Aquaculture 319 (2011) 247–252

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

# Effects of dietary arginine and glutamine on growth performance, immune responses and intestinal structure of red drum, *Sciaenops ocellatus*

Zhenyan Cheng [a,c], Alejandro Buentello [a,b], Delbert M. Gatlin III [a,b,*]

[a] Department of Wildlife and Fisheries Sciences, Texas A&M University System, College Station, TX 77843-2258, USA
[b] Intercollegiate Faculty of Nutrition, Texas A&M University System, College Station, TX 77843-2258, USA
[c] Tianjin Key Lab of Aqua-Ecology and Aquaculture, Department of Fishery Science, Tianjin Agricultural College, Tianjin 300381, PR China

ARTICLE INFO

*Article history:*
Received 13 July 2010
Received in revised form 22 April 2011
Accepted 17 June 2011
Available online 25 June 2011

*Keywords:*
Arginine
Glutamine
Red drum
*Sciaenops ocellatus*
Immune response
Intestinal morphology

ABSTRACT

Both arginine and glutamine play important roles in tissue repair, cell replication and collagen synthesis which impact animal growth and survival. Thus, a 7-week feeding trial was conducted to determine the effects of dietary arginine and glutamine on growth performance, innate immune responses and intestinal structure of juvenile red drum, *Sciaenops ocellatus* (6.9 ± 0.04 g, initial average weight). Protein in all experimental diets was derived from menhaden fishmeal and dehulled, solvent-extracted soybean meal to provide isonitrogenous (44% crude protein) and isolipidic (10%) diets. The basal diet contained 0.14% glutamine and 1.72% arginine on a dry-matter basis, which exceeds the established dietary requirement of red drum. Experimental diets were prepared by supplementing the basal diet with either arginine at 1% of dry weight, glutamine at 1%, glutamine at 2%, or both arginine and glutamine at 1%, with adjustments in glycine to maintain equal nitrogen among all diets.

Feed efficiency was significantly ($P<0.05$) improved by supplementation of glutamine at 2% and the combination of both arginine and glutamine at 1% of diet. Neutrophil oxidative radical production in fish fed the glutamine and/or arginine-supplemented diets was significantly ($P=0.03$) higher compared with that of fish fed the basal diet, with a synergistic effect observed in fish fed the combined arginine–glutamine diet. Significantly ($P<0.05$) higher serum lysozyme activity also was observed in fish fed the diet supplemented with 1% of both arginine and glutamine. Extracellular superoxide anion production by red drum macrophages was significantly ($P<0.05$) higher for fish fed diets with glutamine at either 1 or 2% and the 1% arginine–glutamine combination compared to fish fed the basal diet. Similarly, macrophages from fish fed the diet with glutamine at 2% and the arginine–glutamine combination at 1% produced significantly higher amounts of intracellular superoxide anion.

Morphometric analyses – i.e., measurements of enterocyte, microvillus and fold heights – demonstrated positive effects of both dietary glutamine and arginine in different portions (proximal, mid and distal) of the gastrointestinal tract of red drum. In general, fish fed the diet supplemented with 2% glutamine had the greatest increases in magnitude of the chosen structures, although those fed the diet with arginine at 1% also yielded improved scores for some enteric portions. Therefore, results from the present study establish the importance of both dietary arginine and glutamine supplementation in improving feed efficiency, as well as eliciting positive changes to several components of the innate immune system and intestinal functionality of red drum.



## 1. Introduction

Aquaculture continues to be the fastest growing food-producing activity in the world and, if current trends continue, aquafarms will produce more than 65% of the global supply of aquatic organisms including fish (FAO, 2009). As aquacultural production intensifies, the need for enhanced production efficiency and effective disease control measures becomes more readily apparent. Hence, research on interactions between nutrition and immunity has received increased attention in recent years (Verlhac Trichet, 2010). Among cultured marine fish species the red drum, *Sciaenops ocellatus*, possesses desirable characteristics that have established it as an important segment of the finfish aquaculture industry in the United States, and abroad.

Nutritional research has demonstrated that dietary protein in general, and amino acids in particular, play fundamental roles in the overall activity of the immune system of fish (Gatlin, 2002; Li et al., 2009). Glutamine is an abundant free amino acid in the plasma of

* Corresponding author. Tel.: +1 979 847 9333; fax: +1 979 845 4096.
*E-mail address:* dgatlin@nature.tamu.edu (D.M. Gatlin).

0044-8486/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2011.06.025

animals (Wu et al., 1994). It serves as a precursor for protein synthesis and donates nitrogen for the formation of purines, pyrimidines, nucleotides and amino sugars (Li et al., 2007). As a major energy substrate for leukocytes and a key modulator of cytokine and nitric oxide (NO) production, glutamine is crucial to the immune response in fish (Li et al., 2009). Satoh et al. (2003) indicated that glutamine was a key nutrient for the mammalian gut. Lin and Zhou (2006) also found that glutamine can improve weight gain, digestive enzyme activities and both intestinal structure and function of juvenile Jian carp, *Cyprinus carpio* var. Jian. Glutamine also has been shown to increase weight gain (Tannuri et al., 2000; Wu et al., 1996), exert powerful trophic effects on the gastrointestinal (GI) mucosa (Tannuri et al., 2000), prevent jejunal atrophy and normalize lymphocyte function (Chamorro et al., 2010), as well as activating intestinal epithelial cells in mammals (Wu et al., 1995).

Arginine, which is an abundant amino acid in tissue proteins (Wu et al., 1999), has fundamental roles in nitrogen metabolism, creatine, and polyamine synthesis, and is the major substrate for the production of NO (Jobgen et al., 2006). Thus, arginine plays important roles in the modulation of the immune response (Jobgen et al., 2006) and enhances immune function during immunological challenges (Calder and Yaqoob, 2004; Field et al., 2000). Buentello and Gatlin (1999, 2001) demonstrated that dietary arginine played a major role in the ability of macrophages to produce NO and elevated the resistance of channel catfish to the bacterial pathogen *Edwardsiella ictaluri*. In a different study, Buentello et al. (2007) provided evidence that hematological parameters and some aspects of the innate immune system of channel catfish were sensitive to changes in dietary arginine. Special consideration is due to arginine's unique role over polyamine biosynthesis because numerous studies have shown that the polyamines putrescine, spermidine, and spermine, have a significant effect on the growth of the GI mucosa of a variety of research animals (Larque et al., 2007; Loser et al., 1999), including fish (Péres et al., 1997). In addition, arginine has been shown to improve duodenal, jejunal and ileal weight and mucosal cell proliferation as well as restore intestinal absorptive function after ischemia in rats (Sukhotnik et al., 2005). Likewise, several studies on GI tract morphology of humans and other terrestrial animals have reported that dietary arginine alleviates gut mucosal injury (Liu et al., 2008), increases villus height (Hebiguchi et al., 1997) and decreases crypt depth (Wu et al., 1995). Hurt et al. (2006) demonstrated in rodents that diets supplemented with both glutamine and arginine enhanced the immunity of the gut mucosa and helped maintain intestinal tissue oxygenation and/or brush barrier function, as well as altered inflammatory processes, and improved systemic nitrogen balance. Therefore, the present study was designed to evaluate the effects of dietary supplementation of arginine and glutamine on growth performance, immune responses and intestinal structure of juvenile red drum.

## 2. Materials and methods

### 2.1. Experimental diets

For all diets, menhaden fishmeal and solvent-extracted dehulled soybean meal contributed protein (60 and 40% of total protein, respectively) for a total crude protein content of 44% of diet (Table 1). Dextrin and menhaden oil were included as carbohydrate and lipid sources, respectively, providing a total of 10% lipid and an estimated digestible energy level of 3.5 kcal/g. The basal diet was analyzed via high performance liquid chromatography as described by Buentello and Gatlin (2002) to contain 1.72% arginine and 0.14% glutamine. Four experimental diets were derived from the basal diet by supplementing either L-arginine hydrochloride (HCl) at 1% of diet, glutamine at 1% or 2%, or arginine-HCl and glutamine both at 1%, while adjusting glycine to maintain the diets isonitrogenous. These levels of supplementation were selected to be slightly above the previously determined nutritional requirement for arginine of red drum (1.4% of diet, Barziza et al., 2000) and still within physiological levels. As glutamine is not classified as an essential amino acid, there is no nutritional requirement for maximum growth demonstrated in fish species for this amino acid. Procedures for diet preparation and storage were as previously described by Barziza et al. (2000).

**Table 1**
Formulations and analyzed chemical composition of experimental diets (% dry matter).

| Ingredients | Basal diet | 1% Arginine | 1% Glutamine | 2% Glutamine | 1%Glutamine + 1%arginine |
|---|---|---|---|---|---|
| Menhaden fish meal[1] | 35.3 | 35.3 | 35.3 | 35.3 | 35.3 |
| Soybean meal[2] | 29.6 | 29.6 | 29.6 | 29.6 | 29.6 |
| Menhaden oil[3] | 5.5 | 5.5 | 5.5 | 5.5 | 5.5 |
| Dextrin[4] | 17.0 | 17.0 | 17.0 | 17.0 | 17.0 |
| Cellulose[4] | 0.8 | 1.6 | 0.9 | 0.9 | 1.6 |
| Carboxymethyl cellulose[4] | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Vitamin premix[5] | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Mineral premix[5] | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| Glycine[4] | 2.8 | 1.0 | 1.7 | 0.7 | 0 |
| Arginine-HCl[4] | – | 1.0 | – | – | 1.0 |
| Glutamine[4] | | | 1.0 | 2.0 | 1.0 |
| *Analyzed composition % dry wt.* | | | | | |
| Dry matter | 89.1 | 87.7 | 88.4 | 89.6 | 89.7 |
| Arginine | 1.72 | 2.30 | 1.66 | 1.74 | 2.00 |
| Glutamine | 0.14 | 0.17 | 0.91 | 1.61 | 0.83 |
| Glycine | 3.18 | 1.90 | 2.23 | 1.72 | 1.10 |
| Crude protein | 44.2 | 44.0 | 44.2 | 44.3 | 43.8 |
| Crude lipid | 9.4 | 9.9 | 9.8 | 9.9 | 9.7 |
| Ash | 11.7 | 11.9 | 11.5 | 11.4 | 11.4 |

[1] Special Select, Omega Protein, Houston, TX; Crude protein, 68.0% of dry matter; crude lipid, 10.8% of dry matter.
[2] Soybean meal, crude protein, 54.0% of dry matter; crude lipid, 2.4% of dry matter.
[3] Omega Protein, Reedville, VA.
[4] US Biochemical, Cleveland, OH.
[5] Same as Moon and Gatlin (1991).

### 2.2. Fish and feeding trial

Juvenile red drum were obtained from the Marine Development Center (Lake Jackson, TX) operated by the Texas Parks and Wildlife Department and were maintained indoors at the Texas A&M University Aquacultural Research and Teaching Facility. Fish were conditioned on a commercial diet (Ranger, Buhl, ID) and acclimated to experimental conditions for 1 week prior to the feeding trial. Fish of similar sizes ($6.9 \pm 0.04$ g) were randomly distributed as groups of 12 fish into 15 glass aquaria (38-L each) operated as a closed, recirculating system. Each diet was randomly assigned to three replicate aquaria. All groups of fish were fed their respective diets at the same fixed rate approaching satiation (initially 6% of body weight per day and gradually reduced to 3%) for 7 weeks. Each group of fish was weighed weekly and feed rations were adjusted accordingly.

Water temperature remained at $26 \pm 1$ °C throughout the trial by conditioning ambient air. Salinity was maintained at about 3‰ using well water and synthetic sea salt (Fritz Industries Inc., Dallas, TX). Water flow rate remained at approximately 1 L/min via a recirculating system in which adequate water quality was achieved through biological and mechanical filtration. Low pressure electrical blowers provided aeration to keep dissolved oxygen levels near saturation in each aquarium. A 12 h light:12 h dark photoperiod was maintained with fluorescent lights controlled by timers.

### 2.3. Sample collection and analysis

At the end of the feeding trial, all the fish from each aquarium were collectively weighed to obtain a final biomass. Two fish from each tank