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Dietary arginine surplus does not improve intestinal nutrient absorption capacity, amino acid metabolism and oxidative status of gilthead sea bream (*Sparus aurata*) juveniles

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ABSTRACT

This study aims to evaluate the effects of dietary arginine (Arg) surplus on gilthead sea bream growth, intestinal nutrient absorption capacity, hepatic and intestinal amino acid (AA) metabolism, and oxidative status. For that purpose a 6 weeks growth trial was performed with gilthead sea bream juveniles fed four isolipidic (18%) and isoproteic (44%) diets supplemented with 0, 0.5, 1 and 2% Arg. Dietary Arg did not affect fish growth performance, feed and nitrogen utilization, body composition, and activity of the main AA catabolic enzymes in liver and intestine. Similarly, intestinal nutrient absorption capacity, evaluated by intestinal brush border membrane vesicles technique, was also unaffected by the dietary Arg surplus. On the other hand, enzymatic antioxidant response was modulated by dietary Arg increment, which led to a reduced glutathione peroxidase activity in the liver and the intestine and increased superoxide dismutase activity in the intestine, but did not affect the overall lipid peroxidation values. Some differences in liver and intestine antioxidant enzymatic responses were identified, with the liver showing higher catalase and glucose-6-phosphate dehydrogenase activities, while the intestine presented higher glutathione peroxidase and glutathione reductase activities. Overall, dietary Arg excess showed limited potential to enhance gilthead sea bream performance and intestinal nutrient absorption capacity, but it was shown to modulate hepatic and intestinal antioxidant defences, without affecting overall lipid peroxidation.

Statement of relevance: Recently some fish species have been shown to benefit from dietary arginine surplus, while evidences also exist of an Arg-Lys antagonism in other fish species. This manuscript gives further insight on the potential of dietary arginine supplementation in gilthead sea bream, a major aquaculture species in the Mediterranean.

1. Introduction

In the past decades a great deal of research focused on the development of more sustainable aquafeeds. It is now feasible to formulate diets for several carnivorous fish species with substantial amount of protein of plant origin without impairing growth performance (Oliva-Teles et al., 2015). For instance, gilthead sea bream (*Sparus aurata*), a major aquaculture species in the Mediterranean, has recently been shown to tolerate a 100% plant protein diet without compromising growth (Kissil and Lupatsch, 2004; Watson et al., 2013).

However, even if no decreased performance or pathological signs are observed in fish fed plant protein-based diets, physiological alterations may still occur potentially impairing fish immunity and stress responses. In fact, intestinal inflammation and depressed immune status due to the anti-nutrients found in plant feedstuffs were reported in gilthead sea bream fed plant feedstuff-rich diets (Bonaldo et al., 2008; Kokou et al., 2012; Sitja-Bobadilla et al., 2005). Moreover, disturbances of the intestinal mucosal structure have also been identified in gilthead sea bream fed diets including purified saponins and phytosterols, anti-nutritional factors present in soybean among other feedstuffs (Couto et al., 2014). This, associated with the stressful environment of intensive

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culture conditions and the increasing farmers and consumers concerns with fish health and welfare, has driven attention to the potential of nutritional-based health approaches (Kiron, 2012; Oliva-Teles, 2012; Pohlenz and Gatlin Iii, 2014).

Arginine (Arg) is an indispensable amino acid (IAA) that, besides being required for protein synthesis, is the most abundant nitrogen carrier for tissue proteins and is also essential for the synthesis of biologically important molecules such as proline, creatine, ornithine, polyamines, and nitric oxide (NO) (Wu and Morris, 1998). Moreover, in fish given high Arg doses either by injection or through force-feeding, Arg was shown to be a more potent insulinotropic than glucose, leading to increasing titres of insulin (Andoh, 2007; Baños et al., 1999; Lall et al., 1994; Mommsen et al., 2001; Plisetskaya et al., 1991; Sink and Lochmann, 2007), insulin-like growth factor 1 (IGF-1), and growth hormone (GH) (Baños et al., 1999; Lall et al., 1994; Mommsen et al., 2001).

This remarkable endocrine modulatory capacity of Arg has been pointed as the main cause for the enhanced growth performance and protein efficiency observed in channel catfish (*Ictalurus punctatus*) (Pohlenz et al., 2014), juvenile Atlantic salmon (*Salmo salar*) (Andersen et al., 2013) and hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) (Cheng et al., 2012) fed diets with an Arg surplus. However, results on dietary Arg surplus effects on fish performance are diverse, with studies in black seabream (*Acanthopagrus schlegelii*) (Zhou et al., 2012a), European sea bass (Tulli et al., 2007), Japanese flounder (*Paralichthys olivaceus*) (Alam et al., 2002), red drum (*Sciaenops ocellatus*) (Cheng et al., 2011), grouper (*Epinephelus coioides*) (Luo et al., 2007) and adult Atlantic salmon (Andersen et al., 2014) reporting that dietary Arg surplus did not change growth performance and feed utilization, while studies in rainbow trout (*Oncorhynchus mykiss*) (Kaushik and Fauconneau, 1984; Kaushik et al., 1988) and blunt snout bream (*Megalobrama amblycephala*) (Ren et al., 2013) reported that dietary Arg excess resulted in growth depression, which was associated to an Arg-lysine (Lys) antagonism. This antagonism is yet to be fully understood in fish, although it was suggested to result from competition between Arg and Lys at intestinal transporters (Berge et al., 1999; Kaushik et al., 1988), or to an increased ureagenesis (Kaushik and Fauconneau, 1984). In fact, since the majority of teleosts are ammoneotelic and lack a fully functional ornithine–urea cycle (Ip and Chew, 2010; Ip et al., 2001), argininolysis is of major importance for the overall urea production (Fournier et al., 2003).

Evidences also exist of a protective role of dietary Arg against cell membranes oxidative damage by reactive oxygen species (ROS) (Ren et al., 2013). Wang et al. (2015) suggested that dietary Arg decreases oxidative damage by elevating fish radical scavenging ability, although further studies are required to confirm this hypothesis and to fully understand the potential of dietary Arg supplementation to mitigate fish oxidative stress. Thus, the present work was performed to evaluate the effects of a dietary Arg surplus on gilthead sea bream performance, hepatic and intestinal AA metabolism, and oxidative status. Additionally, an evaluation of the effect of dietary Arg excess on intestinal L-Arg, L-glutamine and D-glucose absorption capacity was performed for the first time in fish.

2. Material and methods

2.1. Diets

Four isolipidic (18%) and isonitrogenous (7%) diets were formulated with an amino acid profile similar to that of the whole-body of gilthead sea bream (Kaushik, 1998). Diets were supplemented with 0, 0.5%, 1% and 2% Arg at the expense of a dispensable amino acid (DAA) mixture. Ingredients and proximate composition of the experimental diets are presented in Table 1 and the AA composition of the experimental diets is presented in Table 2.

Table 1
Ingredient composition and chemical analysis of the experimental diets.

	Control	0.5Arg	1Arg	2Arg
Ingredients (% dry weight)				
Fish meal[a]	24	24	24	24
CPSP G[b]	5	5	5	5
Soybean meal[c]	26	26	26	26
Whole wheat meal[d]	13.6	13.6	13.6	13.6
Fish oil	13.6	13.6	13.6	13.6
Vitamin premix[e]	1	1	1	1
Choline chloride (50%)	0.5	0.5	0.5	0.5
Minerals premix[f]	1	1	1	1
Binder[g]	1	1	1	1
Cellulose	5.9	6.5	7.2	8.4
NEAA premix[h]	6.2	5.0	3.9	1.6
Dibasic calcium phosphate	1.2	1.2	1.2	1.2
Agar	1	1	1	1
L-Arginine	0	0.5	1	2
Chemical analysis (% dry weight)				
Dry matter (%)	86.0	87.1	88.1	91.9
Crude protein	45.0	44.4	44.4	43.1
Crude lipid	18.2	18.3	18.0	16.6
Ash	10.7	10.6	10.5	10.3
Gross energy (kJ g^{-1})	23.0	22.6	22.5	21.8

[a] Pesquera Centinela, Steam Dried LT, Chile (CP: 72.1%; CL 9.51%). Sorgal, S.A. Ovar, Portugal.
[b] Soluble fish protein concentrate, Sopropêche, France (CP: 80.4% DM; GL: 19.7% DM).
[c] Soybean meal (CP: 51.3%; CL: 2.6%), Sorgal, S.A. Ovar, Portugal.
[d] Whole wheat meal (CP: 14.1%; CL: 3.2%), Sorgal, S.A. Ovar, Portugal.
[e] Vitamins (mg kg^{-1} diet): retinol, 18,000 (IU kg^{-1} diet); calciferol, 2000 (IU kg^{-1} diet); alpha tocopherol, 35; menadion sodium bis., 10; thiamin, 15; riboflavin, 25; Ca pantothenate, 50; nicotinic acid, 200; pyridoxine, 5; folic acid, 10; cyanocobalamin, 0.02; biotin, 1.5; ascorbyl monophosphate, 50; inositol, 400.
[f] Minerals (mg kg^{-1} diet): cobalt sulphate, 1.91; copper sulphate, 19.6; iron sulphate, 200; sodium fluoride, 2.21; potassium iodide, 0.78; magnesium oxide, 830; manganese oxide, 26; sodium selenite, 0.66; zinc oxide, 37.5; dicalcium phosphate, 8.02 (g kg^{-1} diet); potassium chloride, 1.15 (g kg^{-1} diet); sodium chloride, 0.4 (g kg^{-1} diet).
[g] Aquacube, (Guar gum, polymethyl carbamide, Manioc starch blend, hydrate calcium sulphate). Agil, England.
[h] NEAA Premix composition (g 100^{-1} g DM NEAA premix): L-alanine: 19.57; L-aspartic acid: 27.95 g; L-glycine: 22.86 g; L-serine: 13.91 g; L-proline: 15.71 g.

2.2. Growth trial

This feeding trial was carried out at the Marine Zoology Station, Porto University, experimental facilities, in a thermo-regulated water semi-recirculation system equipped with 24 cylindrical fiberglass

Table 2
Determined amino acid composition (g 16 g^{-1}N) of the experimental diets[a].

	Control	0.5Arg	1Arg	2Arg
IAA[b]				
Arginine	6.8	8.0	9.3	11.4
Histidine	2.4	2.7	2.5	2.9
Isoleucine	3.5	3.7	3.8	3.8
Leucine	6.4	6.5	6.7	6.4
Lysine	6.6	6.5	6.2	6.4
Threonine	4.0	4.0	4.1	4.1
Valine	4.6	4.4	4.5	4.5
Methionine	2.3	2.4	2.4	2.3
Phenylalanine	4.0	4.0	4.0	4.1
DAA[c]				
Tyrosine	2.8	3.0	3.2	3.7
Alanine	7.4	7.1	6.8	6.4
Aspartic acid	11.9	11.6	11.2	10.2
Glutamic acid	13.1	13.6	13.7	13.5
Glycine	8.3	7.9	7.2	6.8
Serine	6.2	6.4	6.0	5.7
Proline	6.4	6.8	6.3	6.1
∑ IAA	40.6	42.2	43.4	45.9
∑ DAA	56.0	56.4	54.3	52.4
IAA/DAA	0.73	0.75	0.80	0.88

[a] Dietary tryptophan and cysteine levels were not determined.
[b] IAA, indispensable amino acids.
[c] DAA, dispensable amino acids.