafeeds to enhance the performance and meet the needs for a more sustainable aquaculture industry (Burrin and Stoll, 2009). Interestingly, extra dietary glutamate was recently demonstrated to reduce hepatic fat content and hepatosomatic index in Atlantic salmon (Larsson et al., 2014). Based on the published literature, it can be hypothesized that the use of glutamate can be used as an FAA to improve growth, feed utilization and slaughtering quality of Atlantic cod. The aim of the present experiment was to investigate if the supplementation of glutamate to high and low energy fish meal based diets fed to Atlantic cod affected growth, feed utilization, nutrient digestibility, product quality as well as lipid metabolism and lipid distribution.

## 2. Material and methods

### 2.1. Fish and facilities

Atlantic cod (*G. morhua*) with a mean initial weight of 201 g (max 235 g, min 170 g) were fed with four different diets in triplicate 2 $m^2$ fiberglass tanks with a water depth of 60 cm, 50 fish in each tank. Each tank was covered with a lid with a 30 × 30 cm opening in the middle, where the feeding automat was mounted. Each tank was supplied with saltwater (33 g $l^{-1}$ salinity), with a mean temperature of 6.4 °C, ranging from of 5.9 to 7.5 °C. Oxygen level was in the range of 88–92% saturation, with the lowest single observation at 86%. Prior to the experiment, the fish were fed with a commercial Atlantic cod diet (Skretting AS, Stavanger, Norway). All experimental procedures involving animals were conducted in agreement with the provisions enforced by the National Animal Research Authority (NARA).

### 2.2. Diets, feeding and experimental design

Four experimental diets were produced by Nofima, Bergen, Norway (Table 1). The diets were extruded to 4 mm pellet size according to the procedures described by Samuelsen et al. (2013). The diets were based on fishmeal, wheat, soy protein concentrate and differed in terms of lipid added post extrusion and supplementation with (+) or without (−) glutamate. The content of oil (rapeseed oil and fish oil; 50:50) for the high fat (HF) and low fat (LF) diets were per kg DM approximately 140 g and 240 g, respectively (Table 1). The diets were added with $Y_2O_3$ as an inert marker for digestibility determination. The diets were fed to triplicate groups of cod. The feed was distributed by electrically driven band-feeders every 10 min during the course of the day. The tanks were designed to accommodate the collection of waste feed from the effluent water in wire mesh boxes. Uneaten feed was collected to monitor daily feed intake in each tank (Helland et al., 1996). The feeding procedure aimed at optimum voluntary feed intake in all groups of fish.

**Table 1**
Feed composition, g $kg^{-1}$, chemical composition in dry matter.

| Diet | LF | LF + G | HF | HF + G |
|---|---|---|---|---|
| *Ingredients* | | | | |
| Fish meal | 665 | 663 | 555 | 553 |
| Wheat | 130 | 130 | 130 | 130 |
| SPC | 60 | 60 | 60 | 60 |
| 50/50% salmon and rapeseed oil | 50 | 50 | 160 | 160 |
| Monosodium phosphate | 10 | 10 | 10 | 10 |
| Vitamin/mineral mix | 25.2 | 25.2 | 25.2 | 25.2 |
| Glutamate | | 2 | | 2 |
| Yttrium | 0.2 | 0.2 | 0.2 | 0.2 |
| *Chemical composition* | | | | |
| Energy, MJ $kg^{-1}$ | 22.0 | 22.1 | 24.1 | 24.0 |
| Dry matter | 903 | 913 | 957 | 934 |
| Crude fat | 130 | 139 | 246 | 235 |
| Crude protein | 622 | 606 | 543 | 538 |
| Starch | 103 | 101 | 102 | 103 |
| Ash | 98 | 103 | 86 | 88 |
| Yttrium | 0.2 | 0.2 | 0.2 | 0.2 |

### 2.3. Sampling and data collection

At the start of the trial, all fish were individually weighed before distributed to the experimental units. Initial samples of fish (3 × 10 fish) for analyses of the whole body chemical composition were taken randomly from the population used in the experiment. The fish were weighed in bulk after 50 days, and individually weighed after 94 days, when the experiment was terminated. The fish were anesthetized using metacain (MS-222, Sipsy, Avrille, France) (60 mg $l^{-1}$). At termination, fish to be sampled were killed by a sharp blow to the head. Fifteen fish per tank were randomly sampled for different analyses; liver weight was recorded in all sampled fish. Five additional fish per tank were sampled for the whole body proximate chemical analyses. Fecal material for digestibility determination was dissected from the hindgut of sampled fish, and immediately frozen.

### 2.4. Analyses of chemical composition

The fish whole body samples, as well as the whole livers, diets and fecal samples, were homogenized and analyzed for dry matter (oven drying at 105 °C, 16–18 h, to constant weight), total lipid (petroleum ether extraction in a Soxtec HT-6 apparatus, in diets HCl hydrolysis first), nitrogen (Kjeltech Auto Analyser, Tecator, Höganäs, Sweden), ash (flame combustion followed by 3–4 h at 550 °C until constant weight), and energy (bomb calorimetry; Parr 1271, Parr, Moline, IL, USA). Diets were in addition analyzed for starch, and diets and feces for yttrium (ICP spectrometry, as described by Refstie et al. (1997)).

### 2.5. Calculations

Growth rates of the fish were calculated as follows based on fish mean weights. $W_0$ is the initial weight (g), $W_1$ is the final weight (g), d is the number of days in experiment and d° is the sum of day degrees.

Thermal growth coefficient (TGC) (Iwama and Tautz, 1981) = $(W_1^{1/3} - W_0^{1/3}) * 1000/d°$.