DIETARY HISTIDINE REQUIREMENT AND PHYSIOLOGICAL EFFECTS OF DIETARY HISTIDINE DEFICIENCY IN JUVENILE 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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May 2017

Major Subject: Wildlife and Fisheries Sciences

ABSTRACT

In order to develop nutritious and cost-effective diets, the amino acid requirements of cultured fish species must be quantified. Several indispensable amino acid requirements of red drum *Sciaenops ocellatus* have already been reported; however, its requirement for histidine (His) has yet to be quantified. The aims of the present studies were to determine the minimum dietary His requirement of juvenile red drum and the physiological effects of His deficiency. A basal diet was prepared using lyophilized red drum muscle as an intact protein (10.5% of dietary protein) supplemented with crystalline amino acids (excluding His) to provide a total of 35% crude protein (CP) in the diet. Five isonitrogenous and isoenergetic experimental diets were prepared by supplementing the basal diet (0.3 g His/100 g dry diet) with increasing amounts of His (0.5, 0.7, 0.9, 1.1, 1.3 g/100 g dry diet). These diets were fed to triplicate tanks of red drum for 6 weeks in feeding trial 1 to quantify the minimum dietary His requirement. His level had significant effects on relative weight gain, feed efficiency ratio, protein efficiency ratio, and plasma free His. According to quadratic broken-line regression on relative weight gain percentage, the dietary His requirement was 0.59 (± 0.15) g/100 g dry diet (1.6% of CP). Feeding trial 2 was performed to further examine His deficiency in red drum. In that trial, the basal diet and a His-supplemented diet (~1.2 g/100 g dry diet) were fed to juvenile red drum in triplicate tanks for 8 weeks. Cataracts were found in 16.7% of eyes from fish fed the basal diet while no fish in the supplemented group developed cataracts. His-supplemented fish were found to have

higher erythrocyte fragility than those fed the basal diet. Based on these results, the His requirement of red drum was defined and His deficiency was observed to affect blood parameters and increase the possibility of cataract development.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Gatlin, for the opportunity to study under him and the advice and guidance he has given. I would also like to thank Dr. Welsh and Dr. Sink for serving on my committee and helping to direct this thesis.

Thanks also go to my friends and colleagues of the Fish Nutrition Lab at Texas A&M University for their help and for making my time here a wonderful experience. Lastly, I would like to thank my family, most especially my parents, for their support and encouragement. *Soli Deo Gloria*

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a thesis committee consisting of Dr. Gatlin as advisor (Department of Wildlife and Fisheries), Dr. Sink of the Department of Wildlife and Fisheries and Dr. Welsh of the Department of Animal Science.

Dr. Scott of the Department of Small Animal Clinical Sciences conducted the cataract examination and provided the photos of the red drum eyes. All other work conducted for the thesis was completed by the student independently.

Funding Sources

Graduate study was supported by a graduate Merit Fellowship from Texas A&M University. This work was made possible in part by Texas A&M AgriLife Research.

NOMENCLATURE

CP	Crude Protein
FER	Feed Efficiency Ratio
FT1	Feeding Trial 1
FT2	Feeding Trial 2
His	Histidine
IAA	Indispensable amino acids
PER	Protein Efficiency Ratio
PR	Protein Retention
UPLC	Ultra-performance liquid chromatography

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1. INTRODUCTION

As the aquaculture industry continues to grow, its use of fishmeal has increased even though the global supply of this feedstuff remains relatively static. In order to increase the sustainability and affordability of the industry, alternative protein feedstuffs need to be utilized to reduce the dependence on fishmeal. In the past, amino acid requirements of fish were not heavily researched because they were largely met by the dietary inclusion of fishmeal, with its well-balanced amino acid composition. Alternative protein feedstuffs, such as plant-based meals, often are limiting in one or more indispensable amino acids (IAA) and therefore amino acids may need to be supplemented in fish diets containing such feedstuffs (NRC, 2011). Knowing the correct balance of amino acids for each fish species allows researchers to evaluate potential protein feedstuffs and estimate their value. Over supplementing amino acids can cause adverse effects as well as increasing the price of the feed; any excess nitrogenous waste is excreted and can contribute to water quality problems (Ahmed and Khan, 2005). In order to develop nutritious and cost-effective feeds, the amino acid requirements of cultured fish species must be quantified.

Red drum (*Sciaenops ocellatus*) is a euryhaline sciaenid that is native to the eastern seaboard of the US from Massachusetts south to the Gulf of Mexico. In the past, this species boasted a healthy wild population that supported both recreational and commercial fisheries. However, due to overfishing, the wild population significantly declined in the early 1980s which resulted in the closure of the commercial fishery and

sparked an effort to restore the red drum population in the Gulf of Mexico by releasing juvenile fish raised in state-run hatcheries. Hatchery-raised fish are also grown to market size by commercial operations and sold for human consumption. Its ease of production in hatcheries and tolerance to a wide range of salinities make the red drum a good candidate for aquaculture (Craig and Gatlin, 1992; Faulk, 2005).

Many IAA requirements of red drum have already been quantified using 35% CP diets. The arginine requirement was determined to be 1.8 % of dry diet (Barziza et al., 2000), the threonine requirement 0.8 % of dry diet (Boren and Gatlin, 1995), the lysine requirement 1.6 % of dry diet (Craig and Gatlin, 1992), the total aromatic amino acid (phenylalanine and tyrosine) requirement 2.1 % of dry diet (Castillo et al., 2015), the tryptophan requirement 0.3% of dry diet (Pewitt et al., 2016), and the total sulfur amino acid requirement (cysteine and methionine) 1.0 % of dry diet (Moon and Gatlin, 1991). The branched chain AA (valine, leucine, and isoleucine) requirements have been determined to be 1.2, 1.6, and 1.1% respectively (Sergio Castillo, personal communication). However, the His requirement of red drum is the last to be quantified.

His is one of 10 IAAs in fish nutrition. Along with arginine and lysine, His is categorized as a basic amino acid. With a positively charged imidazole group, His can act as an acid or a base and plays roles in osmoregulation, muscle pH buffering, and detoxification of reactive carbonyl species (Farhat, 2013; Waagbø et al., 2010). His and its imidazole derivatives, such as carnosine and anserine, are thought to contribute to improved taste, texture, overall fillet quality and storage performance (Farhat, 2013; Gao et al., 2016; Ogata, 2002). Førde-Skjærvik et al. (2006) reported that supplementation of

dietary His increased muscle His, which increased post-mortem fillet pH, and decreased gaping in the fillet product. As a direct precursor of histamine, His plays a prominent role in allergic and immune responses as well (Ahmed and Khan, 2005). His is also found in hemoglobin and is used as a source of carbon in purine molecules. In salmon smolts, His deficiency causes cataracts; increasing His in the diet has a positive effect on eye lens protein turnover and protects the lens from oxidative stress and variation in osmotic pressure (Breck et al., 2005; NRC, 2011). His also has been found to have an effect on the muscle buffering capacity of fish (Fordeskjarvik et al., 2006; Ogata, 2002). In a study with juvenile grass carp, fish fed a His - deficient diet had significantly higher erythrocyte osmotic fragility (Gao et al., 2016).

For some readily cultured species, the His requirement has been summarized by the National Research Council (NRC, 2011). The requirement for chinook (*Oncorhynchus tshawytscha*), chum (*Oncorhynchus keta*), and coho (*Onchorhynchus kisutch*) salmon was reported at 0.7% of diet (1.8%, 1.6%, 1.8% of CP, respectively) (Klein and Halver, 1970; Akiyama et al., 1985). Channel catfish (*Ictalurus punctatus*) were determined to require 0.4% His (1.5% CP) in their diet and stinging catfish (*Heteropneustes fossilis*) require 1.51-1.56% (3.51-3.63% CP) His for fry but 0.54% of diet (1.4% CP) for juveniles (Ahmed, 2013; Khan and Abidi, 2014; Wilson et al., 1980). African catfish (*Clarias gariepinus)* fry are thought to only require 0.4% His in the diet (1.0% CP) (Khan and Abidi, 2009). The common carp (*Cyprinus carpio*) is recommended to have His levels at 0.8% of diet (2.1% CP) while the mrigal carp (*Cirrhinus mrigala*) has a requirement of 0.9% of diet (2.1% CP), and the Indian major

carp (*Catla catla*) has a requirement of 0.63-0.68% of diet (1.9-2.0% CP) (Ahmed and Khan, 2005; Nose, 1979; Zehra and Khan, 2016; Zhao et al., 2012). The grass carp (*Ctenopharyngodon idella*) has been reported to have a requirement of 1.2% of diet (3.2% CP) (Gao et al., 2016). Nile tilapia (*Oreochromis niloticus*) was reported to have a His requirement of 1.0% of diet (1.7% CP) (Santiago and Lovell, 1988). Rainbow trout were recommended to have between 0.5 and 0.6% His in the diet (1.0-1.2% CP) (Rodehutscord et al., 1997). Large yellow croaker (*Pseudosciaena crocea*) have been reported to require 0.9% His in the diet (2.0% CP) (Yan et al., 2013). Differences in the reported His requirement values of various fish species may be due to numerous factors including differences in metabolic needs, natural feeding habits or experimental procedures, including dietary protein levels.

Due to the importance of red drum to the aquaculture industry and the lack of a complete set of quantified IAA requirements for this species, the goal of this study was to determine the dietary His requirement of juvenile red drum and the effects of His deficiency on this species.

2. MATERIALS AND METHODS

In feeding trial 1 (FT1), lyophilized red drum muscle, to be used as the main protein source in the diet, was analyzed to determine crude protein, lipid, and ash. A basal diet was prepared by including lyophilized red drum muscle and crystalline amino acids, excluding His, to provide a total of 35% crude protein in the diet (Table 1). Dietary lipid and dextrin were included to provide, in combination with protein, a total of 13.4 kJ estimated digestible energy/g diet (Table 1). The amount of His provided by red drum muscle, which contributed 10.5% protein, was determined to be 0.28% of dry diet. Five experimental diets were prepared by supplementing the basal diet (0.3% of dry diet, Table 1) with increasing amounts (0.2 % of dry diet) of His (0.5, 0.7, 0.9, 1.10, 1.30% of dry diet). The range of dietary His levels were chosen based on His requirements previously established for other fish species (NRC, 2011). The experimental diets were kept isonitrogenous by adjusting the amount of an aspartate/glutamate premix as His levels were varied.

All diets were mechanically mixed and pressure pelleted using well established procedures (Castillo et al., 2015). Amino acid levels in all diets were analyzed via ultra performance liquid chromatography (UPLC) with an Acquity UPLC system with integrated TUV detector and MassTrak AAA Solutions Kit (Waters Corporation). Diets were also subjected to proximate analysis to determine crude protein, lipid, and ash (AOAC, 1990). Crude protein was quantified using the Dumas protocol (AOAC, 2005) and crude lipid using the Folch procedure (Folch et al., 1957).

Feeding trials were conducted at the Texas A&M Aquacultural Research and Teaching Facility in 38-L aquaria. The aquaria were connected to a recirculating system with biological and mechanical filters to ensure water quality maintenance. Salinity in the system was maintained at 7 g/L by combining a synthetic seawater mixture with well water. Dissolved oxygen was maintained near air saturation via air stones in each aquarium. Water temperature was maintained at 27ºC by controlling the ambient temperature in the building. A 12-h light/dark photoperiod was maintained in the building by controlling the lights with timers.

Juvenile red drum (Sea Center Texas Marine Hatchery, Texas Parks and Wildlife, Lake Jackson, TX) (initial average weight of 0.98 ± 0.08 g) were stocked at 20 fish/aquarium and acclimated to experimental conditions for 1 week. During the acclimation period, fish were fed a nutritionally adequate semi-purified diet to apparent satiation. After the acclimation period, triplicate aquaria were randomly assigned to each diet. Before the trial began, 11 fish were taken to record initial proximate composition. Fish were fed twice daily to apparent satiation with rations based on a percentage of total fish weight. Throughout the feeding trial, the fish were fed at a rate approaching apparent satiation (initially 7% body weight). Aquaria of fish were collectively weighed each week to adjust rations. FT1 was conducted for 6 weeks.

Table 1. Formulation and analyzed proximate composition of the basal diet.

Ingredient	Dry weight, g/100 g
Red drum muscle meal[1]	12.32
Crystalline Amino acid premix[2]	22.53
Dextrinized Starch	35.00
Menhaden Oil	8.20
Vitamin Premix[3]	3.00
Mineral Premix[3]	4.00
Carboxymethylcellulose	1.00
Calcium Phosphate	1.00
Aspartate/Glutamate premix	2.70
Histidine	0.02
Glycine	3.50
Celufil	6.73
Analyzed composition*	
Crude protein (%)	36.85
Crude lipid (% DM Basis)	11.46
Dry matter (%)	85.97

* Means of 2 replicate analyses

[1]Lyophilized from wild fish

[2]Provided as crystalline L-amino acids (each per 100g diet) as follows: 0.86 g taurine, 2.25 g serine, 1.59 g arginine, 1.23 g threonine, 1.65 g alanine, 2.00 g proline, 0.71 g cysteine, 2.35 g lysine, 1.10 g tyrosine, 0.89 g methionine, 1.98 g valine, 1.69 g isoleucine, 2.26 g leucine, 1.55 g phenylalanine, 0.42 g tryptophan

[3]Same as study by Nematipour and Gatlin, 1993

At the end of FT1, final fish weight was obtained and survival percentage was determined. Three fish/aquarium were randomly sampled at 15 h after the last feeding. Blood was collected (1-1.5 mL) from the caudal vasculature via a heparinized needle and pooled from three fish/tank, then centrifuged (2000 x *g*, 10 min) to collect the

plasma in order to assess plasma His levels. Plasma His levels were analyzed via UPLC. Total body weight, fillet, liver, and intraperitoneal fat weights were recorded to calculate the following body indices: condition factor [g body weight x 100/ (mm body length^3)], muscle ratio (g fillet weight/100 g body weight), hepatosomatic index (g liver weight/100 g body weight), and intraperitoneal fat ratio (g intraperitoneal fat weight/100 g body weight). Three additional fish were taken for whole-body proximate analysis (AOAC, 1990; 2005; Folch, 1957).

The effects of dietary His on buffering capacity of fish fillets was determined by homogenizing 0.6 g of muscle tissue (0.2 g from 3 fish/tank) in 100 mL of salt solution (145 mM KCl, 10 mM NaCl and 5 mM iodoacetic acid), then adjusting the pH to 6.0 and titrating to pH 7.5 with a 0.1 N NaOH solution in 30 µL increments (Ogata, 2002). Fillets were also analyzed for amino acid composition via UPLC.

Due to previous observations of cataracts associated with fish fed His-deficient diets, a second feeding trial (FT2) was conducted to further characterize this deficiency sign. Two diets from FT1 were used in this trial. The basal diet, 0.30 g His/100 g dry diet, was compared with an equal mixture (dry-matter basis) of the 1.1 and 1.3 g His/100 g diets to represent a His-deficient and a His-sufficient diet, respectively. These diets were fed to juvenile fish in triplicate 38-L aquaria for 8 weeks. The stocking density was 20 (1.53 ± 0.08 g) fish/tank. Experimental conditions in FT2 were similar to those described for FT1.

At the end of 8 weeks, six fish/tank were collected, with the lens of the right eye sampled for amino acid composition. Remaining fish were bled to conduct sampling for

erythrocyte osmotic fragility assays and the fish were also examined for cataracts using slit lamp biomicroscopy at 16x magnification. Cataract examination was done by a board certified veterinary ophthalmologist (Dr. Erin Scott, Department of Small Animal Clinical Sciences, Texas A&M University). Erythrocyte osmotic fragility assays were measured via hemolysis using increments of saline solution (0, 0.4, 0.5, 0.6, and 0.8%) to challenge the cells (Gao et al., 2016). Five µL of blood was placed in each well of a u-bottomed microplate and incubated for 30 min at room temperature. After incubation, microplates were centrifuged for 5 min at 3000 x *g*. The supernatant was analyzed spectrophotometrically in a flat-bottomed microplate at 545 nm to determine percent hemolysis of the erythrocytes.

In FT1, relative weight gain [(g final weight - initial weight/g initial weight) x 100], feed efficiency ratio (FER = g weight gain/g dry feed intake), protein efficiency ratio (PER = g weight gain/g dry protein fed), protein retention [PR = (final body protein-initial body protein) x 100/total protein fed], survival, plasma His, muscle His, and muscle buffering capacity were analyzed using a 1-factor ANOVA and Tukey's HSD with significance of $P \leq 0.05$ (JMP). For FT2, a t-test was used to compare relative weight gain, survival, erythrocyte osmotic fragility, and lens amino acids. Before analysis, percentage values were arcsine transformed and relative weight gain percentage values over 100% were log transformed. The minimum dietary His requirement was estimated using a quadratic broken-line regression analysis (SAS) (Robbins et al., 2006).

3. RESULTS

Amino acid analysis revealed diets 1-6 to have 0.33, 0.48, 0.68, 1.01, 1.13, and 1.27 g His/100 g dry diet, respectively. Percentage weight gain from initial weight, FER, PER, PR, and survival for FT1 are presented in Table 2. For weight gain percentage, there was a significant difference among fish fed the basal diet and those fed the five experimental diets. Significant differences also were observed among the basal diet and the experimental diets for FER, PER and PR, excluding diet 5 (1.1% His). Survival of fish fed the various diets was not significantly different. Body condition indices are presented in Table 3. Concerning condition factor, there was a significant difference between fish fed the basal diet and those fed diets containing 0.5% and 0.9% His. There were no significant differences among any diets for hepatosomatic index, intraperitoneal fat ratio, muscle ratio, or proximate composition analyses (dry matter, protein, lipid, ash). No significant differences were observed among diet treatments for muscle His content or muscle buffering capacity.

Plasma His levels showed an increasing trend until the 1.01% His inclusion, at which point there was a plateau (Figure 1). According to a quadratic broken line regression model run on weight gain percentage, the His requirement was estimated at 0.59 (± 0.15) g/100 g dry diet (or 1.6% of CP) (Figure 2).

Table 2. Performance[1] (growth, feed utilization, survival) of red drum fed graded levels of histidine for 6 weeks during feeding trial 1. Superscript letters represent significant differences according to Tukey's HSD (P > 0.05).

Variables	Dietary Histidine, % dry weight						Pr>F	Pooled SE
	0.33	0.48	0.68	1.01	1.13	1.27		
Initial mean fish weight, g	1.0	1.0	1.0	0.9	0.9	1.1	0.39	0.047
Final mean fish weight, g	4.4^{b}	6.8^{a}	6.7^{a}	6.9^{a}	7.1^{a}	7.3^{a}	0.002	0.401
Relative Weight Gain, %	340^{c}	587^{ab}	567^{b}	645^{ab}	664^{ab}	689^{a}	<0.001	0.322
Feed Efficiency Ratio (g gain/ g fed)	0.55^{b}	0.81^{a}	0.71^{a}	0.78^{a}	0.68^{ab}	0.81^{a}	<0.001	0.003
Protein Efficiency Ratio (g gain/ g protein fed)	1.49^{b}	2.19^{a}	1.95^{a}	2.10^{a}	1.86^{ab}	2.23^{a}	<0.001	0.020
Protein Retention, %	20.9^{c}	34.9^{ab}	30.2^{ab}	32.2^{ab}	28.1^{bc}	38.1^{a}	<0.001	0.001
Survival, %	100	86.7	96.7	88.3	90	86.7	0.072	0.004
Plasma-free Histidine (µmol/mL)	3.41^{c}	4.12^{bc}	4.91^{ab}	5.99^{a}	5.70^{a}	5.64^{a}	<0.001	0.282

[1]Values of means of 3 replicate groups.

Table 3. Body condition indices and whole-body proximate composition of red drum fed graded levels of histidine for 6 weeks during feeding trial 1. Superscript letters represent significant differences according to Tukey's HSD (P > 0.05).

Variables	Dietary Histidine, % dry weight						Pr>F	Pooled SE
	0.33	**0.48**	**0.68**	**1.01**	**1.13**	**1.27**		
Condition Factor	0.96^{b}	1.12^{a}	1.07^{ab}	1.10^{a}	1.08^{ab}	1.08^{ab}	**0.02**	**0.031**
Hepatosomatic Index, %	3.29	3.09	2.82	3.21	3.25	3.04	**0.42**	**0.005**
Intraperitoneal Fat Ratio, %	0.23	0.19	0.12	0.16	0.14	0.14	**0.69**	**0.060**
Muscle Ratio, %	30.9	35.4	34.2	34.0	34.6	34.6	**0.17**	**0.013**
Whole-Body Composition								
Moisture, %	78.0	76.3	76.9	76.7	76.6	74.1	**0.18**	**0.011**
Crude Protein, %	14.5	15.7	15.6	15.4	15.6	17.0	**0.88**	**0.012**
Lipid, %	4.1	4.2	4.0	4.3	4.4	4.7	**0.19**	**0.004**
Ash, %	3.7	3.8	3.6	3.5	3.5	3.8	**0.29**	**0.010**

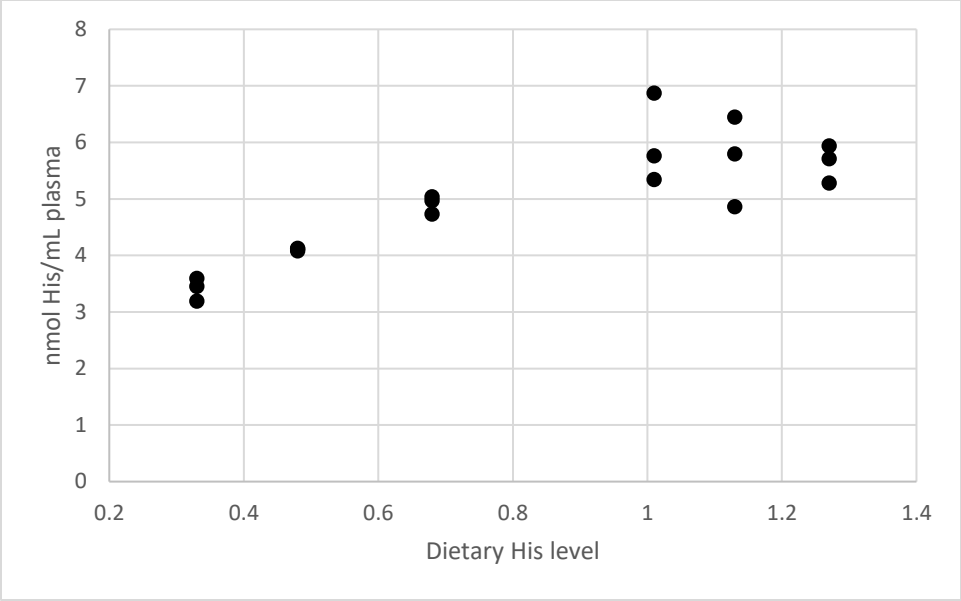

Figure 1. Concentration of histidine (nmol/mL) in plasma of red drum juveniles fed increasing amounts of dietary histidine.

Table 4. Weight gain, survival, and erythrocyte osmotic fragility of juvenile red drum fed a histidine-deficient or histidine-sufficient diet for 8 weeks during feeding trial 2.

	Basal Diet (0.3% His)	**Supplemented Diet (1.2% His)**	**Pr>F**	**Pooled SE**
Weight gain %	566[a]	1341[b]	0.0007	0.0278
Survival %	91.6	70.0	0.0546	0.0890
Erythrocyte Osmotic Fragility %	41.3[a]	69.8[b]	0.0452	0.0724

At the end of 8 weeks in FT2, weight gain of fish fed the deficient diet was only 42% of fish fed the sufficient diet (Table 4). Significant cataracts were observed in 16.7% of eyes examined from fish fed His-deficient diet while eyes examined from fish fed the sufficient diet did not contain any cataracts (Figure 3). Erythrocyte osmotic fragility was significantly higher in fish fed the His-sufficient diet compared to those fed the His-deficient diet (Table 4). Lens amino acids showed significant differences between the basal and supplemented fish in levels of His, taurine (Tau), glycine (Gly), threonine (Thr), valine (Val), isoleucine (Ile), and phenylalanine (Phe) (Table 5).

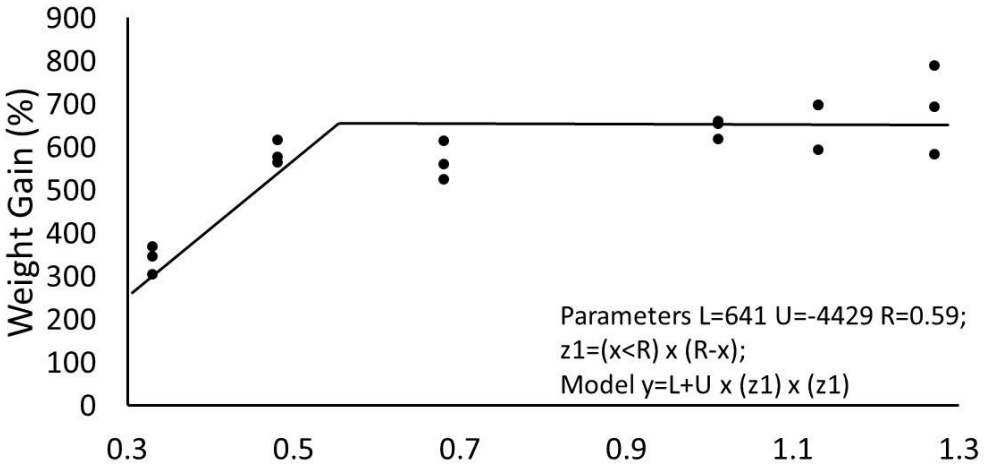

Figure 2. Quadratic broken-line model showing the histidine level at which weight gain reaches a plateau (breaking point).

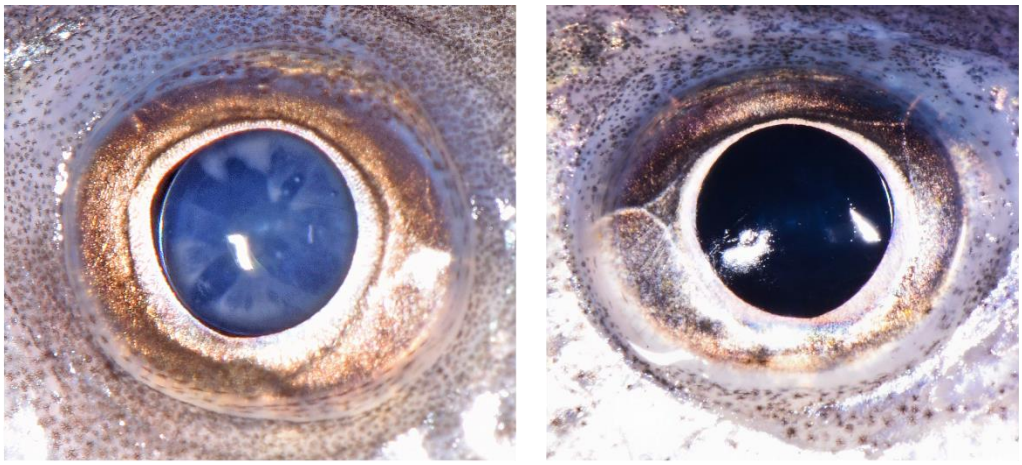

Figure 3. (Left) Histidine-deficient juvenile red drum displaying an incomplete subcapsular cataract. (Right) Histidine sufficient juvenile red drum without cataracts.

Table 5. Levels of lens amino acids in juvenile red drum fed a histidine-deficient or histidine-sufficient diet for 8 weeks during feeding trial 2, * denotes a significant difference.

	g/100 g sample (wet)			
Amino acid	**Basal diet (0.3% His)**	**Supplemented diet (1.2% His)**	**Pr>F**	**Pooled SE**
Histidine	1.45*	2.5*	0.0236	0.2602
Taurine	0.18*	0.29*	0.0377	0.0315
Serine	1.95	2.29	0.2397	0.1902
Arginine	2.53	2.75	0.4784	0.2045
Glycine	1.51*	2.17*	0.0291	0.1841
Asparagine	1.96	2.29	0.2244	0.1840
Glutamate	2.97	3.26	0.3598	0.2087
Threonine	0.45*	0.79*	0.0439	0.1018
Alanine	0.53	0.76	0.1224	0.0950
Proline	1.36	1.47	0.6182	0.1535
Cysteine	0.73	1	0.1180	0.1091
Lysine	0.79	1.03	0.1698	0.1149
Tyrosine	0.74*	0.94*	0.0290	0.0670
Methionine	1.45	1.62	0.4121	0.1427
Valine	0.86*	1.49*	0.0214	0.1893
Isoleucine	0.83*	1.28*	0.0487	0.1429
Leucine	1.22	1.55	0.1497	0.1506
Phenylalanine	2.09*	2.55*	0.0218	0.1163

4. DISCUSSION

Generally, IAA requirement estimates are based on growth response assays, such as weight gain percentage (NRC, 2011). According to a quadratic broken-line regression model run on weight gain, the minimum dietary His requirement of red drum was estimated to be 0.59 (± 0.15) g/100g dry diet (1.6 % CP). Occasionally, plasma amino acid levels have been used to corroborate amino acid requirement results. The theory is that once the amino acid requirement has been met in tissue, excess amino acid will be found in the blood. However, this effect seems to be species, and sometimes amino acid-specific (NRC, 2011). In this experiment an increasing trend in the plasma His levels corresponded with the increasing amounts of dietary His. The plasma His showed an increasing trend up to the 1.01% dietary His inclusion level, at which point it appeared to plateau (Figure 1).

Survival of red drum was not significantly different in either feeding trial. However, in FT2, survival of the His-supplemented group was numerically lower than the His-deficient group (Table 4). This is likely due to the rapid growth of the His-supplemented fish; quick growing red drum can turn cannibalistic if they are not being completely satiated. In FT1, no overt deficiency signs, other than reduced growth, were observed. However, during FT1, in-depth cataract examination was not performed and, therefore, less severe cataracts may have been missed. Reduced growth and cataracts were observed in FT2. Cataracts are classified as an opacification of the lens and/or lens capsule which may or may not be reversible (Taylor et al., 2015).

Cataracts pose a threat to fish welfare and can affect their ability to feed, and cataracts can also decrease the value of a fish at market if the whole fish is sold (Remø et al., 2014). His deficiency in salmon smolts is known to cause cataracts (Breck et al., 2005a; Waagbø et al., 2010). However, His deficiency is not the only nutritional deficiency that has been reported to result in cataracts. Riboflavin, methionine, tryptophan, and zinc deficiencies also may cause cataracts (Breck et al., 2005a). The first severe occurrence of cataracts in aquaculture was seen in European salmon culture due to the exclusion of mammalian blood meal from the diet, a protein feedstuff high in His (Breck et al., 2005a). Other factors, such as changes in salinity and temperature, as well as rapid growth, may contribute to high prevalence of cataracts in commercial salmon culture (Breck and Sveier, 2001). Cataract risk periods are 2-3 months and 8-10 months after transfer of salmon smolts from freshwater to seawater (Breck et al., 2005a; Breck and Sveier, 2001; Taylor et al., 2015). Therefore, it is interesting to note the observance of cataract development in juvenile red drum after only 8 weeks of His deficiency.

The cataract mitigating ability of His is due to N-acetylhistidine that is synthesized in the lens. This molecule works as an osmolyte, maintaining water balance in the lens (Remø et al., 2014). N-acetylhistidine is formed by the acetylation of His, which happens rapidly in the lens (Breck et al., 2005b). Breck et al. (2005b) reported that the concentration of lens free His quickly responded to His uptake from the diet and reflected dietary His levels. Studies published by Breck et al. (2005a), Breck et al. (2005b), and Waagbø et al. (2010) found lens amino acid composition, lens N-

acetylhistidine, and cataract prevalence to reflect dietary His levels. Likewise, the current experiment found significantly higher His in lenses from His-sufficient fish when compared to His-deficient fish. Interestingly, there were also significantly higher levels of Tau, Gly, Thr, Val, Ile, and Phe as well. Lens tissue levels of His and N-acetylhistidine were negatively correlated with cataract scores (Breck et al., 2005b). Also, the His requirement for optimal cataract mitigation was higher than the minimum requirement for growth of Atlantic salmon (Remø et al., 2014; Taylor et al., 2015). No cataracts were seen in His-sufficient red drum in the present study while 16.7% of His-deficient fish eyes contained cataracts. The cataracts observed in this study were not particularly severe but it is possible that they would worsen if the fish were deprived of His for longer than 8 weeks.

Besides having cataract-mitigating effects, His is a major component of non-carbonated buffering against pH changes in fish muscle (Gao et al., 2016). Muscle buffering prevents acidosis caused by anaerobic metabolism in white muscle (Ogata, 2002). The imidazole feature of His means that buffering is not temperature sensitive (Abe et al., 1985; Breck et al., 2005a). Abe et al. (1985) reported that His and its related compounds (carnosine and anserine) were principal compounds in intracellular buffering in rainbow trout (*Oncorhynchus mykiss*) and Pacific blue marlin (*Makaira nigricans*), with increased activity in the white muscle as compared to red muscle. Dark-fleshed, migratory, pelagic, marine fish species have more free His in muscle when compared to fish with alternative life histories (Abe, 1983; Abe et al., 1985; Ogata, 2002). Pacific blue marlin had six time greater muscle His and buffering capacity in red and white

muscle fibers when compared to red and white muscle from the rainbow trout (Abe et al., 1985). The primary His metabolites in muscle vary depending on the species (Abe, 1983). For example, cyprinids primarily use His, while in salmonids, anserine fills the role of muscle buffer. Anguillid muscle, on the other hand, is buffered by carnosine. Clearly, the function His as a muscle buffer relates to the life history, muscle metabolism and swimming style of the species in question.

In some species, dietary His levels directly contribute to muscle His levels. White muscle of juvenile yellowtail (*Seriola quinqueradiata*) and Atlantic cod (*Gadus morhua*) showed an increase in muscle buffering capacity with a His-supplemented diet (Ogata 2002; Førde-Skjærvik et al. 2006). Førde-Skjærvik et al. (2006) reported increased muscle His and increased muscle pH in mature Atlantic cod after only 3 weeks of His supplementation (4.8% of diet). In the present study, there were no significant differences in either muscle His levels or muscle buffering capacity. Although it is primarily a marine species, the red drum is not a dark-fleshed species, nor is it particularly migratory when compared to the Pacific blue marlin. However, the lack of significant differences in muscle His and buffering capacity could also be due to the young age of the fish tested in this study. The Atlantic cod, as well as the Pacific blue marlin previously mentioned, were mature fish when their muscle was analyzed. The yellowtail were juveniles, but the starting weight (22 g) was still much larger than the red drum in FT1 (0.98 g).

In blood, His affects erythrocyte fragility, hemoglobin concentration, hematopoesis, and hematocrit (Farhat, 2013; Gao et al., 2016; Khan and Abidi, 2014;

Zehra and Khan, 2016). His constitutes up to 10% of amino acids in hemoglobin (Zehra and Khan, 2016). The requirement of His for hematopoesis is greater than that for growth. Zehra and Khan (2016) reported that hemoglobin concentration, hematocrit, and red blood cell counts were positively correlated with levels of dietary His in the Indian major carp. Erythrocyte osmotic fragility, which quantifies the amount of hemolysis occurring when erythrocytes are subjected to osmotic stress, can be viewed as a measure of the quality of the erythrocytes and the integrity of their membranes. In grass carp and stinging catfish, erythrocyte fragility was significantly affected by dietary His (Farhat, 2013; Gao et al., 2016). Erythrocyte osmotic fragility decreased with an increase in dietary His up to the requirement of stinging catfish, but fish fed excess His experienced an increase in erythrocyte fragility. In FT2, fish fed the His-deficient diet had lower erythrocyte fragility compared to those fed a sufficient diet. It is possible, but unlikely, that the His-sufficient diet (1.2% of diet) had enough surplus His to cause increased erythrocyte fragility. Another possible explanation is an increase in the amount of circulating histamine due to higher dietary His levels. Increases in free His could cause elevated histamine production, and excess histamine could induce pro-inflammatory cytokines, possibly disturbing the erythrocytes (Jiang et al., 2016).

As a direct precursor of histamine, His also has effects on brain function and the histaminergic response. In the brain, the histaminergic system is involved in wakefulness, the sleep-wake cycle, appetite control, learning, memory, anxiety, and stress response (Cofiel and Mattioli, 2009; Yoshikawa et al., 2014). Histamine itself functions in allergic reactions, gastric acid secretion, and as a neurotransmitter in the

brain (Yoshikawa et al., 2014). Histidyl dipeptides can be found in electrically excitable tissues in the brain; histaminergic neurons are found in the vicinity of the posterior recess (Cofiel and Mattioli, 2009; O'Dowd et al., 1989). For instance, N-acetylhistidine is synthesized in the fish brain from acetyl CoA and L-His and is decomposed by a highly specific brain acylase (O'Dowd et al., 1989). In zebrafish subjected to confinement stress, increased His levels facilitated learning behavior (Cofiel and Mattioli 2009). His-supplemented fish were faster than non-supplemented fish to enter a designated feeding area when signaled, whether they were subjected to stress or not. Cofiel and Mattioli (2009) delivered His intraperitonally but there is reason to believe that increased plasma His, due to supplemented dietary His, would have the same effect. According to Yoshikawa et al. (2014), adult male mice with 60% of normal brain histamine exhibited anxiety-like behavior; those with less than 50% showed signs of neurological disorders. Insufficient dietary intake reduced brain histamine content and resulted in a decrease of brain histamine clearance (Yoshikawa et al., 2014). Dietary His is related to brain histamine level; His supplementation could be used to mitigate the effects of stress in cultured fish species.

Another aspect of His stress mitigation is antioxidant status. His is present at the active site of superoxide dismutase, a vital antioxidant (Forman et al., 1973). In juvenile Jian carp (*Cyprinus carpio* var. Jian), increased dietary His decreased the generation and increased the elimination of reactive oxygen species (Feng et al., 2013). Superoxide scavenging ability also was enhanced by His supplementation. At the optimum dietary His level, less amino acid oxidation residues were detected in Jian carp blood than in

blood from His-deficient Jian carp. Grass carp fed deficient and excessive amounts of His experienced oxidative damage to the gill, due to the depression and impairment of antioxidant production (Jiang et al., 2016). Clearly, the balance of His is imperative to reap the benefits of His supplementation while avoiding the negative impacts of excess His.

5. CONCLUSIONS

His is an indispensable amino acid that has wide ranging physiological effects on the growth and health of red drum. His deficiency manifested itself in red drum in the form of decreased growth and cataract formation. According to quadratic regression analysis based on relative weight gain, the His requirement for optimum growth of juvenile red drum was 0.59 (± 0.15) g/100 g dry diet (1.6 % CP). With the His requirement reported here, the Fish Nutrition Lab at Texas A&M University has defined all of the IAA requirements of juvenile red drum.

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