EVALUATION OF POTENTIAL DIETARY ARGININE-LYSINE

ANTAGONISM IN

RED DRUM *Sciaenops ocellatus*

A Thesis

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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December 2013

Major Subject: Wildlife and Fisheries Sciences

ABSTRACT

Two feeding trials were conducted to evaluate possible dietary arginine-lysine antagonisms in the red drum (*Sciaenops ocellatus*). In the first feeding trial, seven dietary treatments with arginine levels of 1.0, 1.25, 1.5 (previously established requirement), 1.75, 2.0, 2.5 and 3.0% of dry diet with lysine at the previously established requirement of 1.6%, and three diets with arginine at 1.75% and lysine at 1.75, 2.05 and 2.65% of dry diet were evaluated. In the second trial, similar treatments were used except the highest level of arginine (2.8% of dry diet) was combined with a higher level of lysine (2.5% of dry diet) to evaluate low and high levels of arginine and lysine in a factorial arrangement. Each feeding trial was conducted with juvenile red drum in 38-L aquaria connected as a recirculating system with the first trial continuing for 9 weeks and the second trial for 8 weeks. In the first feeding trial, fish were stocked as groups of 12 fish initially averaging 1.57 g/fish and in the second trial groups consisted of 15 fish initially averaging 2.51 g/fish. The feeding rate was initially set at 6% of body weight per day and adjusted equally among dietary treatments as the fish grew to maintain a level close to satiation without overfeeding. Results from both feeding trials indicated that red drum fed the lowest dietary arginine level had the lowest weight gain. Other parameters such as feed efficiency, protein retention and plasma amino acid concentrations also showed that high levels of dietary arginine did not significantly increase fish performance or interfere with lysine utilization. In terms of dietary lysine, increasing concentrations above the previously determined requirement level tended to

increase growth performance of the fish, although a significant difference was only found in the first trial. Therefore, based on the results of both feeding trials, no antagonism between dietary arginine and lysine was evident in red drum.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Gatlin, and my committee members, Dr. Lawrence, and Dr. Bailey, for their guidance and support throughout the course of this research.

Thanks also go to Dr. Pohlenz and Mr. Brian Ray for their laboratory and field assistance, my friends and colleagues of the Fish Nutrition Laboratory and the department faculty and staff for making my time at Texas A&M University a great experience. I also want to extend my gratitude to the Fulbright committee, which provided the funding for my Master's degree here.

Finally, thanks to my parents for their encouragement and support, to Dian for her patience and love, and to my friends in Indonesia; Andrew and Aninda.

TABLE OF CONTENTS

LIST OF TABLES

1. INTRODUCTION

Red drum is an important fish species native to the Atlantic and Gulf coasts of the United States which is produced in aquaculture for food and recreational purposes. This species also is produced in other areas of the world including Ecuador, Israel and China (FAO, 2005). The FAO (2012) reported that in 2010 the global aquaculture production of red drum reached more than 50,000 ton per year.

Because red drum aquaculture has an increasing prospect in global markets, further efforts are required to strengthen this activity. One important aspect that should be thoroughly evaluated is the nutrient requirements for increasing growth efficiency. Determining the nutrient requirements of red drum is an important aspect that can reduce feed costs and thus directly affect general costs of its culture, given the fact that diet costs are typically over 50% of total production costs (Rana et al., 2009).

Red drum is a carnivorous species that eats fish and crustaceans in the wild. This feeding habit of many marine fish species typically means a higher dietary protein requirement. Previous research by Serrano et al. (1992) reported that red drum grow best if fed a diet with 40% crude protein. From this study, it was concluded that the dietary protein requirement of red drum is relatively high compared to other cultured fish such as channel catfish with its 26-32% protein requirement or tilapia with 35% protein in the diet being considered optimum (NRC, 2011).

Because amino acids are building blocks of protein, the amino acid requirements are significantly linked to the protein requirement of a species. In fact, feed formulations for some farmed animals are set to fulfill their essential amino acid requirements as a first priority instead of the crude protein requirement. While all the indispensable (essential) amino acids have important roles in various aspects of fish physiology, one of the amino acids often considered as the most limiting indispensable amino acid is lysine because its level in many feedstuffs is lower than the dietary requirement of the targeted species. Lysine is primarily used for protein synthesis. In addition, lysine is an important substrate for synthesis of carnitine, an amino acid compound which has responsibility for transport of long-chain fatty acids from the cytosol into mitochondria for oxidation. Carnitine has been established as being responsible for promoting growth, protecting against toxicity of ammonia and xenobiotics, and improving acclimatization to extreme temperature changes as well as enhancing reproduction performance (Harpaz, 2005).

Another amino acid that also has many important physiological functions is arginine. Arginine is considered an indispensable amino acid for several fish species (NRC, 2011) including sockeye salmon (Halver and Shanks, 1960), common carp, rainbow trout, and Japanese eel (Tacon, 1987). One of the main physiological actions of arginine is its function in enhancing the immune system. In addition, it possesses some insulinothropic activities which stimulate amino acid intake and protein synthesis in fish (Plisetskaya et al.1991). In rainbow trout, a deficiency of this compound can disrupt metabolism which later causes undesirable effects such as high mortality, fin erosion, and retarded growth (Ketola, 1983).

Assessments of arginine and lysine requirements of red drum were previously conducted by Barziza et al. (2000) and Craig and Gatlin (1992), respectively. It was reported that the arginine requirement of red drum was approximately 4.1 to 4.2% of dietary protein or 1.44 to 1.47% of a 35% crude protein diet (Barziza et al., 2000), and for lysine the requirement was estimated to be 4.43% of dietary protein or 1.55% of a 35% crude protein diet (Craig and Gatlin, 1992). More recently, it has been shown that dietary arginine above the minimum requirement level may have positive effects on immunological responses and disease resistance of certain fish species such as red drum (Cheng et al., 2011), hybrid striped bass (Cheng et al., 2012) and channel catfish (Pohlenz et al., 2012).

It has been observed in some animals that increasing levels of lysine in the diet may also increase the requirement for arginine (NRC, 2011) due to interactions between these two basic indispensable amino acids. Anderson and Dobson (1959) found that lysine was the amino acid that most likely influenced arginine requirements of chickens. A few years later, Jones (1964) showed that toxicity caused by high dietary levels of lysine was due to an arginine-lysine antagonism. Arginine-lysine antagonism caused several negative effects in chickens including depressed weight gain, deterioration of feed conversion ratio, hyper-irritability, leg tremors and leg weakness (Jones, 1961). Arginine-lysine antagonism also was potentially found in several different species such as dogs and rats (Ball et al., 2007). However, in studies that were conducted with pigs (Edmonds and Baker, 1987) and cats (Fascetti et al., 2004), an arginine and lysine antagonism was not found. There are only two arginine-lysine antagonism studies conducted with fish to date, and both of them gave different results. In fingerling channel catfish, Robinson et al. (1981) did not find any antagonist activity between those two amino acids; whereas, Kaushik and Fauconneau (1984) did find indications of arginine and lysine antagonism at least at the level of ureagenesis. Their study was based on the fact that lysine plays a dual role as an indispensable amino acid under certain physiological states and as an important intermediate in urea biosynthesis. Thus, they conducted their study to see the effect of different dietary levels of lysine and arginine on some parameters of nitrogen metabolism. While they found an arginine and lysine antagonism at the level of ureagenesis, Kaushik and Fauconneau (1984) were unable to establish that this antagonism may affect fish performance.

Because these previous studies concerning arginine and lysine antagonisms in different species have yielded variable results, it will be necessary to experimentally evaluate if red drum also may exhibit arginine and lysine antagonism. In addition, conducting this research also may allow re-evaluation of the minimum dietary requirements of the aforementioned amino acids as well as the effects of over supplementation on immunological responses.

2. METHODS

2.1 Experimental design

This study was conducted in two separate feeding trials in which diets containing different levels of arginine and lysine were evaluated. Experimental diets were formulated from semi-purified ingredients including red drum muscle, wheat gluten and crystalline amino acids to contain 35% crude protein and digestible energy at 3.5 kcal/g (Barziza et al., 2000; Webb and Gatlin, 2003).

In the first feeding trial, for the diets with different levels of arginine, the lysine level was maintained at 1.6% of diet (4.57% of dietary protein) which is considered the red drum's minimum dietary requirement according to Brown et al. (1988) and Craig and Gatlin (1992). Different inclusion levels of arginine were set at 1.00, 1.25, 1.50, 1.75, 2.00, 2.50 and 3.00% of dry diet to evaluate the effects of insufficient and excessive dietary arginine. The lowest dietary arginine level was set at 1.00% of diet which is lower than the minimum requirement of 1.44% of diet previously determined by Barziza et al. (2000). To evaluate excessive lysine levels, three diets was produced with lysine levels set at 1.75, 2.05 and 2.65% of diet with the arginine level fixed at 1.75% of diet. All other essential amino acids were formulated to have levels that met or exceeded the red drum's requirement for optimum growth (NRC, 2011).

In order to further evaluate potential arginine-lysine antagonisms, a second feeding trial was conducted with inclusion levels of arginine set at 0.80, 1.00, 1.25, 1.60, 1.80, 2.30 and 2.80% of dry diet with lysine maintained at 1.60% of diet. Two additional diets were prepared with lysine at 1.90 and 2.50% of diet to evaluate excessive lysine levels with the arginine level fixed at 1.60% of diet. Additionally, a 2 x 2 factorial design was achieved with diets containing arginine at 1.60 and 2.80% of diet in combination with lysine levels of 1.60 and 2.50% of dry diet to evaluate the interaction between these amino acids.

2.2 Diet preparation

Red drum muscle, wheat gluten and dextrinized starch were the main dietary ingredients, while a crystalline amino acid premix was formulated to be similar to the amino acid composition of red drum muscle tissue at 35% crude protein (Table 1). Diet formulations for Trial 2 differed slightly from those used in Trial 1 because a different batch of red drum muscle was included in those diets (Table 2). To make all the diets isonitrogenous, a premix with equal proportions of aspartic acid and glycine was used instead of a premix containing glutamate because of the potential synthesis of arginine from glutamate (Buentello and Gatlin, 2000). The amount of glutamate contributed by red drum muscle and wheat gluten was 1.47% of dry weight for the diets in both trials.

Diets were prepared by mixing all the dry ingredients in a V-mixer for approximately 30 minutes, and then mixing oil and water with the dry ingredients using a Hobart mixer. After the entire formulation was mixed homogenously, it was pelleted into 3-mm strands using a Hobart meat grinder attachment and broken into appropriate lengths manually by hand. The pellets were then dried overnight using a fan at 25-27^{0} C. The 3-mm pellets were ground and sieved into different sizes so they could be easily consumed by the fish as they grew.

Diets were analyzed in duplicate using AOAC (1990) procedures for crude protein, lipid, and ash. Diets also were analyzed for amino acids after acid hydrolysis with 6N HCl according to procedures described by Pohlenz et al. (2012). Based on the amino acid analysis of experimental diets (Tables 3 and 4) the targeted arginine and lysine values were achieved with analyzed values no more than 10% of formulated values and generally less than 4%.

2.3 Rearing conditions

The feeding trials were conducted at the Texas A&M University Aquacultural Research and Teaching Facility. Thirty glass aquaria of 38-liter volume were connected as a recirculating system consisting of a biofilter, settling chamber, and sand filter to maintain water quality within acceptable levels. Each diet was fed to three replicate groups of juvenile red drum (*Sciaenops ocellatus*), and the diet and aquarium assignments were made according to a completely random design. In Trial 1, each aquarium was initially stocked with 12 fish (initial group body weight of 21.3 – 26.1 g; average of 1.57 g/fish) and in Trial 2, 15 fish (initial group body weight of 34.3 – 43.1 g; average of 2.51 g/fish) were stocked.

Table 1. Formulations of the experimental diets, Trial 1.

Ingredients (g/100 g dry weight)	Diet designations									
	1% Arg 1.6% Lys	**1.25% Arg 1.6% Lys**	**1.5% Arg 1.6% Lys**	**1.75% Arg 1.6% Lys**	**2% Arg 1.6% Lys**	**2.5% Arg 1.6% Lys**	**3% Arg 1.6% Lys**	**1.75% Arg 1.75% Lys**	**1.75% Arg 2.05% Lys**	**1.75% Arg 2.65% Lys**
Red drum muscle[1]	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5
Wheat gluten[2]	11.3	11.3	11.3	11.3	11.3	11.3	11.3	11.3	11.3	11.3
Amino acid premix[3]	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3
Dextrinized starch[2]	30.3	30.3	30.3	30.3	30.3	30.3	30.3	30.3	30.3	30.3
Menhaden oil[4]	8.7	8.7	8.7	8.7	8.7	8.7	8.7	8.7	8.7	8.7
Vitamin premix[5]	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Mineral premix[6]	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Carboxymethyl cellulose[2]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Asp/Gly premix[7]	3.2	2.9	2.7	2.4	2.1	1.6	1.0	2.3	2.1	1.7
L-Lysine-HCl[8]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.6	0.9	1.5
L-Arginine[8]	0.0	0.3	0.5	0.8	1.0	1.5	2.0	0.8	0.8	0.8
Celufil[9]	14.3	14.3	14.3	14.3	14.4	14.4	14.5	14.3	14.2	14.0

[1] Wild-caught red drum, freeze dried, 91% (dry weight) crude protein; 7.4 % (dry weight) crude lipid
[2] MP Biomedicals LLC, Solon, OH
[3] See appendix A
[4] Omega Protein, Reedville, VA
[5] See appendix B and C
[6] See appendix D
[7] Asp:Gly; 50:50 used to make diets isonitrogenous.
[8] Affymetrix, Cleveland, OH
[9] USB Corporation, Cleveland, OH

Table 2. Formulations of the experimental diets, Trial 2.

Ingredients (g/100 g of dry weight)	Diet designations									
	0.80% Arg 1.6% Lys	**1% Arg 1.6% Lys**	**1.25% Arg 1.6% Lys**	**1.60% Arg 1.6% Lys**	**1.80% Arg 1.6% Lys**	**2.30% Arg 1.6% Lys**	**2.80% Arg 1.6% Lys**	**1.60% Arg 1.9% Lys**	**1.60% Arg 2.5% Lys**	**2.80% Arg 2.5% Lys**
Red drum muscle[1]	12.4	12.4	12.4	12.4	12.4	12.4	12.4	12.4	12.4	12.4
Wheat gluten[2]	11.3	11.3	11.3	11.3	11.3	11.3	11.3	11.3	11.3	11.3
Amino acid premix[3]	11.6	11.6	11.6	11.6	11.6	11.56	11.6	11.6	11.6	11.6
Dextrinized corn starch[2]	29.9	29.9	29.9	29.9	29.9	29.9	29.9	29.9	29.9	29.9
Menhaden oil[4]	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4
Vitamin premix[5]	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
Mineral premix[6]	3.8	3.8	3.76	3.8	3.8	3.8	3.8	3.8	3.8	3.8
Carboxymethyl cellulose[2]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Asp/Gly premix[7]	4.3	3.7	3.2	2.7	2.2	1.1	0.1	2.3	1.6	0
L-Lysine HCl[8]	0.66	0.66	0.66	0.66	0.66	0.66	0.66	1.00	1.66	1.66
L-Arginine[8]	0.00	0.24	0.47	0.71	0.94	1.41	1.88	0.71	0.71	1.88
Celufil[9]	13.0	13.2	13.5	13.8	14.1	14.7	15.3	13.9	14	14.4

[1] Wild-caught red drum, freeze dried, 88.2% (dry weight) crude protein; 10.5% (dry weight) crude lipid
[2] MP Biomedicals LLC, Solon, OH
[3] See appendix A
[4] Omega Protein, Reedville, VA
[5] See appendix B and C
[6] See appendix D
[7] Asp:Gly; 50:50 used to make diets isonitrogenous
[8] Affymetrix, Cleveland, OH
[9] USB Corporation, Cleveland, OH

Table 3. Analyzed amino acid profiles (% dry weight) of the experimental diets, Trial 1.

	Diet designations									
Essential amino acid	**1% Arg 1.6% Lys**	**1.25% Arg 1.6% Lys**	**1.5% Arg 1.6% Lys**	**1.75% Arg 1.6% Lys**	**2% Arg 1.6% Lys**	**2.5% Arg 1.6% Lys**	**3% Arg 1.6% Lys**	**1.75% Arg 1.75% Lys**	**1.75% Arg 2.05% Lys**	**1.75% Arg 2.65% Lys**
His	0.94	1.01	1.08	1.11	1.04	1.06	0.98	1.04	1.07	1.12
Arg	0.87	1.13	1.53	1.72	2.00	2.51	2.97	1.65	1.68	1.86
Thr	1.66	1.72	1.87	1.88	1.80	1.82	1.73	1.74	1.87	1.93
Lys	1.34	1.35	1.45	1.50	1.46	1.53	1.38	1.39	1.78	2.42
Tyr	1.10	1.15	1.28	1.20	1.13	1.16	1.17	1.18	1.24	1.27
Met	0.95	0.97	1.10	1.05	1.03	1.00	0.93	1.00	1.08	1.14
Val	1.84	1.89	2.07	2.13	1.99	2.06	1.89	1.85	2.00	2.12
Ile	1.81	1.85	2.00	1.97	1.90	1.97	1.83	1.79	1.96	2.07
Leu	2.98	3.13	3.42	3.38	3.21	3.36	3.23	3.03	3.38	3.48
Phe	1.39	1.49	1.53	1.55	1.51	1.51	1.43	1.46	1.49	1.61

Means of two replicate analyses

Table 4. Analyzed amino acid profiles (% of dry weight) of the experimental diets, Trial 2.

	Diet designations									
Essential Amino Acid	**0.80% Arg 1.6% Lys**	**1% Arg 1.6% Lys**	**1.25% Arg 1.6% Lys**	**1.60% Arg 1.6% Lys**	**1.80% Arg 1.6% Lys**	**2.30% Arg 1.6% Lys**	**2.80% Arg 1.6% Lys**	**1.60% Arg 1.9% Lys**	**1.60% Arg 2.5% Lys**	**2.80% Arg 2.5% Lys**
His	0.86	0.82	0.82	0.81	0.85	0.82	0.86	0.84	0.89	0.90
Arg	0.80	0.96	1.24	1.65	1.80	2.23	2.80	1.53	1.55	2.88
Thr	1.56	1.50	1.53	1.49	1.57	1.57	1.57	1.54	1.63	1.63
Lys	1.49	1.45	1.55	1.46	1.57	1.61	1.60	1.89	2.47	2.42
Tyr	1.00	0.92	0.90	0.90	0.94	0.90	0.93	0.89	0.98	0.95
Met	0.85	0.77	0.82	0.80	0.83	0.79	0.84	0.81	0.87	0.87
Val	1.69	1.65	1.68	1.61	1.75	1.70	1.68	1.70	1.80	1.74
Ile	1.66	1.60	1.65	1.57	1.68	1.70	1.68	1.65	1.75	1.71
Leu	2.82	2.68	2.76	2.65	2.81	2.86	2.85	2.74	2.93	2.87
Phe	1.22	1.14	1.16	1.14	1.20	1.18	1.22	1.20	1.41	1.26

Means of two replicate analyses

The feeding rate was initially set at 6% of body weight per day. Feed quantities were divided between morning and evening feedings, and the fish were fed 7 days per week. Fishes in each aquarium were collectively weighed weekly to measure biomass and survival. After each weekly sampling, the feeding rate was adjusted equally among all treatments to maintain a level approaching apparent satiation without overfeeding. The first feeding trial continued for 9 weeks and the second trial was terminated after 8 weeks.

2.4 Sample collection and data analysis

At the end of each feeding trial, all fish in each aquarium were weighed collectively to obtain final biomass. Three fish per aquarium were then bled 15 to 20 hours after the final feeding using 1-ml syringes coated with heparin to prevent the blood from clotting. Plasma was separated after centrifugation and stored at -80C until analysis of amino acid composition. Bled fish was then processed by collecting liver and muscle (fillet) samples. The liver was weighed to compute hepatosomatic index (HSI) which is the liver to body weight ratio, while the muscle was weighed to compute muscle ratio as another body condition index. Five additional fish per aquarium were euthanized by immersion in tricaine methanesulfonate (MS-222) at an excessive level and then collected for analysis of whole-body composition. Crude protein was estimated by measuring total nitrogen by the Dumas method (Ebeling, 1968) and multiplying by 6.25. Dry matter was determined by heating at 125°C for 3 h, and ash was quantified after heating at 650 °C for 3 h (AOAC, 1990). Crude lipid was detemined by chloroform and methanol extraction (Folch et al., 1957). Ultra-performance liquid chromatography (UPLC-Acquity system®, Waters™, Milford, MA) was used to analyze whole-body and plasma amino acids according to the procedures of Pohlenz et al. (2012). Similar samples were collected and analyzed in Trials 1 and 2 except whole-body composition was excluded in Trial 2 due to limited effects observed in Trial 1.

Response variables including weight gain, feed efficiency, survival, HSI, muscle ratio, and amino acid composition of whole-body and plasma tissues were subjected to analysis of variance using JMP (version 10.0.2, SAS Institute, Inc., Cary, North Carolina, USA) to determine the effects of excessive and insufficient dietary arginine and lysine levels on red drum. Treatment effects were considered significant at $P \leq 0.05$.

3. RESULTS

3.1 Trial 1

During the course of the 9-week feeding trial, red drum fed all experimental diets grew well and exhibited high feed efficiency and survival values. Analysis of variance did not show a significant effect of dietary arginine on weight gain although there was a tendency for fish fed the diet with the lowest arginine level (1% of diet) to have the lowest weight gain (Table 5). Analysis of feed efficiency values did show significant ($P = 0.0015$) differences among some of the dietary treatments (Table 5). The lowest feed efficiency was observed in fish fed the diet with arginine at 1%, while the highest feed efficiency was observed in fish fed the diet with arginine at 1.25%. In terms of survival, significant ($P = 0.034$) differences were observed with the highest value achieved by fish fed the diet with arginine at 2.50% and the lowest survival was observed in fish fed the diet with arginine at 1% (Table 5). Protein efficiency ratio (PER) values of red drum fed the various diets also were significantly ($P = 0.0041$) different. The lowest PER was observed in fish fed the basal diet with arginine at 1% of diet while the highest PER value was exhibited by fish fed the diet with arginine at 1.25%.

In regard to feeding different lysine levels at a constant arginine level of 1.75%, significant ($P = 0.005$), differences in weight gain were observed but there was no detrimental effect of increasing dietary lysine (Table 6). In fact, fish fed the diet with the highest lysine level (2.65% of diet) had the greatest weight gain and feed efficiency compared to fish fed diets with lysine at 1.65 and 1.75% of diet (Table 6). Survival, protein efficiency ratio (PER), whole-body protein, muscle protein and protein retention values did not show any significant differences in this trial (Table 6).

Plasma concentrations of arginine, ornithine, citrulline, glutamine and glutamate did not show any significant differences among fish fed the various diets with different arginine levels (Table 7). Fish fed the diet with arginine at 1.75% tended to have the highest values for all of these plasma amino acids. Plasma lysine of fish fed diets with different lysine levels also did not show any significant differences (Table 8). The various dietary lysine concentrations did not significantly affect plasma arginine levels.

3.2 Trial 2

In the second trial, diets with graded levels of arginine did not produce significant differences in weight gain of juvenile red drum, although the lowest weight gain was achieved by fish fed the lowest arginine level (0.80% of diet) and the greatest weight gain was produced by fish fed the highest arginine level (Table 9). Similar to the weight gain responses, feed efficiency did not show any significant differences among treatments. Survival of fish fed all dietary treatments was high at over 91.1%.

In terms of responses to different dietary lysine levels, growth performance of red drum correlated positively with higher lysine level in the diet ($p = 0.04$). Feed efficiency responses were generally similar to the weight gain responses but not significantly different (Table 10). Thus, it was apparent that incremental levels of dietary lysine at a constant dietary arginine level did not adversely affect weight gain or feed efficiency of red drum in the present trial.

Table 5. Effect of different arginine levels on weight gain, feed efficiency, survival, protein efficiency ratio (PER), and protein retention of red drum, Trial 1.

Arg (% of diet)[1]	**Weight gain (% initial wt.)**	**Feed efficiency (g gain/g feed)**	**Survival (%)**	**PER (g wt. gain/g protein fed)**	**Protein retention (%)**	**Whole-body protein (% fresh w.t)**	**Muscle protein (% fresh wt.)**
1.00	806	0.73^{a}	84.4^{ab}	1.70^{a}	35.7	16.9	23.4
1.25	1009	0.87^{d}	93.3^{bc}	2.11^{c}	38.7	16.5	23.4
1.50	936	0.79^{b}	84.4^{ab}	1.80^{ab}	38.2	17.0	23.6
1.75	965	0.80^{bc}	88.9^{abc}	1.87^{abc}	35.8	17.2	25.0
2.00	1005	0.84^{cd}	80.0^{a}	1.88^{ab}	37.7	16.0	22.2
2.50	1031	0.84^{bcd}	95.6^{c}	1.99^{bc}	33.5	16.1	23.1
3.00	968	0.81^{bc}	86.7^{abc}	1.79^{ab}	33.8	16.0	22.1
Pooled SE[2]	82.9	0.029	5.25	0.10	2.98	1.89	2.62
$Pr > F$[3]	0.077	0.002	0.034	0.004	0.256	0.959	0.859

Values are means of three replicate groups.
[1]Lysine level in all diets was set 1.6% of diet.
[2] Pooled standard error.
[3] Probability associated with the F statistic.

Table 6. Effect of different lysine levels on weight gain, feed efficiency, survival, protein efficiency ratio (PER), and protein retention of red drum, Trial 1.

Lys (% of diet)[1]	**Weight gain (% initial wt.)**	**Feed efficiency (g gain/g feed)**	**Survival (%)**	**PER (g wt. gain/g protein fed)**	**Protein retention (%)**	**Whole-body protein (% fresh wt.)**	**Mucle protein (% fresh wt.)**
1.60	936[ab]	0.80[a]	88.9	1.87	35.8	17.3	25.0
1.75	810[a]	0.77[a]	84.4	1.84	37.9	16.6	23.0
2.05	1020[bc]	0.84[b]	84.4	1.81	36.5	16.7	22.8
2.65	1120[c]	0.88[b]	91.1	1.97	36.0	17.3	23.9
Pooled SE[2]	73.46	0.21	6.09	0.11	6.34	1.70	2.65
Pr > F[3]	0.005	0.002	0.482	0.402	0.977	0.931	0.910

Values are means of three replicate groups.
[1] All diets had arginine set at 1.75% of diet.
[2] Pooled standard error.
[3] Probability associated with the F statistic.

Table 7. Plasma amino acid concentrations of red drum fed diets with different arginine levels, Trial 1.

Arg (% of diet)	Plasma amino acid concentration (mmol/mL)				
	Arg	Orn	Cit	Gln	Glu
1.00	32.7	7.93	5.41	508	36.5
1.25	35.5	7.05	3.66	359	30.9
1.50	64.8	9.90	5.87	540	47.7
1.75	69.8	13.71	9.49	870	61.5
2.00	56.0	7.94	5.87	411	27.6
2.50	58.0	12.56	6.21	593	42.4
3.00	49.8	11.21	4.12	467	33.1
Pooled SE[1]	25.5	3.06	2.59	174.6	14.95
Pr > F[2]	0.52	0.121	0.22	0.06	0.163

Values are composite samples from three fish in each of three replicate groups.
[1] Pooled standard error.
[2] Probability associated with the F statistic.

Table 8. Plasma arginine and lysine concentrations of red drum fed diets with different lysine levels, Trials 1.

Lys (% of diet)	Plasma lysine concentration (mmol/mL)	Plasma arginine concentration (mmol/mL)
1.6	101.4	69.8
1.75	69.9	37.3
2.05	55.7	38.2
2.65	85.5	44.8
Pooled SE[1]	30.9	28.4
Pr > F[2]	0.26	0.50

Values are means of three replicate groups.
[1] Pooled standard error.
[2] Probability associated with the F statistic.

Factorial analysis also was performed to evaluate any potential main effects and interactions between dietary arginine and lysine on red drum weight gain and feed efficiency (Table 11). There was a significant ($p = 0.009$) effect of increasing lysine on growth performance of red drum but no apparent interaction between the two amino acids based on this factorial analysis.

Table 9. Effects of different arginine levels on weight gain and feed efficiency of red drum, Trial 2.

Arg (% of diet) [1]	**Weight gain (% of initial body weight)**	**Feed efficiency (g gain/g feed)**	**Survival (%)**
0.80	543	0.70	95.6
1.00	623	0.76	100
1.25	570	0.75	93.3
1.60	564	0.73	95.6
1.80	537	0.70	100
2.30	597	0.75	93.3
2.80	616	0.75	93.3
Pooled SE[2]	44.94	0.04	6.51
Pr > F[3]	0.185	0.246	0.702

Values are means of three replicate groups.
[1] All diets had lysine set at 1.60% of diet.
[2] Pooled standard error.
[3] Probability associated with the F statistic.

Table 10. Effects of different lysine levels on weight gain and feed efficiency of red drum, Trial 2.

Lys (% of diet) [1]	**Weight gain (% of initial body weight)**	**Feed efficiency (g gain/g feed**	**Survival (%)**
1.6	565[a]	0.73	95.6
1.9	617[ab]	0.76	97.8
2.5	685[b]	0.80	91.1
Pooled SE[2]	458	0.04	3.84
Pr > F[3]	0.04	0.07	0.178

Values are means of three replicate groups.
[1] All diets had arginine set at 1.75% of diet.
[2] Pooled standard error.
[3] Probability associated with the F statistic.

Table 11. Factorial analysis of weight gain and feed efficiency of red drum fed low and high levels of arginine and lysine in a factorial arrangement, Trial 2.

Arg (% of diet)	**Lys (% of diet)**	**Weight gain (% of initial weight)**	**Feed efficiency (%)**
1.6	1.6	564[a]	73.0[a]
1.6	2.5	686[b]	79.7[ab]
2.8	1.6	616[ab]	75.3[a]
2.8	2.5	716[b]	84.3[b]
Pooled SE[1]		55.7	3.98
		Means of main effect	
1.6		624	76.4
2.8		665	80.0
	1.6	589	74.2
	2.5	700	82.0
		Anova: *Pr* > F[2]	
Arg		0.235	0.174
Lys		0.009	0.009
Arg x Lys		0.7418	0.625

Values are means of three replicate groups.
[1] Pooled standard error.
[2] Probability associated with the F statistic.

4. DISCUSSION

The data from Trials 1 and 2 showed that increasing levels of either arginine or lysine in the diet did not negatively affect growth performance of juvenile red drum. This finding is in accordance with the result from Robinson et al. (1981) with channel catfish. In their research on channel catfish, elevated levels of dietary lysine (up to four times the established requirement) with either adequate or sub-optimal levels of arginine did not have negative effects on weight gain or feed efficiency. Likewise, Robinson et al. (1981) did not observe any negative effects of dietary arginine at levels up to four times the established requirement on growth performance of channel catfish fed diets somewhat limiting (75% of established requirement) in lysine.

In regard to the arginine requirement of red drum, growth responses of fish in both feeding trials in the current study revealed limited effects of the graded levels of dietary arginine. The study of Barziza et al. (2000) reported arginine requirements of red drum ranging from 1.44% to 1.75% in diets containing 35% crude protein depending on whether weight gain, feed efficiency or PER was the measured response variable. Thus, the arginine levels chosen in the current study ranged from marginally deficient to almost twice the previously established requirement. In Trial 1, the highest numerical values for weight gain, feed efficiency and PER were achieved by fish fed the diet with arginine at 1.25% of diet which is somehow lower than the previously determined requirement value for red drum by Barziza et al. (2000). Results from trial 2 also indicated that dietary arginine levels below the previously established requirement supported normal growth responses of juvenile red drum. No overt signs of arginine deficiency were observed in either feeding trial of the current study. In the study of Barziza et al. (2000), signs of severe deficiency were not observed even in red drum fed the basal diet with arginine at 0.65%.

This study also found that excessive dietary levels of arginine did not reduce or enhance growth performance of red drum. This finding was in accordance with study of Pohlenz et al. (2012) who found that arginine up to 4% of diet did not affect growth performance of channel catfish. Interestingly, a study conducted by Cheng et al. (2012) in which different dietary arginine levels were fed to hybrid striped bass showed that diets with arginine at up to 4% of diet did provide significantly better growth performance. Such growth enhancement was not observed by red drum in either trials of the current study. In the study of Barziza et al. (2000), red drum fed the diet with the highest arginine level of 2.75% had the greatest numerical weight gain and feed efficiency compared to fish fed diets with lower levels of arginine, but statistical differences were obtained only for feed efficiency. However, in the present study, feeding diets with excessive levels of arginine with constant lysine, did not reduce or enhance growth performance of red drum.

Regarding the effects of different dietary lysine levels on red drum, it was found that increasing dietary lysine to over 1.6 times the previously established minimum requirement of 1.55% of diet by Craig and Gatlin (1992) at a constant arginine level did not negatively affect the fish. In fact, at the highest lysine level of 2.65% of diet, there as an improvement in weight gain of red drum in both trials. However, given that lysine is typically one of the most limiting amino acids in fish diets and the growth enhancement observed at the highest level of supplementation was moderate, supplementing diets with such a high level of lysine is not likely. There were no obvious negative effects of supplementing higher levels of lysine on red drum in the present study. This was another indication that arginine-lysine antagonism was not present in red drum.

5. CONCLUSIONS

In this study, it was found that excess arginine (up to 3% of diet) and lysine (up to 2.5% of diet) did not resulted in any signs of an arginine-lysine antagonism in juvenile red drum based on growth performance and plasma amino acid levels. Increasing levels of lysine at a constant arginine level did not adversely affect growth performance of the fish but tended to improve it with lysine up to 2.5% of diet.

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APPENDIX A

Amino acid premix.

Ingredients	**g/ 100 g**
Glycine	11.92
Histidine	3.76
Isoleucine	6.16
Leucine	9.6
Methionine	4.08
Phenylalanine	2.16
Tyrosine	4.32
Serine	2.16
Threonine	7.04
Tryptophan	1.92
Valine	5.84
Proline	15.76
Alanine	15.84

APPENDIX B

Vitamin premix.

Complete ingredients	Concentration (g/kg)	Concentration at 3% of diet
Ascorbic acid	50.0	1500 mg/kg
Di-calcium pantothenate	5.0	150 mg/kg
Choline chloride	36.2	3000 mg/kg
Inositol	5.0	150 mg/kg
Menadione Sodium bisulfide	2.0	60 mg/kg
Niacin	5.0	150 mg/kg
Pyridoxine hydrochloride	1.0	30 mg/kg
Riboflavin	3.0	90 mg/kg
Thiamine monocitrate	0.5	15 mg/kg
Di-alpha-tocopherol acetate (250 IU/g)	8.0	60 IU/kg
Vitamin A palmitrate (500,000 IU/g)	0.2	3000 IU/kg
Vitamin micro-mix[1]	10.0	
Cellulose	874.1	

[1] see appendix C

APPENDIX C

Vitamin micro-mix

Complete ingredients	Concentration (g/kg)	Concentration at 3% of diet
Biotin	0.50	1.50 mg/kg
Folic acid	1.80	5.40 mg/kg
Vitamin B-12	0.02	0.06 mg/kg
Cholecalciferol	0.02	2400 mg/kg
Cellulose	97.66	

APPENDIX D

Mineral premix.

Ingredients	**Concentration (g/kg)**
$Ca(C_6H_{10}O_6)\cdot 5H_2O$	348.49
$Ca(H_2PO_4)_2\cdot H_2O$	136.00
$FeSO_4\cdot 7H_2O$	5.00
$MgSO_4\cdot 7H_2O$	132.00
K_2HPO_4	240.00
$NaH_2PO_4\cdot H_2O$	88.00
$NaCl$	45.00
$AlCl_3\cdot 6H_2O$	0.15
KI	0.15
$CuSO_4\cdot 5H_2O$	0.50
$MnSO_4\cdot H_2O$	0.70
$CoCl_2\cdot 6H_2O$	1.00
$ZnSO_4\cdot 7H_2O$	3.00
Na_2SeO_3	0.01