# THE DIETARY TRYPTOPHAN REQUIREMENT OF

# JUVENILE RED DRUM

An Undergraduate Research Scholars Thesis
by
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Submitted to Honors and Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by
Research Advisor: Dr. Delbert M. Gatlin III

May 2015

Major: Wildlife and Fisheries Sciences

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# ABSTRACT

The Dietary Tryptophan Requirement of Juvenile Red Drum. (May 2015)

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The red drum, *Sciaenops ocellatus*, is cultured in the United States for both stock enhancement and food production. Ongoing studies in this laboratory are quantifying optimum dietary levels of indispensable and dispensable amino acids of red drum in order to compile a complete set of nutritional requirements. The present study was conducted to determine the optimum dietary tryptophan requirement of red drum. Groups of twelve red drum fingerlings, of initial average weight of 1.5- 2.5 g each were placed into each of 28, 38-L aquaria containing brackish water (7ppt) prepared from well water and a mixture of stock salt and commercial synthetic seawater. Seven semi-purified diets composed of red drum muscle and crystalline amino acids were supplemented with incremental levels of tryptophan. Each diet was given to triplicate groups of fish which were weighed every week to observe health and growth as influenced by dietary tryptophan. Water quality samples were also observed multiple times per week. At the end of the 39-day feeding period, the optimal tryptophan requirement of red drum was estimated based on weight gain, feed efficiency and protein conversion efficiency. Red drum fed the basal diet without adequate tryptophan exhibited significantly ($P \leq 0.05$) reduced weight gain, feed efficiency and survival compared to fish fed the other diets. However, no other specific signs of

deficiency were observed. All of the diets supplemented with tryptophan from 0.25 to 0.75% supported similar growth performance. Based on these data, the minimum dietary tryptophan requirement of red drum was determined to be 0.25% of dry diet. This information can be used in formulating diets for red drum to ensure adequate levels of tryptophan are provided.

## ACKNOWLEDGEMENTS

Great appreciation goes out to Brian Ray, visiting professors, and graduate students at the Aquacultural Research and Teaching Facility at Texas A&M University. We are also grateful to Texas A&M AgriLife Research, the Texas Sea Grant College Program, the Department of Wildlife and Fisheries Sciences at Texas A&M University, and the Undergraduate Research Scholars program at Texas A&M University.

# CHAPTER I

# INTRODUCTION

The red drum, *Sciaenops ocellatus,* is a popular game fish in the Gulf of Mexico and is receptive to most kinds of bait. They thrive in brackish, relatively shallow warm waters that are found along the Texas coast. Red drum are euryhaline and thus also can live in fresh water and thrive in lakes near Waco and San Antonio, Texas. However, the majority of red drum in Texas are harvested by recreational anglers in estuaries along the Gulf of Mexico (Allen and Southwick, 2007). Recreational anglers may keep three red drum per day between 20 and 28 inches (50 to 70 cm).

An increased demand for red drum in the early 1980s resulted in overfishing of wild populations in the Gulf of Mexico which detrimentally affected their populations. As a result, Texas the commercial harvest of red drum has remained closed since the mid-1980s, and state-operated facilities began production of red drum juveniles for enhancement of the depleted stocks (Matlock, 1987; Gatlin, 2000). The species is also produced in the southeastern United States and in parts of Asia via aquaculture as a food source. Red drum has proven to be an excellent species for aquaculture due to its tolerance of a wide range of salinities and other environmental conditions (Gatlin, 2000). Because of the increase in aquacultural production of this species, there is a demand to understand its dietary requirements to maximize growth, development, and harvesting yields.

Artificial diets are provided to this species during culture to maximize its growth. As such, it is important that the species' requirements for essential nutrients be provided at optimal levels in the diet.

One of the most important nutrient groups is the essential amino acids which are required for normal growth and health due to their involvement in protein synthesis. If the organism does not consume proper amounts of all essential amino acids, it will potentially experience impaired protein synthesis, weight loss, stunted growth, and a multitude of other health problems, ultimately resulting in death. The dietary tryptophan requirement for the red drum is currently unknown. Therefore, the objective of this study was determination of the minimum dietary level of this amino acid to ensure maximum health and growth of juvenile red drum.

# CHAPTER II

# METHODS

## Fish and Facilities

Animal care and experimental protocols were conducted in accordance with the Institutional Animal Care and Use Committee. Juvenile red drum were obtained from a marine hatchery operated by Texas Parks and Wildlife Department. Temperature was maintained at 26 C and fluorescent lighting was set to simulate a 12 hour light: 12 hour dark diurnal cycle. Twelve red drum fingerlings (average weight of 1.5-2.5 g) were stocked into 38-L aquaria containing brackish water (7 ppt) prepared from well water and a mixture of stock salt and commercial synthetic seawater. The aquaria were connected as a recirculating system with biological and mechanical filtration to maintain adequate water quality conditions. Fish were acclimated to the system for 1 week prior to initiation of the feeding trial and fed twice daily to apparent satiation.

## Experimental Diets

Experimental diets were formulated to contain 36% crude protein, 10% lipid, and a 15.7 kJ digestible energy/g. Lyophilized red drum muscle served as the only intact protein source in the experimental diets and made up 10% of the formulated 35% total crude protein and contributed tryptophan at 0.15% of dry weight. Six other semi-purified diets composed of red drum muscle and crystalline amino acids were supplemented with incremental levels of tryptophan to provide 0.25%, 0.35%, 0.45%, 0.55%, 0.65%, and 0.75% of dry weight. The amino acid premix was formulated to simulate 35% crude protein from red drum muscle except for tryptophan which was not included in the premix. All diets were kept isonitrogenous by adjusting the levels of

aspartic acid to glutamic acid. Additionally, diets contained a mineral premix and a vitamin premix to meet or exceed all known nutritional requirements of warm water species (NRC 2011).

Diets were prepared by mixing dry ingredients in a V-mixer for 1 hour. The dry mixture was then mixed together with menhaden oil and water using a Hobart feed mixer. The complete mixture was then extruded into 3-mm strands using the Hobart grinder attachment and broken into appropriate lengths by hand before drying overnight with forced air at 25˚C. The diets were analyzed for dry matter content and stored at -18 C with smaller quantities stored at 4 C until fed. Diets will be analyzed in duplicate using AOAC procedures to confirm the formulated values of crude protein, lipid, ash, and energy contents.

Each diet was randomly assigned to an aquarium of fish that were fed twice daily half of the daily ration. Triplicate groups of fish were fed each experimental diet. Fish in each aquarium were fed twice a day to apparent satiation, equating to 5% and, later, 4% of body weight per day on a dry-matter. Fish were group weighed every week to evaluate health and growth as influenced by dietary tryptophan. The feeding trial continued for a total of 39 days.

**Table 1.** Basal Diet Composition

| Ingredient | g/100g Dry Weight |
|---|---|
| Red drum muscle | 13.1 |
| Amino acid premix | 21 |
| Dex. Starch | 35 |
| Menhaden Oil | 7.4 |
| Vitamin Premix | 3 |
| Mineral Premix | 4 |
| CMC | 1 |
| Calcium Phosphate | 1 |
| Asp/Glu premix | 1 |
| Tryptophan | 0 |
| Glycine | 3.5 |
| Celufil | 10 |

**Sample Collection and Analyses**

At the termination of the 39-day trial period all fish in each aquarium were collectively weighed. Three fish per aquarium were bled, killed, and dissected to determine body condition indices. The condition indices determined included: muscle ratio, hepatosomatic index, and intraperitoneal fat. Blood also was collected using heparinized syringes. Samples were then centrifuged and plasma separated.

All data were subjected to one-way analysis of variance using the Statistical Analysis System. Significance set at $P< 0.05$.

# CHAPTER III

# RESULTS

All initial measurements were not statistically different. The basal diet with 0.15% tryptophan was deficient for red drum based on their reduced growth response (Table 2). Red drum fed the basal diet grew almost half as much as those fed greater levels of tryptophan. No statistical differences were found among fish fed the other dietary tryptophan levels. The survival of fish fed all diets was reduced due to a chronic bacterial infection but differences among the dietary treatments were not apparent. Significant statistical differences were observed in fish fed the basal diet compared to those fed all other diets with regard to feed efficiency ratio (Table 2). There were also significant differences in condition factor, hepatosomatic index, and intraperitoneal fat ratio in response to different dietary tryptophan levels (Table 2).

**Table 2.** Growth performance, feed utilization and survival, and body condition indices

| Variables | Dietary Tryptophan (Percentage of Dry Weight) | | | | | | | P>F | PSE |
|---|---|---|---|---|---|---|---|---|---|
| | 0.15% | 0.25% | 0.35% | 0.45% | 0.55% | 0.65% | 0.75% | | |
| Initial Avg. Fish Weight (g) | 5.90 | 5.55 | 5.34 | 6.01 | 5.34 | 5.69 | 5.94 | 0.0831 | 0.1812 |
| Final Avg. Fish Weight (g) | $16.25^{B}$ | $26.17^{A}$ | $24.66^{AB}$ | $29.98^{A}$ | $25.63^{A}$ | $25.5^{A}$ | $27.09^{A}$ | 0.0031 | 1.753 |
| Weight Gain (%) | $175.68^{B}$ | $363.33^{A}$ | $361.82^{A}$ | $399.00^{A}$ | $379.56^{A}$ | $349.16^{A}$ | $355.96^{A}$ | 0.0008 | 26.60 |
| Feed Efficiency Ratio | $0.37^{B}$ | $0.82^{A}$ | $0.78^{A}$ | $0.73^{A}$ | $0.71^{A}$ | $0.73^{A}$ | $0.80^{A}$ | <0.0001 | 0.0344 |
| | | | | | | | | | |
| Hepatosomatic Index | $4.40^{AB}$ | $4.40^{AB}$ | $3.65^{C}$ | $3.57^{C}$ | $3.70^{BC}$ | $5.10^{A}$ | $4.41^{AB}$ | 0.0027 | 0.2369 |
| IPF Ratio | $0.29^{B}$ | $0.61^{AB}$ | $0.29^{B}$ | $0.48^{AB}$ | $0.40^{AB}$ | $0.90^{A}$ | $0.84^{A}$ | 0.0044 | 0.1073 |
| Muscle Ratio | 26.30 | 30.01 | 28.85 | 29.31 | 30.50 | 30.46 | 31.81 | 0.1155 | 1.1943 |
| Survival (%) | 69.44 | 83.76 | 77.78 | 58.33 | 66.66 | 66.67 | 83.33 | 0.09 | 6.12 |

The minimum dietary requirement of juvenile red drum for tryptophan was estimated to be 0.25% of dry weight (Figure 1).

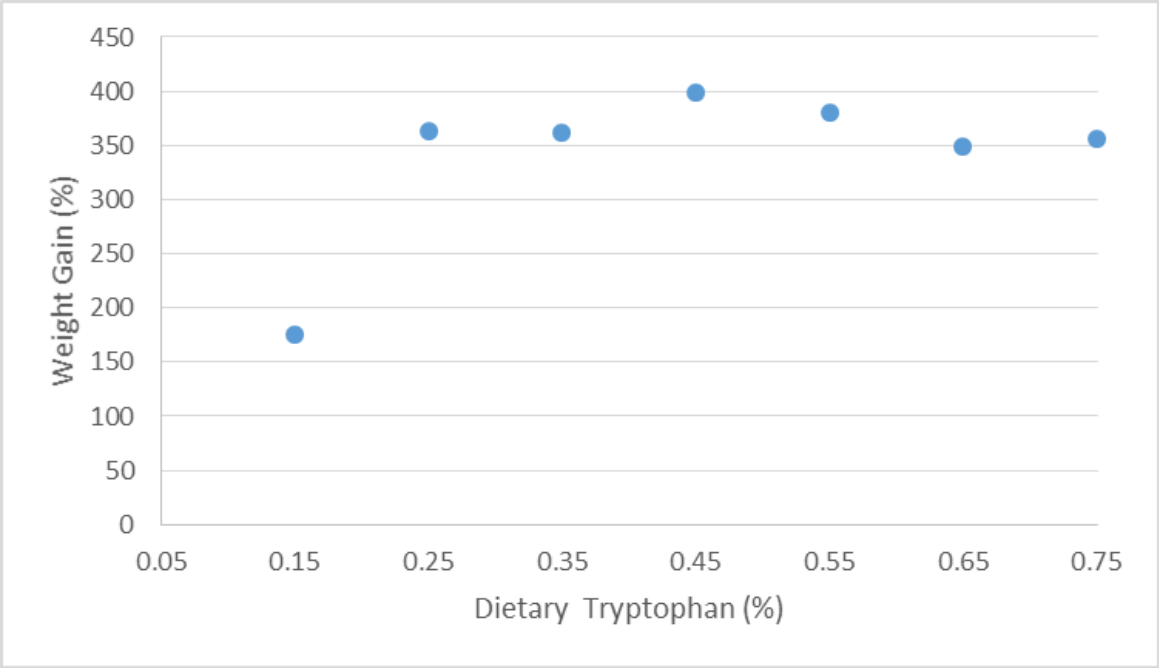


**Figure 1.** Weight gain (%) v. Dietary Tryptophan (%)

Free tryptophan in the plasma of red drum was responsive to dietary tryptophan supplementation (Figure 2). Red drum fed the basal diet did not have detectable levels of tryptophan in their plasma; whereas, fish fed the other diets showed incremental levels of free tryptophan in their plasma.

# CHAPTER IV

# DISCUSSION

The dietary tryptophan requirement in common warm water species varies from 0.1 to 0.4 % of dry weight (NRC, 2011). The estimated tryptophan requirement from the current experiment with red drum falls within this range for all of the response variables examined (0.15-0.75% dry diet). Interestingly, a tryptophan deficiency could be clearly observed with the basal diet. This deficiency began to appear within the first few weeks of the trial.

Coloso et al. (2004) concluded that Asian sea bass (*Lates calcarifer*) had a significantly reduced growth when fed a diet containing tryptophan at less than 0.2%. Below this threshold, feed efficiency decreased and HSI increased. No other signs of deficiency were observed in Asian sea bass in response to graded levels of tryptophan. Coloso et al. (1992) also determined that impaired growth and impaired feed conversion in milkfish (*Chanos chanos*) was attributed to tryptophan deficiency, but mortality was not significantly affected. It was determined by Kim et al. (1987) that the dietary tryptophan requirement of rainbow trout was between 0.2 and 0.25% of diet. In that study, dietary tryptophan concentrations at or above the requirement level resulted in optimal nitrogen retention and feed conversion. Rodehutscord et al. (1997) determined the tryptophan requirement of rainbow trout to be 0.2% of diet. The current trial correlates well with conclusions made by Brown (1995) who concluded that hybrid striped bass responded best to a dietary tryptophan inclusion rate of 0.2 % of diet. Thus, dietary tryptophan requirement of red drum in the current study falls in the range estimated for other carnivorous fish.

# CHAPTER V

# CONCLUSIONS

Red drum were responsive to dietary tryptophan supplementation with fish fed the basal diet exhibiting significantly ($P \leq 0.05$) reduced weight gain, feed efficiency ratio and protein efficiency ratio compared to fish fed the other diets with tryptophan levels of 0.25% and greater. However, no other specific signs of deficiency were observed. All of the diets supplemented with tryptophan from 0.25 to 0.75% supported similar growth performance. Based on these data, the minimum dietary tryptophan requirement of red drum was determined to be 0.25% of dry diet. This information can be used in formulating diets for red drum to ensure adequate levels of tryptophan.

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