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Glutamine and arginine in diets for Nile tilapia: Effects on growth, innate immune responses, plasma amino acid profiles and whole-body composition

R.T. Pereira [a,b,*], P.V. Rosa [b], D.M. Gatlin III [a,*]

[a] Department of Wildlife and Fisheries Sciences and Intercollegiate Faculty of Nutrition, Texas A&M University System, College Station, TX, USA
[b] Department of Animal Science, Federal University of Lavras UFLA, Lavras, Minas Gerais, 37200-000, Brazil

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ABSTRACT

Glutamine (Gln) and arginine (Arg) are functional amino acids (AA) known to improve growth, immunity and nutrient utilization of animals. Juvenile Nile tilapia were fed six experimental diets formulated to contain different levels of supplemental Gln and/or Arg (Control, GLN 1%, GLN 2%, ARG 1%, ARG 2% and GLN + ARG 1%) for a 9-week period. Growth performance, innate immune responses, AA profiles in plasma and whole-body were examined. Dietary Gln and/or Arg supplementation resulted in significant effects on weight gain, feed intake, feed efficiency, protein efficiency and protein retention. Moreover, the concentration of free AAs in plasma at 6 h and 18 h were significantly affected by experimental diets. The AA concentrations significantly affected at the 6 h sampling were Cys, Asp, Ser, Gly and Hyp while at 18 h, differences were observed for Arg, Val, Cys, Ser, Gly and Pro. In contrast, only differences in Gln, Gly and Ser concentrations were observed regarding AA composition of the whole-body tissues. Therefore, the AA profiles of plasma were more affected by the dietary GLN and/or ARG supplementation than whole-body. Most of the immunity indicators were not raised by dietary levels of Gln and/or Arg probably as a reflection of the non-activated state of the immune cells. Although Gly was included to the experimental diets to adjust nitrogen content, this inclusion resulted in effects on the growth performance and physiological parameters. Finally, Nile tilapia fed the combined supplement of GLN + ARG at 1% had more improved growth performance than those fed the diets supplemented individually with Gln or Arg.

1. Introduction

In pursuing a greater understanding of the roles of amino acids (AAs) in animal nutrition and health, numerous studies in recent years have focused on the potential effects of indispensable and dispensable AAs on various physiological and immunological functions. Similarly in fish nutrition, research on both categories of AAs has expanded in the last decade and have reported the importance of various AAs on nitrogen balance, protein and energy utilization, as well as health of species such as channel catfish (Pohlenz et al., 2013), tilapia (Gaye-Siessegger et al., 2007; Mambrini and Kaushik, 1995; Wu et al., 2015) and Atlantic salmon (Larsson et al., 2014).

The gap between traditional AA classification and physiological importance has led to the emergence of the "Functional amino acids" concept proposed by Wu (2010). Functional AAs are defined as those which participate and regulate key metabolic pathways to improve growth and health in mammals and fish. This AA group encompasses arginine, cysteine, glutamine, glutamate, glycine, leucine, proline, and tryptophan regardless of their designation as dispensable or indispensable. Due to many of their metabolic roles, functional AAs are known to improve the efficiency of nutrient utilization by animals (Wu, 2013a, 2010; Wu et al., 2014). The functional AA concept also has led to a paradigm shift regarding the classification of AA as nutritionally dispensable or indispensable. It has been proposed that animals (focused on poultry and swine) have dietary requirements for not only indispensable, but also dispensable AAs to achieve maximum growth and production performance (Wu, 2014; Wu et al., 2014). Indeed, this assumption encourages discussion regarding whether AA requirements in animals could be underestimated under certain conditions and/or if ratios between dispensable or indispensable AAs have been taken into account as they should. Because of the great diversity of fish being cultured along with a lack of understanding regarding AA metabolism in fish and its

Abbreviations: AA, Amino acid; DAA, Dispensable amino acid; IAA, Indispensable amino acid; ARG, Arginine in experimental diet; Arg, Arginine; GLN, Glutamine in experimental diet; Gln, Glutamine; SEC, Superoxide anion extracellular; SIC, Superoxide anion intracellular; NBT, Nitro blue tetrazolium; LYZ–P, Plasma lysozyme activity; LYZ–S, Spleen lysozyme activity; HACS, Hemolytic activity of complement system.

* Corresponding authors at: 216 Heep Laboratory Building, 2258 TAMUS, College Station, TX 77843, USA.
E-mail address: raqueltpr@gmail.com (R.T. Pereira).

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relations to somatic growth and health, additional research is warranted in this field (Kiron, 2012; Pohlenz and Gatlin, 2014; Wu et al., 2014).

As functional AAs, glutamine (Gln) and arginine (Arg) occupy a prominent position. Gln is the major source of nitrogen and carbon in the interorgan metabolism of AAs (Watford, 2008), which reflects a crucial role of this AA in whole-body nutrient metabolism and health of mammals (Wu, 2010, 2009) and fish (Cheng et al., 2012; Pohlenz et al., 2012a, 2012b, 2012c). Moreover, Gln is a major fuel for the mucosal cells of the intestine (Burrin and Stoll, 2009) and it is an AA required for the functions of several cell populations of the immune system in mammals (Dai et al., 2013) and fish (Cheng et al., 2011; Pohlenz et al., 2012a, 2012b, 2012c).

Arg is involved in numerous physiological pathways directly or in the form of derivatives. This AA is the most abundant nitrogen carrier for tissue proteins and it is used in multiple biosynthetic pathways, involving key regulatory enzymes, such as arginase, nitric oxide synthase, arginyl-tRNA synthetase, among others (Wu, 2013a, 2010). As such, Arg serves as a precursor for the synthesis of creatine, ornithine, proline, glutamate, polyamines and nitric oxide, displaying remarkable metabolic and modulatory versatility in animal cells (Dai et al., 2012) and fish (Bogdan, 2015; Buentello and Gatlin, 1999; Pohlenz et al., 2014, 2013, 2012c)

The minimum dietary requirement for Arg, based on weight gain, in different fish species may vary between 1.0 and 3.1% of diet while for Nile tilapia the requirement has been reported at 1.2% of the diet (or 4.2% of crude protein) according to the NRC (2011). However, the fish minimum requirement for Gln is not currently available.

For a number of fish species, dietary supplementation of Gln and Arg has been shown to improve protein optimization and, hence growth performance. Optimization of somatic growth, feed efficiency and/or immune responses supported by dietary supplementation of Gln and/or Arg between 0.5% through 4% of diet have been reported in tilapia (Neu et al., 2016; Yue et al., 2013), blunt snout bream (Liang et al., 2016), Jian carp (Chen et al., 2015; Hu et al., 2015), golden pompano (Lin et al., 2015), yellow catfish (Zhou et al., 2015), channel catfish (Pohlenz et al., 2014, 2012a, 2012b, 2012c), hybrid striped bass (Cheng et al., 2012), and red drum (Cheng et al., 2011). Among these studies, the best results were found with Arg supplementation from 1.4 to 3.6% of the diet and/or when included along with supplemental Gln.

Tilapia *Oreochromis* sp. holds a notable position as it has favorable characteristics already well known to intensive production systems. Robustness, rapid growth, year-round production and great market acceptance make Nile tilapia the second most important fish in global aquaculture, and the most important cultured fish in Brazil accounting for 41% of the national aquaculture production (ACEB, 2014; FAO, 2016).

We designed this study aiming to investigate the effects of dietary glutamine and/or arginine supplementation on growth performance, innate immune responses, circulating AA profiles and whole-body AA composition of Nile tilapia *Oreochromis niloticus*. To the best of our knowledge, this is the first experiment to investigate the effects of Gln and Arg combined in diets for Nile tilapia.

2. Materials and methods

2.1. Experimental diets and feeding trial

A control diet was formulated from menhaden fishmeal and dehulled soybean meal to contain 36% crude protein and meet the tilapia's nutritional requirements based on the most recent publication of the National Research Council (NRC, 2011) (Table 1). That Control diet was analyzed to contain 0.37% glutamine and 2.01% arginine. Six experimental diets composed of the same ingredients were formulated to contain different levels of supplemental Gln and/or Arg (GLN 1%, GLN 2%, ARG 1%, ARG 2% and GLN + ARG 1%) as shown in Table 1. Diets were maintained iso-nitrogenous by adjusting the glycine (Gly) level as previously reported for fish (Buentello and Gatlin, 2000; Cheng et al., 2012). The Gly was chosen because it does not act as a precursor of arginine or glutamine in their metabolic turnover (Buentello and Gatlin, 2001, 2000), and it is structurally the simplest AA. Experimental diets provided arginine at a level above that previously established to meet the minimum requirement of Nile tilapia (NRC, 2011).

Table 1
Formulation and analyzed chemical composition of six experimental diets supplemented with arginine and/or glutamine for Nile tilapia.

Ingredient (g/100 g)	Control	GLN 1%	GLN 2%	ARG 1%	ARG 2%	GLN + ARG 1%
Menhaden fishmeal[a]	11.00	11.00	11.00	11.00	11.00	11.00
Soybean meal[b]	43.26	43.26	43.26	43.26	43.26	43.26
Dextrinized starch[c]	23.25	23.25	23.25	23.25	23.25	23.25
Soy oil[d]	4.58	4.58	4.58	4.58	4.58	4.58
Vitamin premix[e]	3.00	3.00	3.00	3.00	3.00	3.00
Mineral premix[e]	4.00	4.00	4.00	4.00	4.00	4.00
Carboxymethyl cellulose[c]	2.00	2.00	2.00	2.00	2.00	2.00
Calcium phosphate, dibasic[f]	1.00	1.00	1.00	1.00	1.00	1.00
DL-Methionine[g]	0.25	0.25	0.25	0.25	0.25	0.25
Glycine[g]	3.95	2.92	1.89	2.22	0.50	1.20
Cellulose[c]	3.71	3.74	3.77	4.44	5.16	4.46
Glutamine[g]	0.00	1.00	2.00	0.00	0.00	1.00
Arginine[g]	0.00	0.00	0.00	1.00	2.00	1.00
Analyzed composition (g/100 g dry sample)						
Dry matter	89.3	89.5	89.3	89.3	89.3	89.6
Crude Protein	36.0	36.3	36.0	36.3	35.7	35.7
Lipids	8.0	7.6	7.9	7.7	8.3	8.0
Ash	8.7	8.6	9.9	8.6	8.7	8.6
Glutamine[h]	0.37	1.17	1.59	0.27	0.28	0.81
Arginine[i]	2.01	2.37	2.17	3.56	4.38	3.34
Glycine[i]	1.49	1.28	1.01	0.94	0.72	0.85

[a] Crude protein 67.0%, crude lipid 15%, dry matter 92%.
[b] Crude protein 54.0%, crude lipid 4%, dry matter 94%.
[c] MP Biomedicals, Santa Ana, CA, USA.
[d] Commercial refined soybean oil, TX, USA.
[e] Moon & Gatlin III (1991).
[f] Fisher Scientific, Waltham, MA, USA.
[g] USB Corporation, Cleveland, OH, USA.
[h] Analyzed as non-protein bound amino acid.
[i] Analyzed as protein-bound amino acid.

All ingredients were weighed individually and mixed in a commercial V-mixer for 30 min for each experimental diet prior to the addition of oil and water and mixing for another 30 min. Pellets were manufactured by passing the moistened mixture through a 3-mm die using a Hobart meat grinder as described by (Webb and Gatlin, 2003). Lastly, pellets were broken and sieved to 2–3-mm in length and stored at −18 °C during the feeding trial.

The feeding trial was conducted in an indoor recirculation system at the Aquacultural Research and Teaching Facility, Texas A&M University, Texas, USA. The culture system was equipped with a biofilter for ammonia removal and sand filter for mechanical filtration. Water flow rate remained of 1 L min^{-1} throughout the experiment. Juvenile Nile tilapia, genetically modified to be all males, were obtained from a commercial producer (Louisiana Specialty Aquafarms. Robert, LA). A period of 2 weeks was applied to acclimate the fish to the experimental conditions during which all fish were fed the control diet. After the conditioning period, fish initially averaging 7.14 ± 0.15 g each were placed into 24, 38-L glass aquaria at a density of 15 fish. The six dietary treatments were each randomly assigned to four replicate aquaria, for a total of 60 fish per treatment. Fish were fed twice a day (at 8:00 am and 4:00 pm) for 9 weeks. The feeding rate was set at 5% of fish body weight during weeks 1 through 6 and 4% during weeks 7 through 9. Fish in each tank were weighted as a group each week and feed quantities adjusted accordingly.

Water quality parameters were monitored weekly during the feeding trial and measured according to APHA (1992) procedures.