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Dietary phosphorus requirement of juvenile black seabream, *Sparus macrocephalus*

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Abstract

A growth trial was conducted to estimate the optimum requirement of dietary available phosphorus (P) for black seabream (*Sparus macrocephalus*) in indoor net cages (1.5×1.0×1.0 m). Triplicate groups of black seabream (11.45±0.02 g) were fed diets containing graded levels (0.18, 0.36, 0.54, 0.72, 0.89 and 1.07%) of available P to satiation for 8 weeks. The basal diet (diet 1), containing 0.18% available P, was supplemented with graded levels of monosodium phosphate (NaH_2PO_4 $2H_2O$) to formulate five experimental diets. The fish were fed twice daily (08:00 h and 16:00 h) and reared in seawater (salinity, 26–29 g l^{-1}) at a temperature of 28±1 °C. Dissolved oxygen during the experiment was above 5 mg l^{-1}. The specific growth rate (SGR), weight gain (WG), feed efficiency (FE) and protein efficiency ratio (PER) were all significantly improved by dietary phosphorus up to 0.54% ($P<0.05$) and then leveled off beyond this level. Hepatosomatic index (HSI) was inversely correlated with dietary phosphorus levels ($P<0.05$). Efficiency of P utilization stabled in fish fed diets containing 0.18%–0.54% available P and then decreased dramatically with further supplementation of dietary phosphorus. Body composition analysis showed that the whole-body lipid, ash, calcium and phosphorus contents were all significantly affected by dietary available P concentration ($P<0.05$), however, no significance were found in whole-body calcium/phosphorus (Ca/P) ratios among all the treatments ($P>0.05$). Dietary phosphorus levels also affected the mineralization of vertebrae, skin and scale ($P<0.05$). Ca/P ratios in vertebrae and scale were not influenced by dietary P supplementation, while skin Ca/P ratio increased statistically with dietary available P levels (quadratic effect, $P<0.001$). The blood chemistry analysis showed that dietary available P had distinct effects on enzyme activities of alkaline phosphatase (ALP) and plasma lysozyme (LSZ), as well as contents of triacyglycerol (TG) and total cholesterol (T-CHO) ($P<0.05$). Broken-line analysis showed maximum weight gain (WG) was obtained at dietary available P concentrations of 0.55%. Quadratic analysis based on P contents in whole fish, vertebrae or scale indicated that the requirements were 0.81, 0.87 and 0.88%, respectively. Signs of phosphorus deficiency were characterized by poor growth, slightly reduced mineralization and an increase in body lipid content.

Keywords: Diet; Black seabream; *Sparus macrocephalus*; Phosphorus requirement; Feeding and nutrition; Mineralization

1. Introduction

Phosphorus (P) is an essential nutrient for growth (Baeverfjord et al., 1998), skeletal development (Åsgård and Shearer, 1997) and reproduction of fish (Hardy and Shearer, 1985). It plays an important role in the metabolism of carbohydrate, lipid, and amino acids, as well as various metabolic processes involving buffers in body fluids (Lall, 2002). Although fish can absorb minerals from natural water (NRC, 1993), food is the main source of phosphorus because of its low concentration both in freshwater and seawater (0.005–0.07 mg l^{-1}) (Boyd, 1971; Lall, 2002) as well as low absorption rate of phosphorus from the water (Philips et al., 1958).

The optimal amount of phosphorus supplementation in commercial feeds not only important economically, but also for environmental reasons. However, phosphorus concentrations in most practical diets considerably exceeded the estimated requirements

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Table 1
Composition of the basal diet (diet 1) and five experimental diets for black seabream juveniles (% dry matter)

Ingredients:	Diets					
	1	2	3	4	5	6
Casein	47.00	47.00	47.00	47.00	47.00	47.00
Gelatin	2.00	2.00	2.00	2.00	2.00	2.00
Squid meal	7.00	7.00	7.00	7.00	7.00	7.00
Dextrin	21.00	21.00	21.00	21.00	21.00	21.00
Fish oil	6.00	6.00	6.00	6.00	6.00	6.00
Corn oil	3.00	3.00	3.00	3.00	3.00	3.00
Carboxymethyl cellulose	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin premix [a]	4.00	4.00	4.00	4.00	4.00	4.00
Mineral premix [b]	2.75	2.75	2.75	2.75	2.75	2.75
$NaH_2PO_4 2H_2O$	0.00	1.25	2.50	3.75	5.00	6.25
α-cellulose	6.25	5.00	3.75	2.50	1.25	0.00
Chemical composition (%)						
Crude protein	47.10	47.01	47.01	47.07	47.00	47.08
Crude lipid	9.20	9.20	9.18	9.23	9.23	9.18
Total phosphorus	0.49	0.71	0.90	1.12	1.32	1.59
Available phosphorus [c]	0.18	0.36	0.54	0.72	0.89	1.07
Ca/P ratio	1.87	1.83	1.10	0.93	0.67	0.59

[a] Vitamin premix (mg kg^{-1} diet): alpha tocopherol, 20; Na menadione bisulfate, 5; thiamin, 5; riboflavin, 5; calcium pantothenate, 10; nicotinic acid, 100; pyridoxine, 5; folic acid, 2; cyanocobalamin, 0.05; biotin, 0.5; ascorbic acid, 150; *p*-aminobenzoic acid, 50; inositol, 500; choline chloride, 500; (UI kg^{-1} diet): retinol, 10,000, cholecalciferol, 2000.

[b] Composition of the basal mineral premix (g kg^{-1} diet): cobalt sulfate, 0.028; copper sulfate, 0.35; ferric citrate, 2.83; magnesium oxide, 7.08; manganous sulfate, 0.71; $CaCO_3$, 12.50; NaCl, 4.00.

[c] The values were calculated based on the digestibility of basal diet (37%) and monosodium phosphate (72%), as determined in the digestibility trial.

(Rodehutscord and Pfeffer, 1995), which is responsible for the environmental impact caused by surplus phosphorus discharges into the effluents. Therefore, in recent years there has been a trend towards the reduction of dietary phosphorus to levels that satisfy, but do not exceed phosphorus requirements to produce maximum growth of fish and protect water quality (Lall, 1991; Oliva-Teles et al., 1998; Bureau and Cho, 1999). Consequently, estimation of dietary phosphorus requirements in cultured aquatic animals becomes a priority.

Phosphorus requirements have been determined for many marine fish species, such as juvenile milkfish (0.85%; Borlongan and Satoh, 2001), gilthead sea bream (0.75%; Pimentel-Rodrigues and Oliva-Teles, 2001), European sea bass (0.65%; Oliva-Teles and Pimentel-Rodrigues, 2004), Japanese seabass (0.68–0.90%; Zhang et al., 2006), large yellow croaker (0.70–0.91%; Mai et al., 2006) and so on. But for black seabream (*Sparus macrocephalus*), another member of the major commercially important marine fish in China, few studies have been conducted on its nutritional requirements except for the fry production (Hong and Zhang, 2003) and its pharmacokinetics of oxytetracycline (OTC) *in vivo* (Wang et al., 2001, 2004). In addition, trash fish was used for cultured black seabream in China, which could not meet the nutritional requirements of black seabream, and was difficult to store, easy to pollute aquaculture environments. Hence, commercial feeds formulated specifically for black seabream are demanded to meet their nutritional needs, improve productive efficiency and decrease phosphorus discharge. The present investigation was undertaken to determine the dietary available phosphorus requirements of juvenile black seabream.

2. Materials and methods

2.1. Experimental diets

The basal diet (diet 1) and five experimental diets were formulated to contain graded levels (0.18, 0.36, 0.54, 0.72, 0.89 and 1.07%) of dietary available P. Ingredient composition and proximate analysis of the diets are presented in Table 1. Phosphorus in the form of monosodium phosphate (NaH_2PO_4 $2H_2O$) was supplemented in the experimental diets at the expense of α-cellulose.

All the ingredients were homogenized in a mixer after the addition of fish oil and corn oil. Distilled water was included to achieve a proper pelleting consistency, and the mixture was further homogenized and extruded through a 3-mm die. The noodle-like diets were dried at 23 °C for 72 h with air conditioning and fanner working all the time. Dried noodles were broken into particles by a food processor, sieved to remove particles above 3 mm and then stored in refrigerator at −20 °C up to use. A representative sample was taken for proximate analysis.

2.2. Experimental procedure

Juvenile black seabream (*S. macrocephalus*) (initial weight 11.45±0.02 g) were hatched and reared at the Research Institute of Zhejiang Marine Fisheries in Zhoushan. The feeding trial was conducted in two indoor concrete ponds (water volume, 39 m^3). A total of 450 fish were stocked in one pond and conditioned for two weeks by feeding with the basal diet twice daily (08:00 h and 16:00 h) to visual satiation. Prior to the feeding trial, all fish were starved for 24 h, and then weighed after being anesthetized with MS-222 (tricane methanesulphonate, 60 mg l^{-1}). Fish with similar size were distributed into 18 net cages (1.5×1.0×1.0 m) in another pond at a stocking density of 25 fish per cage. All the cages were supplied with sand-filtered seawater at a flowing rate of 3 l min^{-1}. Each diet was fed to triplicate groups of fish twice daily (08:00 h and 16:00 h) to apparent satiation for 8 weeks. During the course of the experiment, water temperature was 28±1 °C, salinity ranged from 26 to 29 g l^{-1} and dissolved oxygen remained above 5 mg l^{-1}. Photoperiod was provided by natural lighting (12-h dark/12-h light).

To determine the phosphorus availability from the basal diet or monosodium phosphate, one hundred and twenty juvenile black seabream (initial weight 38.30±0.06 g) were randomly distributed into six extra cages (1.5×1.0×1.0 m; 20 fish per cage) and both groups with triplicate were adapted to the experimental diets for one week. Chromic (III) oxide (Cr_2O_3) was added (1.0% of diet) to the experimental diets as an inert digestion marker and these diets were fed for two weeks prior to fecal collection. Feces were stripped from all fish by applying gentle pressure in the anal area according to the procedure of Austreng (1978). After a 6-day interval, three samples were collected from each cage. Fecal samples were pooled, dried at 60 °C in an oven, and stored at −20 °C for subsequent analysis.

2.3. Sample collection and analysis

Upon termination of the 8-week growth study, fish were counted and bulk-weighed after a 24-h fast. Five fish randomly selected from each cage were used for whole-body lipid and mineralization analysis. Blood samples were collected from 5 anesthetized (tricane methanesulphonate, 60 mg l^{-1}) fish of each cage with 1-ml syringe by puncture of the caudal vein into a heparinized tube, centrifuged at 3000 ×*g* for 10 min and plasma was removed and frozen at −20 °C for subsequent analysis. After measuring the body weight and length, livers were removed from all the remaining fish and weighed for hepatosomatic index (HSI) calculation. Skins, scales and vertebrae were collected individually from 5 fish of each triplicate for the determination of ash, calcium, and phosphorus concentration. Skins (with scale) and scales were removed from sampled fish, washed with distilled water and then dried at 105 °C. Vertebrae were easily removed from fish after heating in a microwave oven for 60–80 s,