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Diets supplemented with glutamate or glutamine improve protein retention and modulate gene expression of key enzymes of hepatic metabolism in gilthead seabream (*Sparus aurata*) juveniles

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ABSTRACT

The present study evaluates the effect of dietary glutamate and glutamine supplementation on growth performance, body composition and expression of key enzymes involved in the hepatic metabolism of juvenile gilthead seabream (*Sparus aurata*). Fish were fed for 52 days with four diets, which were identical in composition except for a 4% supplementation with glutamate (GLU diet), glutamine (GLN diet), carbohydrate (CHO diet) or bovine serum albumin (BSA diet). Glutamate and glutamine supplementation improved feed conversion ratio and protein retention compared to carbohydrate supplementation, and, in the case of glutamate, protein retention was improved over that of fish fed the protein supplemented diet. Feeding CHO and GLU diets resulted in up-regulation of glucokinase and lipogenic enzymes compared to fish fed GLN and BSA diets. Consistently, fish fed CHO or GLU diets showed higher triglyceride levels in serum and liver, and feeding the GLU diet resulted in higher body fat content than in fish fed GLN or BSA diets. The liver of fish fed GLN or BSA diets showed increased glutamate dehydrogenase activity in the direction of the glutamate oxidation. In contrast to fish fed the BSA diet, increased glutamate oxidation did not reduce free glutamine and glutamate levels in the liver of fish fed the GLN diet, suggesting that glutamine intake exceeded liver's energy requirements. In conclusion, glutamate supplementation improved hepatic glucose metabolism, whereas supplemented glutamine seems to be preferentially oxidized over amino acids derived from dietary protein, thus promoting higher protein retention in both cases. Our findings indicate that glutamate and glutamine could partly replace dietary protein and that glutamate supplementation could also facilitate protein replacement by carbohydrates in fish feeds.

1. Introduction

Glutamine and glutamate are non-essential amino acids (NEAA) with relevant functions in animal physiology and metabolism (Rezaei et al., 2013; Wu, 2010). Glutamate plays a key role in the amino acid metabolism through its conversion to α-ketoglutarate or other NEAA. These conversions are mediated by different transaminases and glutamate dehydrogenase (Brosnan, 2000; Brosnan and Brosnan, 2009). By the action of these enzymes, carbon skeletons from amino acids can be used to replenish TCA cycle or derived to glucose production through the gluconeogenic pathway. Glutamate is an important precursor of glutathione, a major intracellular antioxidant. It also acts as a signaling molecule regulating appetite and digestion. In addition, glutamate is an important neurotransmitter in the central nervous system of mammals (Brosnan and Brosnan, 2013; Burrin and Stoll, 2009; Torii et al., 2013).

Glutamine can be synthesized from glutamate by glutamine synthetase and converted to glutamate by glutaminase (Newsholme et al., 2003). In mammals, glutamine synthesis and catabolism play important roles in regulating ammonia levels and in acid-base homeostasis (Taylor and Curthoys, 2004). Moreover, glutamine is almost essential for cell proliferation both as an energy substrate and as a precursor of purine and pyrimidine nucleotides (Li and Gatlin, 2006; Wu et al., 2011). In mammals, glutamine metabolism activates the mammalian target of rapamycin (mTOR), a Ser/Thr protein kinase involved in cell growth, metabolism and protein synthesis (Durán and Hall, 2012; Fumarola et al., 2005; Wu et al., 2013).

Research on amino acid requirements of reared fish has to date largely focused on essential amino acids (EAA), given that their deficiency have negative effects on fish growth, health and development (Li et al., 2009). Nevertheless, growing evidence points to a significant

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role of NEAA in fish nutrition. Optimum dietary levels of NEAA have been found to improve protein utilization and growth in different fish species (Green et al., 2002; Peres and Oliva-Teles, 2006; Schuhmacher et al., 1995). Indeed, appropriate levels of NEAA in the diet can spare EAA from being catabolized by fish (Abboudi et al., 2009; Conceição et al., 2002; Kim et al., 1992; Rønnestad et al., 2003). Only a few studies have addressed the metabolic role of a specific NEAA on fish nutrition. However, it is already known that dietary NEAA levels have important metabolic effects, such as sustaining gluconeogenic and lipogenic fluxes (Figueiredo-Silva et al., 2010; Kirchner et al., 2003; Moyano et al., 1991).

The effect of supplementing diets with glutamate or glutamine in mammals has been studied mainly in piglets, with little published work on fish. In both mammals and fish, studies to date have focused on the role of these two amino acids as energy sources for intestinal cells and tissues (Ando, 1988; Blachier et al., 2009; Burrin and Stoll, 2009; Reeds et al., 2000), and the derived beneficial effects on the maintenance of the intestinal structure and function (Cabrera et al., 2013; Cheng et al., 2011, 2012; Pohlenz et al., 2012; Qiyou et al., 2011; Rezaei et al., 2013; Shan et al., 2012; Wu et al., 2011; Yan and Qiu-Zhou, 2006). A significant part of the glutamate and glutamine absorbed by the intestine is transported to the liver (Brosnan, 2003), the major organ involved in nutrient assimilation and transformation into oxidizable substrates, such as glucose and fatty acids, that can be utilized by the whole body.

In piglets, glutamine and glutamate supplementation enhance feed efficiency and growth, whereas their reported effects on fish growth performance have to date been inconclusive. Glutamate supplementation has been reported to either increase (Hughes, 1985; Oehme et al., 2010), decrease (Figueiredo-Silva et al., 2010; Mambrini and Kaushik, 1994), or having no effect on fish growth performance (Kirchner et al., 2003). There is a stronger evidence that glutamine promotes growth in fish (Cheng et al., 2012; Qiyou et al., 2011; Schuhmacher et al., 1995; Wang et al., 2011; Yan and Qiu-Zhou, 2006).

The aim of the present study was to analyze the effect of glutamate and glutamine supplementation on growth performance and their role in the expression of key enzymes involved in the liver metabolism of juvenile gilthead seabream (*Sparus aurata*). Such knowledge could be helpful to evaluate the possible role of glutamine and glutamate in sparing dietary protein.

2. Materials and methods

2.1. Diets

Four isoenergetic diets supplemented with 4% glutamate (GLU), glutamine (GLN) or bovine serum albumin (BSA) at the expense of dietary carbohydrate contents (CHO) were fed to *S. aurata* juveniles (Table 1). CHO and BSA diets were designed as controls to assess the effect of substituting dietary carbohydrates and proteins, respectively, by glutamate or glutamine.

Differences in the amino acid composition of diets were negligible (Table 2), except for diets GLU and GLN, which presented higher levels of glutamate and glutamine, respectively.

2.2. Feeding trial and sampling

Gilthead seabream (*S. aurata*) juveniles (3.7 ± 0.5 g body weight, n = 186) were obtained from Piscimar (Burriana, Spain) and distributed in twelve seawater aquaria of 260 l, 15–17 fish per aquarium. Fish maintenance conditions were as previously described in Fernández et al. (2007). Fish were acclimated for two weeks to facility conditions and fed a commercial diet Microbaq-15 (Dibaq Group, Spain; 51% crude protein, 21% crude fat, 14% carbohydrates). Each experimental diet was randomly assigned to three aquaria. The fish were fed for 52 days with a daily ration equivalent to 4% body weight (BW), divided into two meals (at 9.30 and 15.30 h) from Monday to Friday. On weekends, the fish were fed a single meal of 2% BW. Fish well accepted all diets and consumed the ration within 10–15 min completely. Fish from each aquarium were individually weighed every 7–10 days to adjust feed ration. Fish were not fed the days they were weighed.

For initial and final whole-body composition analysis, 12 fish were sacrificed in the beginning, and another 5 fish were taken from each aquarium and sacrificed at the end of the experiment. Prior to sampling, fish were fasted for 24 h to ensure complete evacuation of the gut. Fish were sacrificed by anesthesia overdose (MS-222), ground and kept at −20 °C until analysis. The remaining fish were anesthetized by immersion in seawater with 70 mg/l of MS-222. Once anesthetized, fish were sacrificed by cervical section, and immediately dissected to obtain blood and liver samples. Serum from blood samples was recovered after centrifugation (4000 rpm for 15 min) and stored at −20 °C.

Table 1
Composition and proximate analysis of the experimental diets.

	CHO	GLU	GLN	BSA
Formulation (%)				
Brown fish meal[a]	70.0	70.0	70.0	70.0
Fish oil[b]	12.7	12.7	12.7	12.7
Starch[c]	14.2	10.2	10.2	10.2
Glutamate[d]	–	4.0	–	–
Glutamine[d]	–	–	4.0	–
Albumin[d]	–	–	–	4.0
Carrageenan[d]	2.0	2.0	2.0	2.0
Mineral mixture[e]	0.9	0.9	0.9	0.9
Vitamin mixture[f]	0.2	0.2	0.2	0.2
Chemical analysis (% dry weight, DW)				
Crude protein	51.9	55.6	56.1	55.9
Carbohydrate[g]	18.3	14.0	13.6	14.1
Crude fat	18.4	18.8	18.9	18.9
Ash	11.3	11.6	11.4	11.1
Gross energy (kJ/g DW)[h]	22.8	23.0	23.1	23.2

[a] Corpesca S.A. Super-Prime fish meal (Santiago de Chile, Chile).
[b] Fish oil from A.F.A.M.S.A. (Vigo, Spain).
[c] Pregelatinised corn starch from Brenntag Química S.A. (St. Andreu de la Barca, Barcelona, Spain).
[d] L-glutamic acid, L-glutamine, bovine serum albumin and iota carrageenan from Sigma-Aldrich (St Louis, MO, USA).
[e] Mineral mixture (mg/kg): $CaHPO_4 \cdot 2H_2O$, 7340; MgO, 800; KCl, 750; $FeSO_4 \cdot 7H_2O$, 60; ZnO, 30; MnO_2, 15; $CuSO_4 \cdot 5H_2O$, 1.7; $CoCl_2 \cdot 6H_2O$, 1.5; KI, 1.5; Na_2SeO_3, 0.3.
[f] Vitamin mixture (mg/kg): choline chloride, 1200; myo-inositol, 400; ascorbic acid, 200; nicotinic acid, 70; all-rac-tocopherol acetate, 60; calcium pantothenate, 30; riboflavin, 15; piridoxin, 10; folic acid, 10; menadione, 10; thiamin-HCl, 8; all-trans retinol, 2; biotin, 0,7 cholecalciferol, 0.05; cyanocobalamin, 0.05.
[g] Carbohydrates were calculated by difference.
[h] Calculated from gross composition (protein 24 kJ/g, fat 39 kJ/g, carbohydrates 17 kJ/g).

Table 2
Amino acid composition (% DW) of the experimental diets.

	CHO	GLU	GLN	BSA
Arg	3.2	3.0	3.0	3.3
His	1.9	1.8	1.8	1.9
Lys	3.9	3.7	3.7	4.2
Thr	2.2	2.1	2.1	2.3
Ile	2.0	1.9	1.8	2.0
Leu	3.6	3.4	3.4	3.9
Val	2.4	2.3	2.3	2.5
Met	1.4	1.3	1.4	1.4
Phe	1.9	1.7	1.8	2.1
Cys	0.1	0.2	0.2	0.4
Tyr	1.5	1.4	1.4	1.6
Asx[a]	4.5	4.2	4.3	4.7
Glx[b]	6.3	9.9	10.1	6.9
Ala	2.9	2.7	2.8	3.0
Gly	2.9	2.7	2.8	2.9
Ser	1.9	1.8	1.9	2.0
Pro	1.8	1.6	1.7	1.9

[a] Asx is the sum of Asn and Asp, since Asn is converted to Asp during hydrolysis.
[b] Glx is the sum of Gln and Glu, since Gln is converted to Glu during hydrolysis.