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Short communication

Characterization of pantothenic acid deficiency and the dietary requirement of juvenile hybrid striped bass, *Morone chrysops* × *M. saxatilis*

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ABSTRACT

An 8-week feeding trial was conducted to characterize the pantothenic acid deficiency signs and quantify the dietary requirement of juvenile hybrid striped bass, *Morone chrysops* × *Morone saxatilis*. A basal diet composed of fish muscle and crystalline amino acids was analyzed to contain 5 mg of pantothenate/kg and supplemented with calcium D-pantothenate resulting in five diets with graded pantothenate levels of 5, 10, 20, 30 and 40 mg/kg diet. Each diet was fed to triplicate groups of hybrid striped bass juveniles (initial average weight 1.6 g) in a recirculating system at 26.0 ± 1 °C. Fish fed the unsupplemented basal diet performed poorly in terms of growth parameters and exhibited typical signs of pantothenic acid deficiency such as hemorrhages, sluggishness, high mortality, anemia and severe hyperplasia of the epithelial cells of gill lamellae. Fish fed the diet containing 10 mg of pantothenate/kg diet performed significantly better than fish fed the basal diet but poorly when compared with the responses of fish fed diets containing 20, 30 and 40 mg of calcium D-pantothenate/kg diet. Also, pantothenic acid-deficiency signs were apparent in fish fed the diet containing 10 mg/kg diet; whereas, no deficiency signs were observed in fish fed diets supplemented with the higher levels. Based on these data, the dietary pantothenic acid requirement of hybrid striped bass was quantified at 18.8 mg/kg diet based on broken-line regression analysis of weight gain data.

Statement of relevance:

Pantothenic acid deficiency in hybrid striped bass was characterized and the minimum dietary pantothenic acid requirement was determined to be 18.8 mg/kg based on weight gain. This information will assist in refining diet formulations for hybrid striped bass.

1. Introduction

Pantothenic acid (PA) is a water-soluble vitamin that functions as a part of the coenzyme A molecule in the metabolic release of energy from all three energy-providing nutrients, carbohydrate, fat and protein, by way of the tricarboxylic acid (TCA) cycle (NRC, 2011). Dietary essentiality of this nutrient has been established in numerous animals including various fish species (NRC, 2011). Response parameters used for PA requirement estimates are often based on the absence of deficiency signs, normal growth rate, liver tissue saturation and/or metabolic biomarker responses. The sign most frequently reported in fish caused by PA deficiency is severe hyperplasia of the epithelial cells of gill lamellae (clubbed gills), as well as hemorrhages, sluggishness, high mortality, and anemia (NRC, 2011). Due to this vitamin's essentiality in the normal growth and health of fish, various researchers have attempted to determine qualitative and quantitative dietary requirements for PA which have ranged from 10 to 45 mg PA/kg diet (Cho and Woodward, 1990; Masumoto et al., 1994; Masumoto, 2002; Murai and Andrews, 1979; Ogino, 1967; Roem et al., 1991; Shimeno, 1991; Soliman and Wilson, 1992; Wen et al., 2009; Wilson et al., 1983).

Hybrid striped bass, crosses between white bass, *Morone chrysops*, and striped bass, *Morone saxatilis*, have shown considerable potential for aquaculture (Smith et al., 1985). The hybrids have desirable characteristics such as rapid growth and wide salinity tolerance (Smith et al., 1986). Some nutritional requirements have been determined for these hybrids (Brown et al., 1993; Keembiyehetty and Gatlin, 1992; NRC, 2011), but not that for PA, which may assist in the development of suitable diets. The present study was conducted to characterize PA deficiency signs and quantify the dietary requirement of juvenile hybrid striped bass, by evaluating growth performance and histopathological responses.

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2. Materials and methods

2.1. Experimental diets

Semi-purified diets were formulated to provide 35% crude protein from solvent-extracted red drum (*Sciaenops ocellatus*) muscle and a crystalline amino acid premix. The red drum muscle was prepared in this laboratory by lyophilizing muscle tissue from adult red drum, as well as extracting three times in a boiling solution of hexane:ethanol (4:1 *v*/*v*) which reduced the endogenous lipid level so that it contributed approximately 0.65% lipid to the diet and 22.5% crude protein (Craig and Gatlin, 1997). The crystalline amino acid premix was formulated to provide the remaining 12.5% crude protein. Menhaden fish oil was supplemented to the lipid in the red drum muscle to provide a total of 9.7% dietary lipid.

The basal diet (Table 1) was analyzed to contain pantothenate at 5 mg/kg, and thus supplemental levels of calcium D-pantothenate (92% PA active) were adjusted to achieve total pantothenate levels of 10, 20, 30 and 40 mg/kg diet in the experimental diets. Samples of the experimental diets were sent to Eurofins Scientific (Des Moines, IA) for microbial analysis of PA according to AOAC (1990) procedures.

Preparation of the diets began with all dry components being individually weighed into 3.8-L plastic bags. Once all dry ingredients had been weighed, diets were thoroughly mixed using a commercial V-mixer for 30 min. Diets were then placed in a commercial Hobart mixer where the fish oil and water were added. Diets were then pressure pelleted through a 3-mm die using a Hobart meat grinder. Pellets were then crumbled by hand and dried at 25 °C. After drying, pellets were screened to remove fines and stored at −20 °C until fed.

2.2. Experimental procedures

Juvenile hybrid striped bass were obtained from Keo Fish Farms (Lonoke, Arkansas) and transported by truck to the Texas A&M Aquacultural Research and Teaching Facility. Prior to the beginning of the feeding trial, fish underwent a l-week conditioning period to adjust to a purified basal diet and standardized experimental conditions. Each of the five dietary treatments was randomly assigned to triplicate 110-L aquaria in a recirculating system at the Texas A&M University Aquacultural Research and Teaching Facility. Each aquarium contained 15 fish with an initial average weight of 1.6 g per fish. Water flow through each culture chamber was maintained at approximately 0.65 L/min via a 1.5 HP pump that recirculated water through biological and mechanical filters to maintain appropriate water quality (total ammonia nitrogen < 0.6 mg/L). Salinity was maintained at 6.5 to 8‰ using well water and synthetic sea salt (Fritz Industries Inc., Dallas, TX, USA). Low-pressure electrical blowers provided aeration via air stones and constantly maintained dissolved oxygen (DO) levels at or near saturation. Water temperature was regulated by controlling ambient air temperature and remained at 26 ± 1 °C throughout the trial. A daily light:dark cycle of 12:12 h was provided by fluorescent lighting.

Fish were fed each diet at 8% of body weight per day for the first week, and then feeding rate was progressively reduced to minimize overfeeding while maintaining a level close to satiation. Fish were weighed every week and the amount of diet fed was adjusted accordingly. Dead fish were removed and not replaced during the experiment. Fish were fed the experimental diets for 8 weeks.

Table 1
Composition of the basal diet.

Ingredient	% (dry weight)
Red drum muscle (lipid extracted)	21.9
Amino acid mix[a]	12.5
Dextrin[b]	30.0
Menhaden oil[c]	9.7
Vitamin premix[d]	3.0
Mineral premix[d]	4.0
Carboxymethylcellulose[b]	2.0
Celufil[b]	14.4
$CaHPO_4 \cdot 2H_2O_2$	1.0
Aspartate/glutamate premix[b]	1.5
Calcium D-pantothenate[b]	0.0

[a] Contains (g/100 g): arginine 9.44; glycine 11.92; histidine 3.76; isoleucine 6.16; leucine 9.60; methionine 4.08; phenylalanine 2.16; tyrosine 4.32; serine 2.16; threonine 7.0.4; tryptophan 1.92; valine 5.84; proline 15.76; and alanine 15.84.
[b] US Biochemical Corp., Cleveland, OH, USA.
[c] Omega Protein Corporation, Inc. Houston, TX, USA.
[d] Same as Moon and Gatlin (1991).

2.3. Growth performance and histopathology

Percentage of body weight gain (WG) [100 × (final body weight − initial body weight) ⁄ initial body weight], feed efficiency (FE) [(final body weight − initial body weight) ⁄ feed intake], and survival [100 × (final fish number ⁄ initial fish number)] were calculated.

Fifteen fish were randomly selected from the initial group before assignment to the various diets and fixed in 10% neutral buffered formalin to serve as control histological samples. At the end of the feeding trial, gills from fish fed the diet supplemented with 15 mg PA/kg and the basal diet were dissected, placed in 10% neutral buffered formalin, embedded in Paraplast (Lancer Co., St. Louis, MO), sectioned and stained with hematoxylin and eosin. The gill sections were examined microscopically for histological changes.

2.4. Statistical analysis

All data were subjected to analysis of variance and Duncan's multiple-range test (Duncan, 1955) to determine differences in treatment means ($P < 0.05$). Weight gain data also were analyzed by least-squares regression using the broken-line model (Robbins et al., 2006) to estimate the minimum dietary PA requirement of hybrid striped bass for optimal growth. Data analyses were performed using the SAS/PC statistical software (SAS Inst. Inc., Cary, NC).

3. Results and discussion

The importance of dietary PA for hybrid striped bass was evident in the present study. The response of fish to increasing levels of dietary calcium D-pantothenate is presented in Table 2. Significantly reduced weight gain, anemia, anorexia, low survival, and sluggishness were observed in fish fed the basal diet. Feed efficiency and percent survival increased stepwise up to a dietary level of 15 mg calcium D-pantothenate/kg diet and plateaued. Dietary pantothenic acid had no effect on condition factor of the fish.

Marked interlamellar proliferative lesions were observed in the gills of fish fed the basal diet. The lesions were visible as early as week 3 and were most pronounced at the distal end of the gill filaments giving a

Table 2
Responses of fingerling hybrid striped bass to graded levels of dietary calcium D-pantothenate levels[1,2].

Dietary pantothenate	Initial weight	Weight gain	Feed efficiency	Survival
mg/kg diet	g/group	% of initial wt.	g gain/g feed	%
5	19.3 ± 1.4	212 ± 40.7[c]	0.35 ± 0.04[c]	17.8 ± 8.0[b]
10	20.2 ± 1.5	628 ± 35.0[b]	0.54 ± 0.05[b]	75.6 ± 24.4[a]
20	20.6 ± 0.6	1358 ± 57.2[a]	0.75 ± 0.01[a]	97.8 ± 2.2[a]
30	20.1 ± 1.3	1373 ± 25.1[a]	0.75 ± 0.01[a]	100 ± 0.0[a]
40	20.8 ± 1.6	1338 ± 93.3[a]	0.75 ± 0.03[a]	100 ± 0.0[a]

[1] Values are means ± standard error of three groups of fish fed the same experimental diet ($n = 3$) with 15 fish per group.
[2] Numbers with different superscripts in the same column indicate significant ($P \le 0.05$) differences according to Duncan's multiple-range test.

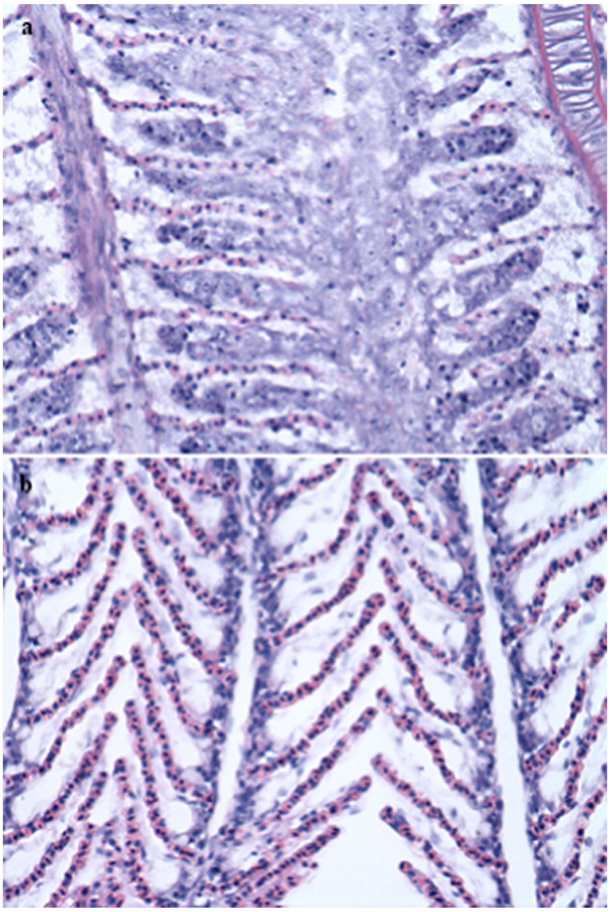

Fig. 1. Histological images (×40) of gills from hybrid striped bass fed diets with 5 and 20 mg PA/kg diet for 8 weeks. (a) Clubbed gills with marked interlamellar proliferative lesions were observed in fish fed the diet with 5 mg PA/kg diet; (b) no lesions were found in gills of fish fed the diet with 20 mg PA/kg diet.

"clubbed" appearance (Fig. 1). A similar deficiency sign also has been reported in blue tilapia (Soliman and Wilson, 1992), channel catfish (Murai and Andrews, 1979; Wilson et al., 1983), grouper (Lin et al., 2012), and rainbow trout (Karges and Woodward, 1984; Masumoto et al., 1994; Olsvik et al., 2013) when fish were fed a PA-devoid diet. These gill lesions may result in reduced oxygen uptake, consequently leading to high mortality (Table 2). Lesions were not observed in the control group of fish fed the diet with 20 mg pantothenate/kg for the 8-week period.

Some authors have suggested that anemia is associated with anorexia in fish fed a PA-deficient diet (Lin et al., 2012; Murai and Andrews, 1979; Soliman and Wilson, 1992). Similar results were observed in the current study, with fish fed the basal diet and the diet with 5 mg calcium D-pantothenate/kg diet. In addition, reduced feed consumption was evident along with reduced feed efficiency in fish fed the basal diet.

The high mortality observed in the present study was not strictly related to the PA deficiency, but there was an unidentified bacterial epizootic that began at week 3. Fish fed the basal diet had the highest mortality, which decreased as the dietary calcium D-pantothenate increased (Table 2). Survival and feed efficiency values increased up to a level of 20 mg pantothenate/kg and then plateaued at higher levels. The plateauing of these values provides additional evidence to support the requirement based on weight gain. When broken-line analysis of weight gain data was conducted, the regression equation $Y = 83.10X + 203.28$, was obtained, with an R^2 of 0.97, and break point at 18.8 as the estimate of the minimum dietary PA requirement. This value is similar to the requirements reported in the NRC (2011) for channel catfish (10–15 mg/kg), blue tilapia (10 mg/kg) and grass carp (10–20 mg/kg). However, some higher requirement values were reported in the NRC (2011) for Pacific salmon (40–50 mg/kg), yellowtail (35.9 mg/kg), common carp (30–50 mg/kg), grass carp (25 mg/kg) and Jian carp (23 mg/kg). This range of requirement values for several cultured fish species may be species specific, but also may vary depending on experimental conditions including fish size, age, and water temperature.

Modern feed processing using high temperature and pressure may lead to loss of vitamins, including PA (Barrows et al. 2008). However, calcium D-pantothenate, which was used in the present study, is a stable form of PA, with no more than 20% lost after extrusion and storage (Gaubadan and Hardy, 2000).

The inclusion of plant feedstuffs as replacements for fishmeal in diets of aquatic species may affect both level and availability of several vitamins, as well as the growth of the targeted species. Hansen et al. (2015) expressed concern that vitamin requirements quantified with fish consuming semi-purified diets, which may confer lower weight gain than the species' potential, could yield inaccurate requirements. In the present study, juvenile hybrid striped bass fed the semi-purified diet composed of red drum muscle and crystalline amino acids had excellent weight gain comparable to that of fish fed practical diets containing 40% crude protein from fishmeal and soybean meal (Castillo et al., 2014). Therefore, the quantified value of 18.8 mg/kg diet determined in the present study should provide an accurate and conservative estimate of the minimum dietary PA requirement of juvenile hybrid striped bass.

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