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Dietary Phosphorus Requirement of Juvenile Red Drum *Sciaenops ocellatus*

DONALD A. DAVIS AND EDWIN H. ROBINSON[1]

Department of Wildlife and Fisheries Sciences, Texas A&M University
College Station, Texas, 77843 USA

Abstract

Eight isoenergetic and isonitrogenous diets containing graded levels of inorganic phosphorus were fed to juvenile red drum *Sciaenops ocellatus* for an 11 week period. Red drum were maintained in a brackish water (5-6 ppt) recirculating system. There were no significant differences in weight gain or feed conversion. However, survival was generally lower for fish fed deficient diets. Phosphorus supplements up to 0.86% increased bone calcium and scale ash, calcium, and phosphorus values. Bone ash and phosphorus were not significantly affected above 0.71% dietary phosphorus. It appears that approximately 0.86% dietary phosphorus is needed for maximum tissue mineralization of juvenile red drum.

The red drum or redfish, *Sciaenops ocellatus,* is currently being investigated as a potential euryhaline culture species. Recent studies have demonstrated that red drum accept and grow well on artificial feeds (Crocker et al. 1981; Davis and Stickney 1982). Although red drum grow well on commercial feeds formulated for other species, a feed formulated specifically to meet the red drum's nutritional needs could increase production efficiency, thus increasing the feasibility of profitable commercial culture. In order to formulate an efficient and economical feed, the nutritional requirements of the red drum must be defined.

The dietary protein (Lin and Arnold 1983), protein : energy ratio (Daniels and Robinson 1986), and lipid requirement (Williams and Robinson, submitted) have been reported for juvenile red drum. Little is known concerning remaining constituents needed to formulate a complete diet.

Minerals are important dietary components which are necessary for various physiological processes. Calcium and phosphorus are predominant minerals in the animal body and occur largely in the skeletal tissues and scales. Fish can absorb all or a large amount of their calcium requirement from the water (NRC 1983); however, because of the low rate of absorption of phosphorus (Phillips et al. 1957) and its low concentration in natural waters (Boyd 1971), phosphorus is obtained primarily from dietary sources. Dietary phosphorus requirements ranging from 0.33 to 0.80% have been reported for warmwater fish (NRC 1983). Since the phosphorus requirement for red drum has not been reported, the present study was designed to determine the dietary phosphorus requirement for juvenile red drum.

Methods

Experimental Design and Diets

Eight semipurified diets containing graded levels of phosphorus, supplied as monobasic sodium phosphate, were fed to juvenile red drum. Total dietary phosphorus (calculated) ranged from 0.26 (basal) to 1.31% of the diet in 0.15% increments. The basal diet (Table 1) was formulated to contain 40% crude protein and 3,500 kcal/kg of diet (Daniels and Robinson 1986). Egg albumin, deboned fish meal and shrimp-head meal served as protein sources. Shrimp-head meal and deboned fish meal

[1] Corresponding author's current address: Dr. Edwin H. Robinson, Delta Branch Experiment Station, P.O. Box 197, Stoneville, Mississippi 38776

TABLE 1. *Composition of basal diet.*

Ingredient	% Dry weight
Egg albumin[a]	20.25
Shrimp-head meal[b]	5.00
Fish meal[c] (deboned)	25.00
Dextrin[a]	18.62
Vitamin mix[d]	0.50
P-free mineral mix[e]	4.00
Menhaden fish oil[c]	8.0
Cellulose[a]	18.63

[a] U.S. Biochemical Corp., Cleveland, Ohio.
[b] Blum and Bergeron, Inc., Houma, Louisiana.
[c] Zapata Haynie Corp., Reedville, Virginia.
[d] Same as reported by Robinson et al. 1984.
[e] Contains (as grams per kilogram of mix): calcium carbonate, 53.9; calcium sulfate, 204.0; ferrous sulfate, 29.7; magnesium sulfate, 132.0; potassium sulfate, 239.9; sodium sulfate, 44.88; sodium chloride, 43.5; aluminum chloride, 0.15; potassium iodide, 0.15; cupric chloride, 0.10; manganous sulfate, 0.8; cobalt chloride, 1.0; zinc sulfate, 3.0.

also served as attractants to ensure the palatability of the diets. Vitamin and phosphorus-free mineral supplements were added to equal or to exceed recommended levels for warmwater fish (NRC 1983).

All diets were prepared by mixing the dry ingredients in a twin-shell dry blender (Patterson-Kelley Co., East Straudsburg, Pennsylvania) for 30 minutes. The lipid source was then blended with the dry ingredients using a Hobart mixer (Hobart Corp., Troy, Ohio). Water was added at a rate of 45% to give the mixture a consistency that would facilitate pressure pelleting. Each diet was pressure pelleted using a meat grinder and a 4.5 mm die. After pelleting, all diets were air dried to approximately 10% moisture then frozen until needed. Portions, as needed for feeding, were mechanically crumbled and sieved to the appropriate size and refrigerated.

Juvenile red drum, which were provided by the Texas Parks and Wildlife Department, Palacacios, Texas, were transported to the Texas A&M Aquacultural Research Center and acclimated to low-salinity water by slowly adding fresh water to the hauling tank. Acclimation was completed in approximately 3–4 hours. Following acclimation, red drum (1.3 grams average weight) were stocked at a rate of 21 fish per 110 L aquaria. For two weeks prior to initiation of the experiment, fish were fed a conditioning diet which was the basal diet with 0.30% supplemental phosphorus (0.56% total phosphorus). After conditioning, triplicate random groups of fish were fed one of the eight experimental diets at an average rate of 8% of their wet weight per day. The fish were fed twice daily (8 a.m. and 5 p.m.) for a period of 11 weeks. All fish were weighed biweekly in order to adjust feed quantity. Weight gain, feed conversion, and survival data were collected at each weighing. During the first two weeks of the experiment dead fish were replaced.

Water Quality

Water was supplied via a closed brackish water (4.5–6.0 ppt) recirculating system which delivered water at a rate of 4 L/min. Supplemental air was supplied to each aquaria by a low-pressure blower. Salinity was monitored daily with a salinity refractometer (American Optical, South Bridge, Massachusetts) and adjusted as needed using artificial sea salts (Instant Ocean, Aquarium Systems, Mentor, Ohio). Ammonia and nitrite were monitored biweekly by spectrophotometric methods (HACH-DREL/5 spectrophotometer, Hach Chemical Company, Loveland, Colorado). A Digi-sense pH meter (Cole Parmer, Chicago, Illinois) was used to measure pH biweekly. Dissolved oxygen and temperature were monitored weekly using a YSI 51B oxygen/temperature meter (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio).

Sample Collection and Analysis

At the conclusion of the experiment, blood from all fish in an aquarium was pooled for mineral analysis. However, blood minerals were not determined because the samples were inadvertently discarded. Eight fish from each tank were randomly selected and frozen for subsequent mineral analysis (P, Ca,

TABLE 2. *Initial weight, final weight, weight gain, feed conversion, and survival of juvenile red drum fed diets containing graded levels of phosphorus. Data represent the mean of three replicates. Means not sharing a common letter are significantly different (P < 0.05).*[a]

Diet no.	Dietary phosphorus (%)	Initial weight (g)	Final weight (g)	Weight[b] gain (%)	Feed[c] conversion	Survival (%)
1	0.26	1.2	13.9	1,026.5 z	1.8 z	60.3 yz
2	0.41	1.2	16.1	1,178.9 z	1.8 z	63.5 yz
3	0.56	1.3	16.0	1,143.7 z	1.8 z	47.6 y
4	0.71	1.2	16.3	1,208.4 z	1.8 z	66.7 yz
5	0.86	1.3	14.9	1,049.8 z	1.9 z	69.8 z
6	1.01	1.3	15.6	1,118.9 z	1.7 z	73.0 z
7	1.16	1.3	16.4	1,195.5 z	1.8 z	80.9 z
8	1.31	1.2	17.4	1,311.0 z	1.6 z	73.0 z

[a] The pooled standard errors for weight gain, feed conversion, and survival were 33.1, 0.023, and 2.35, respectively.
[b] ((Final weight − initial weight)/initial weight)·100.
[c] Dry weight feed/wet weight gain.

and Mg) of bone, scale, skin and muscle tissue. Four fish were randomly selected from frozen samples for tissue analysis. Fish were weighed, scaled, skinned, fileted, and the vertebrae removed. Vertebrae were placed in hot distilled water, scrubbed to remove excess flesh, dried, and extracted with ether. Prior to drying, scales were washed with deionized water to remove mucus. Tissue samples from fish in each aquarium (bone, scale, muscle, and skin samples) were dried and frozen for subsequent wet ashing and mineral analysis. Ashed samples were prepared for mineral determination using AOAC methods (AOAC 1980). Calcium and magnesium were analyzed by atomic absorption spectrophotometry (Perkin-Elmer model 4000 aa/ae spectrophometer, R and E Corp., Norwalk, Connecticut). Phosphorus was determined photometrically (Fiske and Subbarow 1925). All analyses were conducted in duplicate on each pooled sample and averaged for each aquarium.

Statistical Analysis

Data were analyzed using a one-way analysis of variance and Duncan's multiple range test (Steel and Torrie 1960) using the statistical analysis system, SAS (Helwig and Council 1979). Results were considered significant at the 0.05 level.

Results

Water Quality

Water temperature and dissolved oxygen ranged from 25–30 C and 6.7–7.5 mg/L, respectively. Ammonia nitrogen ranged from 0 to 0.5 mg/L with an average of 0.2 mg/L. Nitrite nitrogen values ranged from a minimum value of 0.005 mg/L to a maximum value of 0.045 mg/L near termination of the study. Salinity ranged from 4.5 to 6.0 ppt.

Growth Parameters

Weight gain, feed conversion, and survival data are presented in Table 2. Weight gain ranged from 1,311.0 (Diet 8) to 1,026.5% (Diet 1). Although fish fed Diet 1 had consistently poorer weight gain than fish fed other diets, there were no significant differences between treatments. Feed conversions ranged from 1.7 (Diet 5) to 1.6 (Diet 1). Regardless of dietary treatments, there were no significant differences or trends observed in the feed conversion data. At the conclusion of the experiment (week 11), survival ranged from 80.9 (Diet 7) to 47.6% (Diet 3). At weeks five, seven, and nine,

TABLE 3. *Bone mineralization of juvenile red drum fed diets containing graded levels of phosphorus. Data represent the mean of three replicates expressed on a dry, fat free basis. Means not sharing a common letter are significantly different (P < 0.05).*[a]

Diet no.	Dietary phosphorus (%)	Ash (%)	Phosphorus (%)	Calcium (%)	Ca/P	Magnesium (%)
1	0.26	23.4 w	4.0 w	6.1 v	1.6 z	0.22 z
2	0.41	31.9 x	5.7 x	9.9 w	1.7 z	0.27 z
3	0.56	48.1 y	9.2 y	11.6 wx	1.2 z	0.29 z
4	0.71	52.1 z	9.8 yz	13.7 wxy	1.4 z	0.30 z
5	0.86	52.7 z	10.2 z	13.2 wxy	1.3 z	0.36 z
6	1.01	52.5 z	10.2 z	16.3 yz	1.6 z	0.38 z
7	1.16	53.8 z	10.3 z	18.3 z	1.8 z	0.38 z
8	1.31	53.8 z	10.4 z	15.0 xyz	1.5 z	0.40 z

[a] The pooled standard errors for ash, phosphorus, calcium, Ca/P, and magnesium were 0.316, 0.092, 0.422, 0.046, and 0.011, respectively.

ANOVA indicated significant ($P < 0.05$) differences in survival among treatments. For these weeks, Duncan's multiple range test ($P < 0.05$) indicated Diets 1–3 produced significantly lower survival than Diets 7 and 8. Although by week 11 no significant differences were observed.

Bone Mineralization

In the present study, no physical deformities were noted from external observation. However, fish fed diets containing low levels of phosphorus (Diets 1 and 2) had noticeably smaller and softer vertebrae than fish fed diets higher in phosphorus.

Bone ash and mineral data are presented in Table 3. Bone ash and phosphorus increased significantly as dietary phosphorus was increased to 0.71%. Higher levels of phosphorus did not significantly affect ash or phosphorus. Bone calcium levels generally increased as dietary phosphorus increased. Fish fed Diets 1 and 2 had significantly lower levels of bone calcium than fish fed Diets 6–8, however, no clear plateau was observed. There were no significant differences observed in bone Ca/P ratio or in bone magnesium levels, though magnesium did increase somewhat as dietary phosphorus increased.

TABLE 4. *Scale weight and mineralization of juvenile red drum fed diets containing graded levels of phosphorus. Data represent the mean of three replicates, expressed on a dry weight basis. Means not sharing a common letter are significantly different (P < 0.05).*[a]

Diet no.	Dietary phosphorus (%)	Scale weight[b] (%)	Ash (%)	Phosphorus (%)	Calcium (%)	Ca/P	Magnesium (%)
1	0.26	0.65 y	13.6 v	2.6 v	4.8 v	2.2 z	0.18 x
2	0.41	0.72 y	21.9 w	4.1 w	7.5 w	2.1 z	0.24 x
3	0.56	0.84 y	39.7 x	7.3 x	13.7 x	2.0 z	0.34 y
4	0.71	1.35 z	46.3 y	8.8 y	15.8 y	1.9 z	0.36 y
5	0.86	1.42 z	49.0 yz	9.7 z	17.4 yz	1.9 z	0.36 yz
6	1.01	1.35 z	51.1 z	9.9 z	18.9 z	2.0 z	0.40 yz
7	1.16	1.66 z	48.6 yz	10.1 z	19.0 z	1.8 z	0.41 yz
8	1.31	1.60 z	49.5 z	10.1 z	18.0 z	1.8 z	0.37 yz

[a] The pooled standard errors for scale weight, ash, phosphorus, calcium, Ca/P, and magnesium were 0.0004, 0.013, 0.108, 0.232, 0.031, and 0.007, respectively.

[b] (Dry scale weight/wet fish weight)*100.

TABLE 5. *Skin mineralization of juvenile red drum fed diets containing graded levels of phosphorus. Data represent the mean of three replicates, expressed on a dry weight basis. Means not sharing a common letter are significantly different (P < 0.05).*[a]

Diet no.	Dietary phosphorus (%)	Ash (%)	Phosphorus (%)	Calcium (%)	Ca/P	Magnesium (%)
1	0.26	3.8 z	0.66 w	0.69 z	1.07 z	0.07 z
2	0.41	4.0 z	0.69 wx	0.63 z	0.88 z	0.07 z
3	0.56	4.4 z	0.75 wxy	0.86 z	1.15 z	0.08 z
4	0.71	4.8 z	0.86 yz	1.00 z	1.14 z	0.08 z
5	0.86	5.1 z	0.97 z	1.02 z	1.06 z	0.08 z
6	1.01	4.9 z	0.90 yz	0.90 z	1.00 z	0.08 z
7	1.16	4.3 z	0.84 xyz	1.03 z	1.25 z	0.07 z
8	1.31	5.1 z	0.97 z	1.03 z	1.07 z	0.08 z

[a] The pooled standard errors for ash, phosphorus, calcium, Ca/P, and magnesium were 0.112, 0.018, 0.043, 0.043, and 0.001, respectively.

Scale Weight and Mineralization

The dry weight of scales per gram wet weight of fish is presented in Table 4. Fish fed Diets 1–3 had significantly lower scale weights than fish fed Diets 4–8. Differences in scales were apparent upon visual inspection; fish fed Diets 1–3 had smaller and more fragile scales than fish fed Diets 4–8.

In general, scale ash, phosphorus, calcium, and magnesium increased as dietary phosphorus increased up to 0.86%; higher supplemental levels of phosphorus produced no further changes (Table 4). The Ca/P ratio was not affected by increasing dietary phosphorus. Scale ash values ranged from 13.6 (Diet 1) to 51.5% (Diet 6). Ash values for fish fed Diets 1–3 were significantly lower than fish fed Diets 4–8. Fish fed Diet 4 had significantly lower ash levels than fish fed Diets 6 and 8, but they were not significantly different from fish fed Diets 5 and 7. Scale phosphorus increased significantly with dietary phosphorus in fish fed Diets 1–4 as compared to fish fed Diets 5–8. Magnesium ranged from 0.18 (Diet 1) to 0.41% (Diet 7). Fish fed Diets 1 and 2 had significantly lower scale magnesium levels than fish fed all other diets.

Skin Mineralization

Skin ash, calcium, Ca/P ratio, and magnesium (Table 5) varied only slightly and no significant differences were observed. However, there was a general increase in ash and calcium as dietary phosphorus increased. Though there was some statistical overlap, skin phosphorus generally increased in fish fed up to 0.75% dietary phosphorus, then leveled off.

Muscle Mineralization

Percentage muscle ash, phosphorus, calcium, Ca/P ratio, and magnesium levels averaged 6.0, 1.2, 1.0, 1.0, and 0.13, respectively. Ash and mineral content did not vary significantly between treatments.

Discussion

Water Quality

Holt and Arnold (1983) reported that red drum eggs and larvae could tolerate concentrations of 0.55 mg/L of un-ionized ammonia and nitrite levels of 100 mg/L without significant increases in mortality or growth depression. Based on these data, it is unlikely that the ammonia or nitrite levels observed in the present study (average of 0.2 mg/L and 0.029 mg/L, respectively) exhibited any significant influence on fish performance. Furthermore, since a closed system was used, any water quality change would affect each treatment similarly.

Growth Parameters

In general, phosphorus deficiencies in fish result in a depression of weight gain and feed conversion as well as reduction in survival (NRC 1983). In the current experiment, there were no significant differences in weight gain or feed conversion. Robinson et al. (1987) also observed that weight gain and feed conversion data gave no clear indication of the phosphorus requirement of *Tilapia aurea*. A phosphorus deficiency may not have been reflected in the weight gain of red drum in the present study, because a low level of phosphorus may be adequate for good weight gain in the short term. A longer experimental period may have resulted in a significant depression of weight gain. Furthermore, a relatively high level of mortality within treatments contributed to rather large variations in weight gain.

From week five to nine of the experiment, fish fed low-phosphorus diets (1–3) had significantly lower survival rates than fish fed high-phosphorus diets (7–8). This pattern changed at the end of the experiment with no clear trend exhibited (Table 2). The decrease in survival of fish fed phosphorus complete diets at the conclusion of the experiment was presumably caused by intensified fish aggression due to crowding.

Bone Mineralization

In the present study, no external physical deformities were noted. However, fish fed low-phosphorus diets (1 and 2) had noticeably smaller and softer vertebrae than fish fed diets containing higher levels of phosphorus. This response was apparently due to the improper mineralization of bone in fish fed phosphorus deficient diets. Similar results have been reported for other species (NRC 1983).

Bone ash, phosphorus, and calcium levels observed in the present study were similar to those reported for *Tilapia aurea* (Robinson et al. 1987), channel catfish, red sea bream, but slightly higher than those reported for the chum salmon and rainbow trout (NRC 1983). Bone ash, phosphorus, and calcium levels indicate that 0.71% phosphorus is adequate for proper mineralization of red drum bone.

There were no significant differences ($P < 0.05$) observed in Ca/P ratio or bone magnesium. However, magnesium tended to increase as dietary phosphorus increased up to 0.86% phosphorus and then leveled off. Similar results have been reported in the carp (Ogino and Takeda 1976), rainbow trout (Ogino and Takeda 1978), chum salmon (Watanabe et al. 1980), and *Tilapia aurea* (Robinson et al. 1987).

The phosphorus requirement of 0.71% based on bone mineralization of red drum is higher than the 0.29% requirement for the Japanese eel and 0.4–0.5% reported for channel catfish (NRC 1983). However, it is similar to the 0.6–0.8% phosphorus requirement reported for rainbow trout, Atlantic salmon, carp, chum salmon, and red sea bream (NRC 1983). It appears that scaled fish generally have a higher phosphorus requirement than scaleless fish, such as the Japanese eel and channel catfish.

Scale Weight and Mineralization

Differences in scale size were visually apparent, with fish fed Diets 1–3 having smaller and more fragile scales. Although scale weight, as a percentage wet weight of the fish, did not increase in fish fed levels of phosphorus greater than 0.71%, complete mineralization of the scales occurred at a dietary phosphorus level of 0.86%. Ash, phosphorus, calcium and magnesium generally increased as dietary phosphorus increased up to 0.86%. It should be noted that this level is higher than the level of phosphorus required for bone ash or bone phosphorus deposition. This indicates that bone mineralization occurs preferentially over scale mineralization, which would be expected if the scale tissue is being utilized as a physiological reserve. Reabsorption of scale minerals was originally described by Crichton (1935). The phenomenon has been demonstrated in several species, but is most

conspicuous in salmon and sea trout because of their particular life cycles (Simkiss 1974).

Scale magnesium increased as dietary phosphorus increased up to 0.56%. There are no data currently available on the effects of varying dietary phosphorus levels on the magnesium content of fish scales. Robinson et al. (1987) found that scale magnesium decreased in *Tilapia aurea* as dietary calcium increased.

Muscle Mineralization

Muscle mineralization was unaffected by dietary phosphorus levels. Muscle calcium and phosphorus levels were comparable to those reported by Snodgrass and Halver (1971) for muscle tissue of adult chinook salmon reared in salt water. Since muscle tissue is not a particularly important storage site for minerals, effects of diet should be minimal.

Skin Mineralization

Skin contains appreciable amounts of minerals, for example, up to 40% of stored calcium in trout can be found in the skin (Podoliak and Holden 1965; Simkiss 1967). Thus, skin mineralization may be an important indicator of the mineral status of fish. Calcium, Ca/P ratio, magnesium, and ash values of the skin of red drum did not change significantly in response to varying levels of dietary phosphorus. Skin phosphorus generally increased in fish fed up to 0.71% dietary phosphorus, then leveled off. Though skin phosphorus did not reflect the dietary requirement as clearly as bone or scale phosphorus, it did indicate that the requirement was near 0.71–0.86%.

Conclusion

Based on scale, bone, and skin mineralization, it appears that approximately 0.86% dietary phosphorus is needed for proper tissue mineralization in juvenile red drum. Bone and scale minerals appear to be relatively sensitive indicators of phosphorus status. It is recommended that commercial red drum feeds contain approximately 0 to 0.9% phosphorus.

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