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Effects of dietary phosphorus on growth, body composition, and blood chemistry of juvenile taimen *Hucho taimen*

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Abstract To determine the optimum P requirements of taimen (*Hucho taimen*), the present study investigated the effects of diets supplemented with different levels of inorganic P (NaH_2PO_4) on growth, proximate composition, blood chemistry, and mineralization in juvenile taimen. Triplicate groups of fish (mean initial weight 15.39 ± 0.28 g) were fed, until satiation, with different diets containing graded levels of digestible P (0.22, 0.40, 0.55, 0.74, 0.90, and 1.07%), for 84 days. Weight gain, feed conversion ratio, and protein retention were significantly ($P < 0.05$) improved with 0.40% dietary P, with values 210.69 ± 16.08%, 1.13 ± 0.11, and 31.73 ± 3.34%, respectively. However, no significant increase in these parameters was obtained with increasing dietary P levels ($P > 0.05$). With increasing dietary P levels, P retention and whole-body lipid content decreased from 75.41 ± 7.06 to 36.41 ± 3.30% and from 3.23 ± 0.13 to 2.74 ± 0.25%, respectively, whereas vertebrae ash and plasma P content increased from 43.68 ± 0.23 to 52.09 ± 0.28% and from 2.18 ± 0.14 to 3.98 ± 0.23 mmol L^{-1}, respectively. Using a broken-line regression model that incorporated measurements of P digestibility, feed efficiency, and normal whole-body and vertebrae P content, the dietary requirement of digestible P was determined to be approximately 0.5% for taimen.

Keywords Salmonids · Mineral nutrition · Proximate composition · Hematology

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Abbreviations

IBW	Initial body weight
FBW	Final body weight
WG	Weight gain
FCR	Feed conversion ratio
CF	Condition factor
PPV	Protein productive value
FL	Fork length
ALP	Alkaline phosphatase
TP	Total protein
ALB	Albumin
GLB	Globulin
GLU	Glucose
CHO	Cholesterol
TG	Triglyceride
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase

Introduction

Phosphorus is a major constituent of nucleic acids and cell membranes; it is a structural component of skeletal tissues and is directly involved in all energy-producing cellular reactions in fish (NRC 2011). Although fish can absorb minerals from natural water, food is the main source of P because of the low P content (0.005–0.07 mg L^{-1}) in freshwater and seawater as well as low P absorption rate from water (Lall 2002).

Insufficient dietary P may cause poor growth, bone deformities, sluggish movements, lower feed efficiency, and high lipid and low ash content in the body (Roy and Lall 2003; Yang et al. 2006). Moreover, body lipid accumulation can result in cellular hypoxia, inhibition of oxidative phosphorylation, and other syndromes (Sugiura et al. 2004). In aquaculture, the amount of P in fish feed must be carefully balanced to prevent signs of P deficiency while minimizing P discharge in natural waters by urinary and fecal excretions (Roy and Lall 2003). A high P content of fish diets may cause heavy P loading in freshwater farms that leads to eutrophication and causes serious threats to sustainable aquaculture (Wiesmann et al. 1988). Therefore, there is a need to optimize the dietary P level as well as to develop nutritional strategies for this element.

Taimen (*Hucho taimen*) is a promising salmonid species for aquaculture in China. To optimize the production of this species, it is essential to use commercial feeds formulated specifically for taimen. Currently, there are no clear reports on optimum dietary requirements for taimen, except for proteins and lipids (Xu et al. 2007), and no information is available on the digestible P requirement for this species. Therefore, the present study was designed to investigate the effects of different levels of dietary P on growth, body composition, nutrient digestibility, mineralization, and blood chemistry in juvenile taimen, to find the optimum digestible P content for taimen culture and to keep effluent P at a relatively low level.

Materials and methods

Experimental diets

Isonitrogenous and isocaloric purified diets were formulated with monosodium phosphate (NaH_2PO_4) to contain 0.22 (basal diet), 0.40, 0.55, 0.74, 0.90, and 1.07% digestible P (based on the apparent P digestibility determined), respectively (Table 1).

Dry ingredients were sieved through a 60-mesh screen and then were homogenized and blended thoroughly in a feed mixer for 10 min. The required amount of fish oil and an appropriate amount of water were added to the blended mixture to obtain a dough that could be made into pellets. Diet pellets (diameter 1.5 mm) were air-dried until reaching 8–10% moisture and then were stored at −20 °C. Methods of feed composition analysis followed the AOAC (1995) procedures.

Experimental procedure

Juvenile taimen (*H. taimen*) was obtained from Bohai Coldwater Fish Hatchery, Chinese Academy of Fishery Sciences, Mudanjiang City, Heilongjiang Province, China. For 14 days, fish were acclimatized and fed with a low P diet without supplemental P. From the stock, 540

Table 1 Composition of the experimental diets for juvenile *H. taimen* (% dry matter)

Ingredients	Diets					
	1	2	3	4	5	6
Casein[a]	40.6	40.6	40.6	40.6	40.6	40.6
Gelatin[b]	10.0	10.0	10.0	10.0	10.0	10.0
Dextrin[c]	30.5	30.5	30.5	30.5	30.5	30.5
Fish oil	10.3	10.3	10.3	10.3	10.3	10.3
$CaCO_3$	1.5	1.5	1.5	1.5	1.5	1.5
Cr_2O_3	0.5	0.5	0.5	0.5	0.5	0.5
Premix[d]	1.6	1.6	1.6	1.6	1.6	1.6
α-Cellulose	5.0	4.2	3.4	2.6	1.8	1.0
NaH_2PO_4	0.0	0.8	1.6	2.4	3.2	4.0
Chemical composition (%)						
Crude protein	45.1	45.0	45.0	45.0	45.1	45.0
Crude lipid	10.0	10.0	10.1	10.0	10.0	10.0
Total phosphorus	0.43	0.68	0.85	1.06	1.23	1.42
Digestible phosphorus[e]	0.22	0.40	0.55	0.74	0.90	1.07
Ca/P ratio	2.00	1.26	1.01	0.81	0.70	0.61
Gross energy (MJ kg^{-1})	19.98	19.95	20.02	19.96	19.98	20.02

[a] Hualing Food Company, Zhengzhou, China

[b] Zhiyuan Chemical Agent Company, Tianjin, China

[c] Huludao Chia Tai Feed Corporation, Huludao, China

[d] Premix (mg kg^{-1} diet), including choline chloride 200; antimildew agent 50; magnesium sulfate 200; betain 100; antioxidant 50; potassium chloride 100; sodium chloride 400; vitamin premix 3000 (ascorbic acid 100; alpha tocopherol 60; Na menadione bisulfate 5; retinol acetate 15,000 IU; cholecalciferol 3000 IU; thiamin 15; riboflavin 30; pyridoxine 15; cyanocobalamin 0.5; nicotinic acid 175; folic acid 5; *myo*-inositol 300; biotin 2.5; pantothenic acid 50); mineral premix 2000 (ferrous sulfate 50; copper sulfate 10; manganous sulfate 40; zinc sulfate 50; potassium iodide 1.0; cobalt chloride 5; sodium selenite 1)

[e] Values were based on the apparent P digestibility determined

fish, with similar size (15.39 ± 0.28 g), were selected and randomly distributed into 18 tanks corresponding to six treatments with triplicate groups of 30 fish per tank. Fish were fed by hand four times per day, until apparent satiation. All uneaten feed was collected 30 min after each meal, for feed intake calculation. The experimental trial was performed during 84 days. The fish were kept in tanks (500 L per tank), fed by spring water flow. Spring water was filtered through zeolum, corallite, and activated carbon, and the water quality parameters were monitored daily. Water temperature, dissolved oxygen, pH value, and ammoniacal nitrogen were measured with a multi-parameter water quality sonde (YSI 6600 V2-2). During the experimental period, average water temperature ranged from 10.9 to 11.5 °C, dissolved oxygen was >8.0 mg L^{-1}, pH value ranged from 7.1 to 7.3, and ammoniacal nitrogen was <0.02 mg L^{-1}. Approximately, 2 L s^{-1} of freshwater was provided to the fish. The photoperiod was 12-h light/12-h dark. Prior to the experimental trial, an initial sample of 18 fish was collected for body composition analysis (Table 2).

For calculating the P digestibility in this growth trial, chromium III oxide (Cr_2O_3) was used as an indigestible marker in all experimental diets. Feces were collected 5–6 h after the first meal at 08:00 from each tank over a period of 6 days. Feces were stripped from fish by applying gentle pressure in the anal area, according to the procedure of Austreng (1978). For a single fecal sample, feces were collected from each tank (fish were returned to their tank) and quickly frozen, oven-dried at 65 °C, and stored at −20 °C, until analysis.

Sample collection and analytical methods

At the end of the trial, fish were individually weighed and measured for body length, after 24-h starvation. Six whole-body samples from each tank were kept at −80 °C, until further use for proximate composition determination. Another six fish from each tank were selected randomly and anesthetized (clove oil, 30 mg L^{-1}), and their blood was collected from the caudal vein into heparin tubes. The plasma was separated from the blood cells by centrifugation at 3000*g* for 10 min at 4 °C. Subsequently, plasma was stored at −80 °C, until analysis. After plasma collection, fish were killed with a sharp blow to the head, and livers were removed and weighed to determine the hepatosomatic index (HSI). Muscle tissue was cut from the dorsal part of the left fillet, and the vertebrae were removed to determine the contents of Na, K, Ca, Mn, Fe, Cu, Zn, Se, Co, Mg, and P. The vertebrae were dissected from microwave oven-cooked fish, were cleaned of connective tissues, and then were dried for 24 h at 60 °C.

Feed powder, whole-body, and vertebrae samples were dried at 110 °C to a constant weight. Lipids were extracted from the vertebrae by treating with 10 mL chloroform

Table 2 Initial whole-body composition of juvenile *H. taimen*

Item	Body composition (%)			
	Moisture	Crude protein	Crude lipid	Ash
Initial body	77.88 ± 0.28	15.67 ± 0.20	2.98 ± 0.52	2.62 ± 0.35

Values are represented as mean ± standard deviation of pooled data (n = 18)

and methanol (1:1, *v*/*v*) for 12 h and then were dried. The chemical composition of whole-body and vertebrae samples was determined using the following procedures (AOAC 1995): dry matter, by oven-drying at 110 °C; crude protein (N × 6.25), by the Kjeldahl method using a KDN-812 nitrogen determinator (Shanghai Xianjian Instrument, China); crude lipid, by extraction with ether for 8 h in a Soxhlet extractor; gross energy, measured in an adiabatic bomb calorimeter (Model HWR-15E, Shangli Instruments, China); ash, incinerated in a muffle furnace at 550 °C for 18 h; and chromium content of experimental diets and feces, analyzed according to Czarnocki et al. (1961). Muscle and ash (whole-body or vertebrae) were weighed and wet-digested with HCl and HNO_3. P in all samples, including plasma, was analyzed by a spectrophotometric method using molybdovanadate reagent (Taussky and Shorr 1953). The contents of Na, K, Ca, Mn, Fe, Cu, Zn, Se, Co, and Mg were measured by inductively coupled plasma mass spectrometry (ICP-MS, Xseries 2, Thermo Scientific, USA), as described by Forrer et al. (2001). The activities of alkaline phosphatase (ALP) of plasma were analyzed spectrophotometrically using diagnostic reagent kits, purchased from the Nanjing Jiancheng Bioengineering Institute. Total protein (TP), albumin (ALB), globulin (GLB), glucose (GLU), cholesterol (CHO), triglyceride (TG), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) of plasma were enzymatically analyzed using an automatic chemistry system (HITACHI 7180 and 7600-210, Hitachi, Japan), based on the manual of Daiichi Pure Chemicals Co. Ltd. (2005).

Calculation and statistical analysis

Weight gain (WG), feed conversion ratio (FCR), condition factor (CF), hepatosomatic index (HSI), protein productive value (PPV), and efficiency of P utilization were calculated according to the following equations:

$$\mathrm{WG} = 100 \times (\textit{weight gain}, \mathrm{g}) \Big/ (\textit{initial weight}, \mathrm{g}) \;;$$

$$\mathrm{FCR} = 100 \times (\mathrm{dry}\ \textit{diet intake}, \mathrm{g}) \Big/ (\textit{weight gain}, \mathrm{g}) \;;$$

$$\mathrm{CF} = 100 \times (\textit{body weight}, \mathrm{g}) \Big/ (\textit{body length}, \mathrm{cm})^3 \;;$$

$$\mathrm{HSI} = 100 \times (\textit{weight of liver}, \mathrm{g}) \Big/ (\textit{total weight of fish}, \mathrm{g}) \;;$$

$$\mathrm{PPV} = 100 \times (\textit{body protein gain}, \mathrm{g}) \Big/ (\textit{protein intake}, \mathrm{g}) \;;$$

$$\mathrm{P}\ \textit{retention} = 100 \times [(\textit{final fish}\ \mathrm{P}, \mathrm{g}) - (\textit{initial fish}\ \mathrm{P}, \mathrm{g})] \Big/ (\mathrm{P}\ \textit{intake}, \mathrm{g}).$$

Apparent P digestibility in the experimental diets (*Dp*) was calculated according to the equation $Dp\ (\%) = 100 \times [1 - (Cr_d \times P_f) / (Cr_f \times P_d)]$, where Cr_d was the content of Cr_2O_3 in diet, P_f was the content of P in feces, Cr_f was the content of Cr_2O_3 in feces, and P_d was the content of P in diet (NRC 1993).

All data were averaged on a per tank basis and analyzed using one-way ANOVA, expressed as a mean ± standard deviation of replicates ($n = 3$) by Duncan's multiple range test. $P < 0.05$ was regarded as statistically significant. All statistics were performed using the SPSS package (version 19.0). The plots were drawn using Sigma plot software version 12.5 (Jandel Scientific, San Rafael, CA). The broken-line model was used to estimate the requirement of dietary digestible phosphorus (Robbins et al. 1979).

Results

P digestibility, growth, and feed efficiency

According to the apparent P digestibility trial, digestible P contents in the experimental diets were estimated to be 0.22 (control diet), 0.40, 0.55, 0.74, 0.90, and 1.07%, respectively (Table 1). No fish died during the study. Fish fed with the P-supplemented diets had significantly higher weight gain (WG) and protein retention than fish fed with basal diet ($P < 0.05$) (Table 3). WG increased up to 0.40% digestible P and then leveled off beyond this level. The broken-line analysis for WG indicated that the dietary requirement is 0.43% digestible P (Fig. 1). Feed conversion ratio (FCR) decreased significantly with dietary P supplementation ($P < 0.05$). P utilization decreased with increasing dietary P levels. There were no significant differences in condition factor (CF) among all groups ($P > 0.05$).

Body composition

There were no significant differences in moisture and crude protein of whole-body samples among all treatments ($P > 0.05$) (Table 4). The whole-body lipid content decreased significantly from 0.55 to 0.90% digestible P levels. Ash, Ca, or P content in the whole-body increased linearly to 2.54, 0.94, and 0.53%, respectively, and then leveled off ($P > 0.05$). Based on the broken-line analysis for whole-body P content, the dietary requirement is 0.55% digestible P (Fig. 2). There was no difference in Ca/P ratio in the whole-body among all treatments ($P > 0.05$). Hepatosomatic index (HSI) linearly decreased from 1.92 to 1.30% with increasing dietary P levels.

Plasma biochemical parameters

Plasma P content showed an increasing trend (Table 5). The alkaline phosphatase (ALP) activity increased linearly from 95.43 to 128.62 IU L^{-1} with increasing digestible P level from 0.22 to 0.74% and then gradually decreased. In all groups, no significant changes were recorded in plasma TP, ALB, GLB, and GLU ($P > 0.05$). CHO content ranged from 2.50 to 3.57 mmol L^{-1} in the treatments and was significantly lower to those of the control group ($P < 0.05$). TG content decreased with dietary P supplementation ($P > 0.05$). Dietary P levels had no significant effects on plasma AST and ALT ($P > 0.05$).

Mineralization in vertebrae

Vertebrae mineralization was affected by dietary P supplementation (Supplementary Tables 1 and 2). Ash content in the vertebrae significantly increased with increasing digestible P level from 0.22 to 0.74% and then leveled off ($P > 0.05$). Ca and P content in the vertebrae showed an increasing trend. The dietary requirement is 0.56% digestible P based on the broken-line analysis for vertebrae P (Fig. 3). The Ca/P ratio of the vertebrae remained close to 2:1 in all groups ($P < 0.05$).

Fe, Zn, Mn, and Mg contents of vertebrae linearly decreased with increasing digestible P level from 0.40 to 1.07%. The lowest Cu content in vertebrae was found in a diet containing 0.55% digestible P. Se content decreased with P supplementation ($P < 0.05$). No significant differences were observed in Na, K, and Co contents of vertebrae ($P > 0.05$).

Table 3 Growth performances of juvenile *H. taimen*-fed diets with graded levels of phosphorus for 84 days

Parameters	Digestible phosphorus in the diet (%)					
	0.22	0.40	0.55	0.74	0.90	1.07
IBW (g)	15.50 ± 0.17	15.39 ± 0.35	15.39 ± 0.42	15.39 ± 0.35	15.28 ± 0.25	15.39 ± 0.35
FBW (g)	39.39 ± 2.27 a	47.78 ± 1.55 b	49.28 ± 1.70 b	49.83 ± 0.50 b	49.44 ± 1.09 b	49.78 ± 1.51 b
WG (%)	154.24 ± 17.22 a	210.69 ± 16.08 b	220.33 ± 12.38 b	223.90 ± 5.45 b	223.62 ± 1.92 b	223.46 ± 5.82 b
CF	0.76 ± 0.06	0.86 ± 0.13	0.79 ± 0.06	0.78 ± 0.02	0.79 ± 0.04	0.85 ± 0.07
FCR	1.59 ± 0.08 b	1.13 ± 0.11 a	1.12 ± 0.12 a	1.12 ± 0.07 a	1.16 ± 0.09 a	1.11 ± 0.06 a
PPV (%)	22.82 ± 1.60 a	31.73 ± 3.34 b	32.12 ± 3.01 b	32.28 ± 2.48 b	30.57 ± 2.65 b	32.28 ± 2.31 b
P retention (%)	75.41 ± 7.06 d	67.97 ± 4.60 cd	62.35 ± 5.46 c	48.44 ± 1.61 b	39.70 ± 3.58 a	36.41 ± 3.30 a

Values are represented as mean ± standard deviation of pooled data from triplicates per treatment ($n = 3$). Means in the same row with different letters are significantly different ($P < 0.05$)

IBW initial body weight, *FBW* final body weight, *WG* weight gain, *CF* condition factor, *FCR* feed conversion ratio, *PPV* protein productive ratio

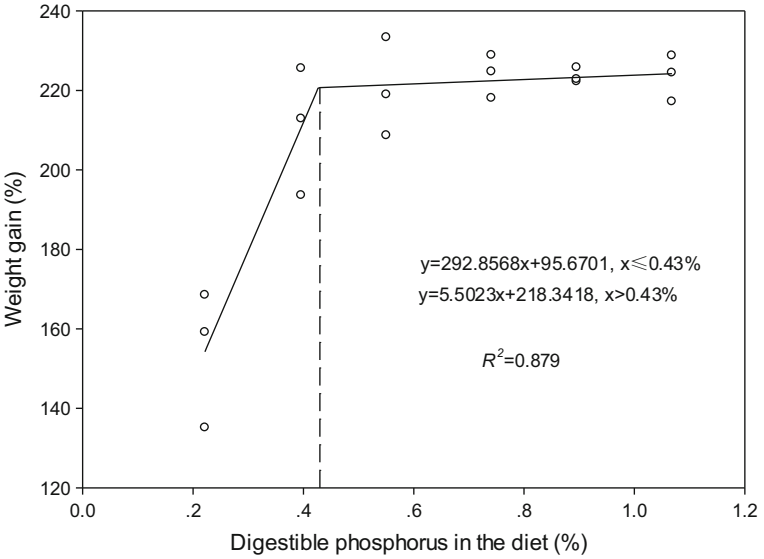

Fig. 1 Relationship between weight gain and digestible phosphorus levels for juvenile *H. taimen* as described by broken-line regression ($n = 3$). The breakpoint in the *broken line* is 0.43% digestible phosphorus

Mineralization in muscle

Dietary P levels significantly affected the muscle mineral contents of taimen (Supplementary Table 3). Ca or P content of muscles linearly increased with increasing digestible P level from 0.22 to 0.74%, whereas no significant differences were observed in 0.74, 0.90, and 1.07% digestible P groups ($P > 0.05$). Mg, Cu, Zn, and Se contents of muscles decreased with increasing P levels. K, Na, and Co contents of muscles were similar in all groups ($P > 0.05$).

Discussion

In fish, suboptimal P supply at subtoxic levels can lead to external symptoms. The reduced growth, poor feed efficiency, and changed body composition observed in taimen (*H. taimen*) fed with P-deficient diet verified in our research were similar to those reported in other studies (Yang et al. 2006; Ye et al. 2006; Yuan et al. 2011). This might be due to the low level of P, which was insufficient for normal growth and other physiological processes (Brown et al. 1993). In contrast, several researchers reported that growth of fish fed with P-deficient diet did not differ from those of fish fed with P-adequate diets (Chaimongkol and Boonyaratpalin 2001; Luo et al. 2010). Several factors including the stage of development, duration of the experiment, diet composition, health, and rearing conditions may be attributable to these differences in different experiments (Luo et al. 2010).

Due to the function of P in bone structure, the vertebrae P content is considered the most sensitive criterion for P availability in fish (Jahan et al. 2001). In this study, dietary P levels significantly affected ash and P content in vertebrae or in the whole body, which indicates that these values could be used to estimate the P requirement of taimen. However, the P requirement, based on vertebrae P content and the whole-body

Table 4 Whole-body composition of juvenile *H. taimen*-fed diets with graded levels of phosphorus for 84 days

Parameters	Digestible phosphorus in the diet (%)					
	0.22	0.40	0.55	0.74	0.90	1.07
Moisture (%)	78.08 ± 0.41	77.73 ± 0.19	77.85 ± 0.12	77.72 ± 0.41	78.12 ± 0.17	77.80 ± 0.18
Crude protein (%)	16.12 ± 0.11	15.97 ± 0.04	15.96 ± 0.23	16.03 ± 0.17	15.92 ± 0.15	16.04 ± 0.16
Crude lipid (%)	3.23 ± 0.13 c	3.15 ± 0.03 bc	2.92 ± 0.03 ab	2.74 ± 0.25 a	2.77 ± 0.17 a	3.01 ± 0.12 abc
Ash (%)	2.20 ± 0.19 a	2.35 ± 0.28 ab	2.54 ± 0.14 bc	2.77 ± 0.05 cd	2.90 ± 0.12 d	2.89 ± 0.09 d
Ca (%)	0.82 ± 0.09	0.94 ± 0.07	0.88 ± 0.04	0.96 ± 0.12	0.93 ± 0.11	0.93 ± 0.11
P (%)	0.48 ± 0.02 a	0.49 ± 0.02 a	0.53 ± 0.01 b	0.52 ± 0.02 b	0.53 ± 0.02 b	0.53 ± 0.02 b
Ca/P ratio	1.72 ± 0.25	1.93 ± 0.21	1.65 ± 0.08	1.68 ± 0.16	1.86 ± 0.26	1.77 ± 0.26
HSI (%)	1.92 ± 0.07 c	1.83 ± 0.11 c	1.64 ± 0.05 b	1.39 ± 0.09 a	1.33 ± 0.18 a	1.30 ± 0.06 a

Values are represented as mean ± standard deviation of pooled data from triplicates per treatment ($n = 3$). Means in the same row with different letters are significantly different ($P < 0.05$)

HSI hepatosomatic index

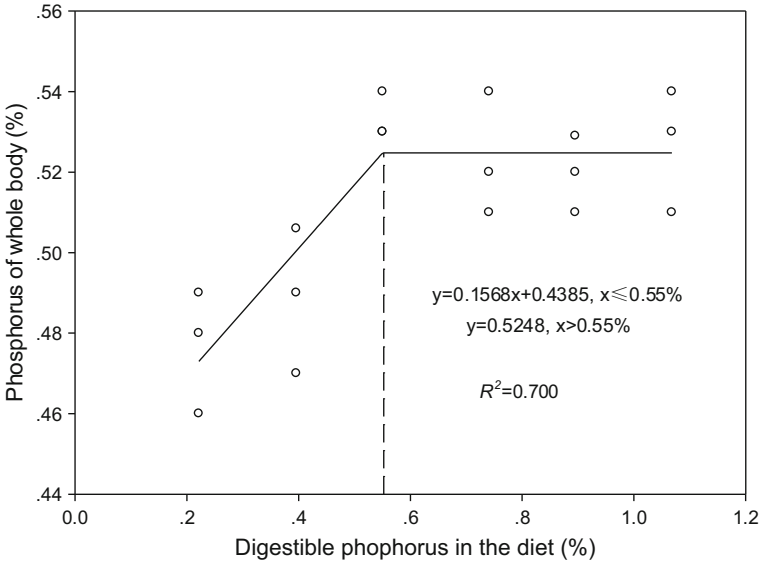

Fig. 2 Relationship between whole-body phosphorus and digestible phosphorus levels for juvenile *H. taimen* as described by broken-line regression (n = 3). The breakpoint in the *broken line* is 0.55% digestible phosphorus

P content, is not in accordance with that reported in other reports (Jahan et al. 2001; Zhang et al. 2006), which could mean that the P requirements might be species-specific.

Our results for dietary P requirement value of juvenile taimen was relatively close to those reported for chum salmon (*Oncorhynchus keta*) (0.5–0.6% digestible P) (Watanabe et al. 1980) and rainbow trout (*Oncorhynchus mykiss*) (0.60% digestible P) (NRC 1993), but lower than that reported for Atlantic salmon (*Salmo salar*) (0.90% digestible P) (Åsgård and Shearer 1997). These differences may be due to inherent differences in species and to variation in intestinal P absorption rate (Avila et al. 2000), P source (Satoh et al. 2002), fish size, health condition and development stage (Rønsholdt 1995), dietary energy content, feed efficiency, and differences in experimental design (Shearer 2000).

The metabolic effect of dietary P has been little researched in fish. Sugiura et al. (2000) suggested that fish and higher animals might have similar metabolic responses to P. Similar to rainbow trout (Sugiura et al. 2007), no significant changes in plasma AST and ALT activity were recorded for taimen, which indicates that slightly lower or higher dietary P content could not induce hepatic damage. However, in fish, dietary P could influence lipid metabolism. In the present study, plasma CHO content significantly decreased with dietary P supplementation. Similarly, El-Zibdeh et al. (1995) found that plasma TG and CHO in redlip mullet (*Liza haematocheila*) reached a maximum at the level of 0.65% dietary P. Blood CHO and TG content in black seabream (*Sparus macrocephalus*) decreased with dietary P supplementation and then leveled off beyond the level of 0.54 and 0.72%, respectively (Shao et al. 2008). Moreover, the whole-body lipid content and HSI decreased with increasing dietary P levels. This result is in accordance with previous findings (Shao et al. 2008; Nwanna et al. 2010). Transport of extra-mitochondrial fatty acids into mitochondria involves an

Table 5 Biochemical composition of plasma of juvenile *H. taimen*-fed diets with graded levels of phosphorus for 84 days

Parameters	Digestible phosphorus in the diet (%)					
	0.22	0.40	0.55	0.74	0.90	1.07
Ca (mmol L^{-1})	1.39 ± 0.07 a	1.66 ± 0.02 b	1.67 ± 0.05 b	1.76 ± 0.10 b	1.70 ± 0.09 b	1.68 ± 0.07 b
P (mmol L^{-1})	2.18 ± 0.14 a	2.55 ± 0.21 a	3.13 ± 0.41 b	3.26 ± 0.20 b	3.93 ± 0.39 c	3.98 ± 0.23 c
ALP (IU L^{-1})	95.43 ± 4.43 a	124.77 ± 2.98 b	125.82 ± 3.84 b	128.62 ± 2.98 b	123.60 ± 3.50 b	125.47 ± 8.99 b
TP (g L^{-1})	39.31 ± 1.04	39.48 ± 1.54	39.63 ± 1.46	39.05 ± 0.73	39.73 ± 0.54	39.53 ± 0.77
ALB (g L^{-1})	17.84 ± 1.43	18.30 ± 1.05	17.64 ± 0.62	18.18 ± 0.74	17.64 ± 0.73	18.83 ± 0.43
GLB (g L^{-1})	21.46 ± 1.16	21.19 ± 1.91	21.99 ± 1.75	20.86 ± 1.37	22.09 ± 0.29	20.70 ± 0.90
GLU (mmol L^{-1})	3.83 ± 1.01	4.41 ± 0.41	4.31 ± 0.08	4.58 ± 0.56	4.34 ± 0.43	4.88 ± 1.09
CHO (mmol L^{-1})	4.72 ± 0.23 c	2.51 ± 0.59 a	2.47 ± 0.21 a	2.50 ± 0.38 a	3.29 ± 0.28 b	3.57 ± 0.48 b
TG (mmol L^{-1})	7.49 ± 0.67 b	6.81 ± 0.55 ab	7.01 ± 0.20 ab	5.78 ± 1.02 a	6.61 ± 0.60 ab	6.75 ± 0.83 ab
AST (IU L^{-1})	324.64 ± 14.10	344.17 ± 11.94	335.92 ± 15.35	331.63 ± 26.96	335.90 ± 19.90	318.96 ± 14.79
ALT (IU L^{-1})	34.34 ± 2.71	33.53 ± 0.86	35.05 ± 2.14	32.75 ± 1.03	34.50 ± 0.87	33.58 ± 1.13

Values are represented as mean ± standard deviation of pooled data from triplicates per treatment ($n = 3$). Means in the same row with different letters are significantly different ($P < 0.05$)

ALP alkaline phosphatase, *TP* total protein, *ALB* albumin, *GLB* globulin, *GLU* glucose, *CHO* cholesterol, *TG* triglyceride, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

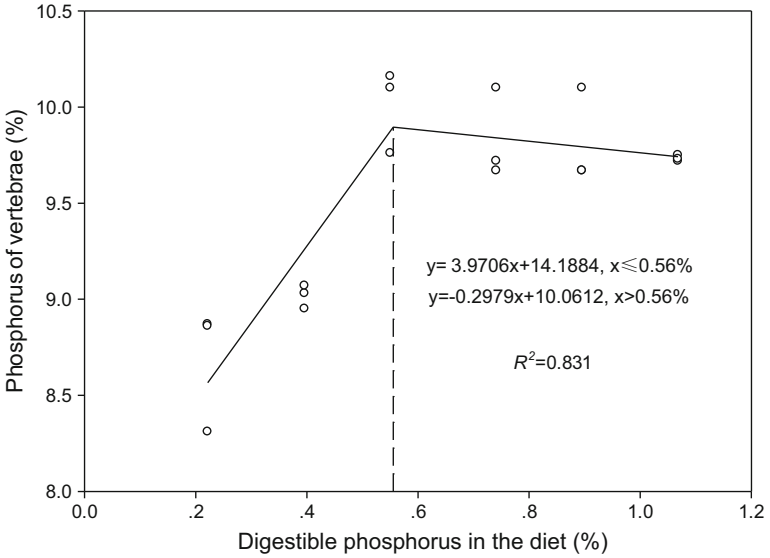

Fig. 3 Relationship between vertebrae phosphorus and digestible phosphorus levels for juvenile *H. taimen* as described by broken-line regression ($n = 3$). The breakpoint in the *broken line* is 0.56% digestible phosphorus

enzyme ATP-driven esterification of the free fatty acid with extra-mitochondrial CoA to yield fatty acyl-CoA, and this step utilizes two high-energy bonds of ATP to activate one molecule of fatty acid; insufficient P may have inhibited this process, leading to lower lipid utilization (Roy and Lall 2003).

The effect of dietary P on the mineralization in fish is poorly known. In the present study, P was necessary for Ca mineralization of taimen, which is similar to results obtained in chum salmon (Watanabe et al. 1980), Atlantic salmon (Baeverfjord et al. 1998), and rainbow trout (Bureau and Cho 1999). Muscle Ca in taimen varied with dietary P level, in which adequate dietary P could help Ca absorption and transportation, but excessive or deficient dietary P reduces fish muscle Ca deposition (Zhou et al. 2004). Taimen has a balanced Ca/P ratio in the body and vertebrae. Similarly, chum salmon (Watanabe et al. 1980), rainbow trout (Ogino and Takeda 1978), and haddock (*Melanogrammus aeglefinus*) (Roy and Lall 2003) had balanced the Ca/P ratio of the body by controlling the absorption or excretion of Ca.

Dietary P levels also affected the content of other elements (Fe, Zn, Mn, Mg, and Se) in the vertebrae and muscles of taimen. These results were in accordance with findings in rainbow trout (Hardy and Shearer 1985; Vielma et al. 2002). A high P diet may chelate other trace elements and reduce their absorption and metabolism in fish (Lall 2002). For instance, high dietary P also decreased Mg and Zn levels in rainbow trout (Satoh et al. 2002), common carp (*Cyprinus carpio*) (Satoh et al. 1992), and grouper (*Epinephelus coioides*) (Zhou et al. 2004) and reduced body Zn content in rainbow trout (Satoh et al. 2002). In addition, fish fed with P-deficient diet also decreased Mg level in scales, vertebrae, and opercula (Ye et al. 2006). These results indicated that excessive or deficient dietary P had a negative effect on bone and muscle mineralization. Therefore, appropriate dietary P may help mineral absorption

and transportation, but excessive or deficient dietary P reduces bone and muscle element deposition in taimen.

Conclusion

P is essential for growth, feed efficiency, and bone mineralization of juvenile taimen. Based on WG, P content in the whole body, and vertebrae P content, the digestible P requirement was determined to be approximately 0.5%. Because deficiency symptoms were observed, there is a need for histological characterization of bone deformities associated with dietary P deficiency. Additional studies based on biochemical mechanisms, particularly the role of hormones in Ca and P metabolism, may provide some clues to improve P retention and minimize the excretion of this element in natural waters from aquaculture operations.

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Compliance with ethical standards All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. The authors declare that they have no conflict of interest.

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