Aquaculture 479 (2017) 690–695

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Dietary arginine supplementation does not improve nutrient utilisation in gilthead seabream

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ARTICLE INFO

Keywords:
Ornithine-urea cycle
Enzymes activity
Arginine
Growth performance
N excretion

ABSTRACT

The aim of this study was to evaluate the beneficial effects of dietary arginine supplementation on growth performance, urea biosynthesis, and N excretion of juvenile gilthead sea bream (*Sparus aurata*) fed fish meal and plant-feedstuffs based diets. For that purpose, five isonitrogenous (45% crude protein) diets were formulated as follows: two diets were fish meal-based and contained 3.0 and 4.0% arginine; three diets were plant feedstuff-based and contained 1.6, 3.0, and 4.0% arginine. Triplicate groups of gilthead seabream juveniles (24 g) were fed each diet for 10 weeks. At the end of the trial, growth performance of fish fed the fish meal-based diets was higher than that of the other groups. Dietary arginine content did not affect growth performance.

Ammonia excretion was not affected by diet composition, while urea excretion was directly related with arginine intake. Carbamyl phosphate synthase activity was not detected in the liver, while arginase activity increased and ornithine carbamyl-transferase decreased with dietary arginine content.

Overall, an excess of dietary arginine had no beneficial effects on growth performance and feed utilisation of gilthead seabream juveniles fed plant feedstuffs-based diets. A functional urea cycle was not detected in the liver of gilthead sea bream juveniles.

1. Introduction

Given its high protein content and amino acid composition similar to the ideal amino acid profile required by fish (Oliva-Teles et al., 2015), fish meal remains a major protein source in diets for marine finfish such as gilthead sea bream. It is, however, well recognised that sustainable development of fish farming requires that fish meal in the feeds be replaced by alternative protein sources. Among these, plant feedstuffs have received much attention. However, protein content of plant feedstuffs is usually lower than that of fish meal, its amino acid composition is less well balanced and some amino acids are limiting (Gatlin et al., 2007; Oliva-Teles et al., 2011). Lysine and methionine are generally the first limiting amino acids in plant feedstuffs, although arginine may be limiting in plant protein concentrates such as corn or wheat gluten meals (Sauvant et al., 2004).

In gilthead sea bream, total fish meal replacement by plant feedstuffs was first achieved by Kissil and Lupatsch (2004) using corn gluten, wheat gluten and soy protein concentrate as protein sources. In that study, although authors found that fish meal replacement with plant protein sources was successful the alternative diet formulations included high amounts of crystalline arginine. Thus, it is of interest to elucidate the potential beneficial effects on fish performance and feed utilisation of supplementing with arginine plant feedstuffs-based diets rich in corn gluten or wheat gluten, which are among the most cost-competitive plant feedstuffs to include in aquafeeds (Oliva-Teles et al., 2015).

Arginine is an essential amino acid for fish (NRC, 2011), necessary not only for protein synthesis but also for the synthesis of several biologically important compounds such as creatine, polyamines, and nitric oxide, thus potentially having a central role in energy metabolism, cell proliferation, regulation of blood pressure and immune response (Morris, 2006; Wu et al., 2009). Arginine is also associated to the release of hormones involved in fish growth, such as glucagon, glucagon like peptide (GLP-1), somatostatin, growth hormone, and insulin growth factor (IGF-1) (Mommsen et al., 2001; Lind, 2004). When administered intra-peritoneally, arginine is an efficient insulin secretagogue in fish, being more efficient than glucose (Moon, 2001; Enes et al., 2009). It was also suggested that excess dietary arginine might contribute to improve glucose utilisation for energy purposes and to increase amino acid uptake by tissues through an insulin-mediated

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[1] Work was undertaken as part of EU funded project under FAIR program, CT 98-3408, when the author was with INRA, St Pée sur Nivelle, France.

http://dx.doi.org/10.1016/j.aquaculture.2017.07.016
Received 28 March 2017; Received in revised form 6 June 2017; Accepted 9 July 2017
Available online 11 July 2017
0044-8486/

mechanism (Tulli et al., 2007). Feeding excess arginine had a transitory stimulatory effect in growth of Chinook salmon and rainbow trout fingerlings (Plisetskaya et al., 1991). However, excess dietary arginine did not induce any increase in circulating levels of insulin or growth hormone in Atlantic salmon fed graded levels of arginine (Lall et al., 1994). Some studies have shown that arginine can improve growth of Atlantic salmon (Oehme et al., 2010), feed efficiency in red drum (Cheng et al., 2011, 2012), and both growth and feed efficiency in hybrid striped bass and catfish (Cheng et al., 2012; Pohlenz et al., 2014). Excess arginine seems to have even a general inhibitory effect on immunity and disease resistance in European sea bass (Azeredo et al., 2015). In gilthead sea bream, it was recently shown that dietary arginine surplus did not affect growth performance, intestinal nutrient absorption capacity or oxidative status (Coutinho et al., 2016).

According to Ball et al. (2007), with the exception of Pacific salmon, arginine requirement in fish is relatively similar among species, requirement being met with an inclusion of 1.2–1.5% arginine in the diets, corresponding to 3.7–3.9% of dietary protein. Available data on quantitative requirement for arginine in different teleost appear however to show much variability (NRC, 2011) and reported to be higher in marine fish, such as European sea bass, gilthead sea bream or turbot, than in rainbow trout (Fournier et al., 2002).

Variation of arginine requirement among species or studies may be related to the possible synthesis of arginine from glutamate-derived citrulline. Possible endogenous synthesis of arginine from glutamic acid was reported in channel catfish fed arginine deficient diets (Buentello and Gatlin, 2000). In rainbow trout given diets with low arginine concentration, arginine deposition exceeds the quantity fed (Rodehutscord et al., 1995) and endogenous arginine synthesis seems to be sufficient to maintain whole-body N balance (Fournier et al., 2002).

The aim of the present study was to evaluate the effect of arginine supplementation to plant feedstuffs based diets on performance and nutrient utilisation of gilthead sea bream juveniles.

2. Material & methods

2.1. Experimental diets

We used five isonitrogenous (45% crude protein) diets: two diets were formulated based on fish meal to contain arginine levels of 3.0 and 4.0% and three others based on plant feedstuffs (PF). Diet FM1 was a standard fish meal (FM) based diet supplemented with arginine to provide a moderate excess of this amino acid, while diet FM2 was further supplemented with arginine. The diets based on plant feedstuffs (PF) were formulated to contain graded levels of arginine: 1.6, 3.0 and 4.0% (diets PF1, PF2 and PF3, respectively) and their amino acid composition adjusted to meet the estimated requirements for gilthead sea bream (Kaushik, 1998; Peres and Oliva-Teles, 2009) by supplementation with lysine and methionine. The dietary arginine level in diet PF1 was lower than that of sea bream requirement (circa 2.4% of the diet; Kaushik, 1998; Peres and Oliva-Teles, 2009) while arginine levels in diets PF2 and PF3 were similar to levels of diet FM1 and FM2, respectively, and corresponded to an excess of arginine. Arginine supplementation to the diets was done by replacing with glutamate on an equal nitrogen basis. Before incorporation into the basal diet, amino acids were coated in agar (1%). Amino acid analysis of all ingredients used in diets formulation was done by Eurolysine (Amiens, France).

After homogenisation of all raw materials, diets were pelleted, dried for 10 min at 80 °C and 10 min at room temperature in a ventilated drier and sieved in two diameters: 2 and 3 mm. The ingredient composition, analysed proximate composition of the experimental diets are presented in Table 1.

2.2. Feeding trial

The feeding trial was undertaken during January–Mars 2001 at the Marine Zoology Station, University of Porto. The experimental facilities consisted of an indoor partial recirculation marine water system provided with 15 tanks of 80 l of water capacity. During the experiment, water temperature was regulated to 20 °C, water salinity averaged 26.4‰, and flow rate was set at approximately 5 l/min.

Juvenile gilthead sea bream (*Sparus aurata*) were obtained from a commercial fish farm and were kept in quarantine tanks to recover from transport and to adapt to the new environmental conditions and experimental facilities. Thereafter, 15 groups of 40 fish with an average body weight of 24 g were established and to triplicate groups of these fish was randomly assigned each experimental diet. The trial lasted 10 weeks and the fish were fed by hand twice a day (at 10 and 16 h), to apparent visual satiety, 6 days a week. During the trial fish were bulk weighted every 2 weeks, after applying a mild anaesthesia (ethylene glycol monophenyl ether). The fish were unfed the day before weighing, and feed distributed during the period was registered at the weighing days.

Table 1
Composition and proximate analysis of the experimental diets.

	FM1	FM2	PF1	PF2	PF3
Arg (% diet)	3.0	4.0	1.6	3.0	4.0
Arg (% protein)	6.4	9.1	3.7	6.4	9.1
Composition (g/kg diet)					
Fish meal[a]	380.0	380.0	–	–	–
Fish protein concentrate[b]	50.0	50.0	50.0	50.0	50.0
Corn gluten[c]	138.3	138.3	140.0	140.0	140.0
Amygluten 110	–	–	293.8	293.8	293.8
Extruded wheat	250.0	250.0	158.6	158.6	158.6
Cod liver oil[a]	76.6	76.6	152.4	152.4	152.4
Cellulose[d]	14.6	36.6	–	31.2	53.2
$CaHPO_4{\cdot}2H_2O$ (18% P)	10.9	10.9	55.2	55.2	55.2
Basal mix[e]	40.15	40.15	40.15	40.15	40.15
L-Arg	1.9	11.9	2.3	16.5	26.5
L-Lys HCl	–	–	10.3	10.3	10.3
DL-Methionine	–	–	1.4	1.4	1.4
L-Glu	37.6	5.6	95.9	50.4	18.4
Proximate analysis (% dry matter)					
Moisture	7.9	7.7	6.3	6.0	6.6
Crude protein	43.7	43.3	43.9	43.5	43.5
Crude lipid	13.0	12.8	17.0	17.2	16.8
Fiber	3.0	4.6	1.9	4.0	5.5
Ash	8.2	8.2	6.6	6.5	6.4

[a] La lorientaise des produits de la pêche, Lorient, France.
[b] CPSP G, Sopropêche, France.
[c] Roquette frères, Lestrem, France.
[d] Arbocel B00, J. Rettenmaier and Söhne, Deutshland.
[e] Basal mix (g/kg diet): vitamin mix[2]: 10; mineral mix[3]: 10; guargel (Louis François, St. Maur, France): 10; Agar (Louis François, St. Maur, France): 10; ethoxyquine (Sigma E8260, St. Quentin Fallavier, France): 0.15. [2]Supplied the following: as per NRC, 1993 (to provide mg/kg diet): retinyl acetate (250,000 U/g), 0.5; cholecalciferol (240,000 U/g), 2.4; ascorbyl phosphate (25%): 200; tocopheryl acetate, 50; menadione, 10; thiamin, 1; riboflavin, 4; pyridoxine, 3; Ca-pantothenate, 20; vitamin B12, 0.01; niacin, 10; biotin, 0.15; folic acid, 1; choline, 1000; inositol, 300. [3]Supplied the following (to provide mg/kg diet, except as noted): magnesium carbonate, 1.24 g; calcium carbonate, 2.15 g; potassium chloride, 0.90 g; sodium chloride, 0.40 g; potassium iodide, 0.4; copper sulphate, 30; cobalt sulphate, 0.2; ferric sulphate, 0.20 g; manganese sulphate, 30; zinc sulphate, 40; dibasic calcium phosphate, 5 g; sodium fluoride: 10.

2.3. Ammonia and urea excretion measurements

Ammonia and urea nitrogen were measured for two consecutive days in each tank during the growth trial. For that purpose, water flow rate was reduced to 2–3 l/min the day before and during the sampling days. Water samples were collected in the outlet of each tank at 0, 2, 4, 6, 8, 10, 12, 18 and 24 h after the first meal. Water collected in the outlet of a tank without fish was used as a blank.