

# Effects of the supplementation of vitamin D<sub>3</sub> on the growth and vitamin D metabolites in juvenile Siberian sturgeon (*Acipenser baerii*)

Liansheng Wang · Hong Xu · Yang Wang · Chang'an Wang · Jinnan Li · Zhigang Zhao · Liang Luo · Xue Du · Qiyu Xu

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**Abstract** The objective of this study was to evaluate the effects of the supplementation of vitamin D<sub>3</sub> on the growth, vitamin D metabolites, and osteocalcin secretion in juvenile Siberian sturgeon (*Acipenser baerii*). A 90-day growth trial was conducted with juvenile Siberian sturgeon (initial body weight  $3.47 \pm 0.14$  g) fed seven isonitrogenous and isoenergetic practical diets (45% CP and 13% lipid) containing 60 (basal diet), 240, 450, 880, 1670, 3300, or  $1.0 \times 10^5$  IU/kg feed (D60~D  $1.0 \times 10^5$ ) vitamin D<sub>3</sub>. The results showed that weight gain and specific growth rate increased as the dietary vitamin D<sub>3</sub> levels increased from 450 to 3300 IU/kg ( $P < 0.05$ ). The fish fed with D1670 and D3300 diets had higher crude lipid and ash levels than the fish fed the D60 diet ( $P < 0.05$ ). The fish fed D880, D1670, or D3300 diets had higher 25-OH-D<sub>3</sub> and 1,25-(OH)<sub>2</sub>-D<sub>3</sub> levels than the fish fed the D60 diet ( $P < 0.05$ ). The fish fed D880, D1670, D3300, or D  $1.0 \times 10^5$  diets had higher osteocalcin levels than the fish fed the D60 diet ( $P < 0.05$ ). Based on the broken line method analysis of weight gain and osteocalcin, the dietary vitamin D<sub>3</sub> requirement of juvenile Siberian sturgeon was estimated to be 1683.30 and 1403.27 IU/kg per diet, respectively.

**Keyword** Juvenile Siberian sturgeon · Vitamin D<sub>3</sub> · Growth · Vitamin D metabolites · Osteocalcin

## Introduction

Vitamin D, a fat-soluble vitamin, is crucial for the preservation of calcium and phosphorus homeostasis and to protect skeletal integrity (Darias et al. 2011). The two major natural sources of vitamin D are ergocalciferol (vitamin D<sub>2</sub>, which occurs predominantly in plants) and cholecalciferol (vitamin D<sub>3</sub>, which occurs in animals). Cholecalciferol is at least three times more effective than ergocalciferol in meeting the vitamin D requirement of rainbow trout (Barnett et al. 1982). Formulated diets are used in aquaculture, and vitamin D intake can be manipulated. Vitamin D studies of different types have been performed in juveniles of several fish species, and the dietary requirements of this vitamin have been reviewed by Lock et al. (2010).

Most fish species contain a large amount of vitamin D<sub>3</sub> in their livers (Takeuchi et al. 1984). After hydroxylation in the liver into 25-OH-D and kidney into 1,25-(OH)<sub>2</sub>-D<sub>3</sub>, the active metabolite can enter the cell and bind to the vitamin D-receptor and subsequently to a responsive gene such as that of calcium binding protein or osteocalcin. Osteocalcin regulates the incorporation of calcium phosphates within the bone thus playing a mineralization and remodeling role (Fraser and Price 1988). Teleosts inhabiting freshwater and seawater are

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L. Wang · H. Xu · Y. Wang · C. Wang · J. Li · Z. Zhao · L. Luo · X. Du · Q. Xu (✉)  
Heilongjiang River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Harbin 150070, China  
e-mail: xuqiyu@sina.com

able to convert vitamin D<sub>3</sub> to 25-OH-D<sub>3</sub>, 1,25-(OH)<sub>2</sub>-D<sub>3</sub>, and 24,25-(OH)<sub>2</sub>-D<sub>3</sub>, which are found in the plasma of several species (Rao and Raghuramulu 1995; Horvli et al. 1998; Darias et al. 2011).

Sturgeon is the common name used for some 25 species of fish in the family *Acipenseridae*, including the genera *Acipenser*, *Huso*, *Scaphirhynchus*, and *Pseudoscaphirhynchus*. The Siberian sturgeon (*Acipenser baerii*) is a species of sturgeon in the *Acipenseridae* family. While wild catches have been generally declining, the Siberian sturgeon is increasingly farmed for both the meat and to produce caviar from its roe; the largest meat producers are Russia and China. Some studies have been performed on the nutrient requirement of Siberian sturgeon such as protein (Hung 1991; Médale et al. 1995), amino acids (Kaushik et al. 1991), ascorbic acid (Moreau et al. 1996), phosphorus (Xu et al. 2011), and vitamin K<sub>3</sub> (Wang et al. 2015). To our knowledge, no information has been published concerning the vitamin D requirement of Siberian sturgeon. Some studies showed that vitamin D deficiency decreases growth, body Ca and P levels and bioavailability for lipid, and delay on skeletogenesis in fish (Lovell and Li 1978; Barnett et al. 1982; Darias et al. 2010). Therefore, the purpose of the present study was to determine the effects of the supplementation of vitamin D<sub>3</sub> on the growth, vitamin D metabolites, and osteocalcin secretion in juvenile Siberian sturgeon and to determine the dietary vitamin D requirement.

## Materials and methods

### Experimental design and diets

Fishmeal-soybean-based meals supplemented with seven levels of vitamin D<sub>3</sub> (233,000 IU/g, Yuxin Chemical Products Ltd., Dalian, China) were used at levels of 60 (basal diet), 240, 450, 880, 1670, 3300 or  $1.0 \times 10^5$  IU/kg feed (D60~D  $1.0 \times 10^5$ ) vitamin D<sub>3</sub>. All of the ingredients were mixed homogeneously, pelleted into a 2.0-mm-diameter size, and stored at  $-20^\circ\text{C}$ . The formula and analyzed proximate composition of the basal diet are shown in Table 1. The dietary vitamin D<sub>3</sub> content was measured using high-performance liquid chromatography (HPLC) (Agilent 1100, California, USA) (GB/T 17818–2010). The separation was performed on an Agilent C<sub>18</sub> column (150 mm  $\times$  4.6 mm, 5  $\mu\text{m}$ ) using methanol–water (95:5 v:v<sup>-1</sup>) for the mobile

**Table 1** Composition of the basal diet (percent dry weight)

Diet ingredients	Air-dry basis, %
Fish meal	50
Soybean meal	20
Wheat middlings	15
Fish oil	4
Soy oil	3
Phospholipid	3
Choline	3
CM-cellulose	2
Mineral mixture <sup>a</sup>	2
Vitamin mixture <sup>b</sup>	0.5
Choline chloride	0.4
Lysine	0.3
Methionine	0.2
Total	100
Nutrients	<i>Proximate composition (as fed %)</i>
CP (%)	45.72
EE (%)	13.97
Ash (%)	8.68
Calcium	2.07
Phosphorus	1.84
DE MJ/Kg <sup>c</sup>	16.93

<sup>a</sup> Mineral premix provides the following for (g/kg) diet: MgSO<sub>4</sub>·7H<sub>2</sub>O (2); Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> (7); NaH<sub>2</sub>PO<sub>4</sub> (5); FeSO<sub>4</sub>·H<sub>2</sub>O (0.12); CuSO<sub>4</sub>·5H<sub>2</sub>O ( $3 \times 10^{-3}$ ); MnSO<sub>4</sub>·H<sub>2</sub>O (0.013); ZnSO<sub>4</sub>·H<sub>2</sub>O (0.03); 1% Na<sub>2</sub>SeO<sub>3</sub> ( $4 \times 10^{-4}$ ); 1% KI ( $1.44 \times 10^{-3}$ ); 1%CoCl<sub>2</sub> ( $1 \times 10^{-4}$ )

<sup>b</sup> Vitamin mixture (VD<sub>3</sub> free) per kg of the diet: VA 5000 IU; VE 60 mg; VB<sub>1</sub> 15 mg;VB<sub>2</sub> 30 mg;VB<sub>6</sub> 15 mg;VB<sub>12</sub> 0.5 mg; niacin 75 mg; folic acid 5 mg; inositol 1000 mg; biotin 2.5 mg

<sup>c</sup> DE is calculated value. Other nutrient levels are measured values

phase. The flow rate was 1.0 mL/min, and the column temperature was  $25^\circ\text{C}$ .

### Fish and feeding

Juvenile Siberian sturgeon from the Fangshan Station of the Chinese Academy of Fishery Sciences was transferred to 21 tanks (220 L of water, 30 fish/tank, and three replicates), which were connected to an automatically controlled recirculation system. The experimental fish from the broodstock originated from cultured Siberian sturgeons from Russia and reared in flowing-water ponds with 25 m  $\times$  4 m, water depth of 1 m. Every three aquaria were connected to a separate recycling system

and supplied with aerated water, which was filtered through zeolum, corallite, and activated carbon. The water temperature and dissolved oxygen during this period averaged  $21.4 \pm 1.0$  °C and  $8.0 \pm 0.5$  mg/L, respectively. The pH values were  $7.6 \pm 0.1$ , the ammonia-N concentrations were  $0.3 \pm 0.05$  mg/L, and the N-NO<sub>2</sub> concentrations were  $0.1 \pm 0.02$  mg/L. Approximately one third of the water in each aquarium was replaced daily with aerated freshwater. The fish were fed with a basal diet three times daily for 2 weeks. With an average initial weight of  $3.47 \pm 0.14$  g, all of the groups of fish were fed their respective diets at the same fixed rate to apparent satiety for 90 days. The fish were weighed every fortnight and feed quantities were adjusted according to fish body weight. The fish were fed three times daily (09:00, 13:00, and 17:00). Any uneaten food was collected 1 h after feeding, and the dry matter content was determined for both supplied and uneaten food (AOAC 1995).

#### Sample collection

At the end of the feeding trial, all of the fish from each aquarium were collectively weighed to obtain a final biomass. The growth and feed conversion were determined according to Li et al. (2015). A total of 18 fish from each treatment (six fish per tank) were anesthetized with tricaine methane sulfonate (MS-222, 200 mg/L, Western Chemical Inc., Ferndale, WA).

Blood was collected from three fish from each tank at the caudal vasculature location using a 1-mL heparinized syringe. The plasma was separated by centrifugation ( $3500 \times g$  for 30 min at 4 °C). The other three fish per tank were killed to collect the liver and the bone for biochemical measurement. All of the samples were stored at -40 °C until analysis.

Weight gain (WG, %) =  $100 \times (\text{final total weight} - \text{initial total weight}) / \text{initial total weight}$

Feed conversion ratio (FCR, %) =  $100 \times \text{dry feed intake} / \text{wet weight gain}$

Specific growth rate (SGR, %) =  $100 \times (\ln(\text{final body weight}) - \ln(\text{initial body weight})) / \text{days}$ .

#### Carcass and bone composition

The carcass samples (three per tank) were collected and assayed for dry matter (at 105 °C for 24 h), crude protein

(Kjeldahl apparatus, nitrogen  $\times 6.25$ ), crude fat (extraction with petroleum ether by a Soxhlet apparatus), and ash (incineration at 550 °C for 6 h) (AOAC 1995). The bones were defatted by ether influxing overnight and were then measured and ashed at 550 °C for 8 h; the bone ash weight was determined and then dissolved in 6 N hydrochloric acid and diluted 100-fold. The vanadium molybdenum yellow colorimetric method was used to determine the bone phosphorus content. The bone calcium content was determined by the ethylene diamine tetraacetic acid (EDTA) method.

#### Biochemical measurements

The 25-OH-D<sub>3</sub> and 1,25-(OH)<sub>2</sub>-D<sub>3</sub> concentrations in the serum and liver samples were measured simultaneously using a Nichols Advantage chemiluminescent assay developed by Nichols Institute Diagnostics (San Clemente, CA, USA) (Roth et al. 2001). The total osteocalcin was analyzed by commercially available ELISA (enzyme-linked immunosorbent assay) kits (Biorbyt Ltd., UK) for fish osteocalcin using the following steps: 50 µl of diluted (1:1) fish serum sample was added to a 96-well microtitration polystyrene plate, and then 50 µl of biotinylated anti-OC was added to all of the wells. The plate was covered and incubated for 1 h at 37 °C. The plate was washed with 0.3 mL of washing solution in each well three times. Next, 60 µl of streptavidin-HRP solution was distributed to all of the wells, and the plate was covered and incubated for 30 min at 37 °C. The well strips were washed according to the step mentioned above, and 50 µl of substrate was immediately added and incubated for 10 min at 37 °C. The enzyme-substrate reaction was stopped by quickly pipetting 50 µl of H<sub>2</sub>SO<sub>4</sub>, and the absorbance was immediately read on a spectrophotometer using 450 nm as the primary wavelength.

#### Statistical analysis

The data are presented as treatment means  $\pm$  SE. The data were analyzed by one-way ANOVA, and the least significance difference (LSD) was used for comparison of the means using SPSS 19.0 (SPSS Inc., Chicago IL) software. Multiple regression analyses (step-wise method) were used to determine the vitamin D<sub>3</sub> requirement of juvenile Siberian sturgeon based on weight gain, and  $P < 0.05$  was considered significant.

## Results

### Growth and feed utilization

The results of the growth and feed utilization of the juvenile Siberian sturgeon fed with graded concentrations of dietary vitamin D<sub>3</sub> are shown in Table 2. Dietary vitamin D<sub>3</sub> levels significantly affected growth ( $P < 0.05$ ). The fish fed with D60 to D450 diets had lower weight gain and specific growth rate than the fish fed the D1670 diet ( $P < 0.05$ ), and the fish fed the D1670 diet presented the highest WG. The lowest weight gain rate was observed in fish fed the D60 and D240 diets ( $P < 0.05$ ). There was no significant difference between the  $1.0 \times 10^5$  and the D1670 diet ( $P > 0.05$ ). The feed conversion ratio was not significantly affected among the different dietary groups ( $P > 0.05$ ). As shown in Fig. 1, based on the broken line method analysis of weight gain, the dietary vitamin D<sub>3</sub> requirement of juvenile Siberian sturgeon was estimated to be 1683.30 IU/kg diet.

### Body composition

The whole body composition of juvenile Siberian sturgeon fed graded concentrations of dietary vitamin D<sub>3</sub> for 90 days is shown in Table 3. There was a significant effect of diet on body crude lipid and ash content ( $P < 0.05$ ). There was no effect of dietary vitamin D<sub>3</sub> supplementation on body crude protein and moisture contents ( $P > 0.05$ ).

### Vitamin D metabolite concentrations in the liver and serum

The concentration of vitamin D<sub>3</sub> metabolites in the serum and liver of Siberian sturgeon fed with graded concentrations of dietary vitamin D<sub>3</sub> is shown in Table 4. There was a significant effect of diet on 25-OH-D<sub>3</sub> and 1,25-(OH)<sub>2</sub>-D<sub>3</sub> in the serum ( $P < 0.05$ ). The fish fed the D880, D1670, or D3300 diets had higher 25-OH-D<sub>3</sub> and 1,25-(OH)<sub>2</sub>-D<sub>3</sub> than the fish fed the D60 diet ( $P < 0.05$ ). The 1,25-(OH)<sub>2</sub>-D<sub>3</sub> in the liver increased as the dietary vitamin D<sub>3</sub> levels increased from 450 to 3300 IU/kg ( $P < 0.05$ ). There was no effect of dietary vitamin D<sub>3</sub> supplementation on the 25-OH-D<sub>3</sub> concentration in the liver ( $P > 0.05$ ). The fish fed with the D880, D1670, D3300, or  $1.0 \times 10^5$  diets had higher osteocalcin levels than the fish fed the D60 diet ( $P < 0.05$ ). As shown in Fig. 2, based on the broken line method analysis of osteocalcin, the dietary vitamin D<sub>3</sub> requirement of juvenile Siberian sturgeon was estimated to be 1403.27 IU/kg per diet.

### The calcium and phosphorus levels in the serum and bone

No significant differences were found among the different dietary groups regarding calcium and phosphorus levels in the serum or bones of the fish ( $P > 0.05$ ). The calcium and phosphorus levels in the bones with average values  $1.02 \pm 0.02$  or  $0.95 \pm 0.03\%$  and  $2.72 \pm 0.21$  or  $5.71 \pm 0.35$  mmol/l in the serum, respectively.

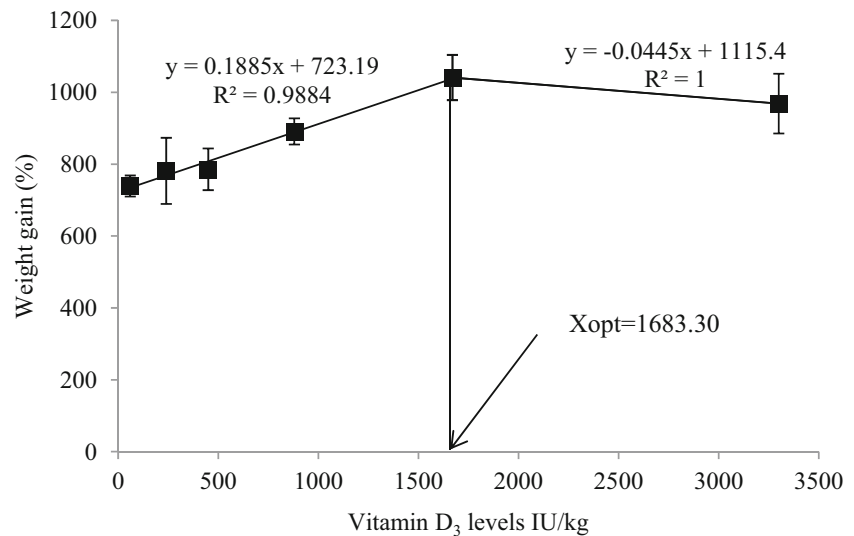
**Table 2** Effect of dietary vitamin D<sub>3</sub> on the growth performance of Siberian sturgeon

Vitamin D (IU/kg)	IBW (g)	FBW (g)	WG (%)	FCR (%)	SGR (%)
60	3.46 ± 0.21	29.03 ± 0.57 <sup>c</sup>	739.13 ± 29.19 <sup>c</sup>	1.08 ± 0.11	3.80 ± 0.01 <sup>c</sup>
240	3.48 ± 0.18	29.78 ± 2.16 <sup>bc</sup>	781.13 ± 92.03 <sup>bc</sup>	1.07 ± 0.03	3.89 ± 0.15 <sup>bc</sup>
450	3.50 ± 0.21	31.00 ± 2.23 <sup>b</sup>	785.82 ± 57.97 <sup>b</sup>	1.02 ± 0.16	3.90 ± 0.12 <sup>b</sup>
880	3.45 ± 0.15	34.19 ± 1.59 <sup>ab</sup>	890.92 ± 36.29 <sup>ab</sup>	1.05 ± 0.16	4.10 ± 0.02 <sup>ab</sup>
1670	3.52 ± 0.08	40.16 ± 1.95 <sup>a</sup>	1041.02 ± 62.82 <sup>a</sup>	1.02 ± 0.16	4.35 ± 0.08 <sup>a</sup>
3300	3.51 ± 0.12	37.50 ± 2.18 <sup>ab</sup>	968.44 ± 83.28 <sup>ab</sup>	1.07 ± 0.12	4.23 ± 0.06 <sup>ab</sup>
$1.0 \times 10^5$	3.45 ± 0.19	34.85 ± 0.84 <sup>ab</sup>	910.47 ± 25.67 <sup>ab</sup>	1.09 ± 0.07	4.13 ± 0.03 <sup>ab</sup>

Values are means from triplicate groups of juvenile fish where the means in each column with a different superscript are significantly different ( $P < 0.05$ ) by one-way ANOVA ( $n = 3$ ), means ± SE

IBW initial average body weight, FBW final average body weight, WG weight gain, SGR specific growth rate (% d<sup>-1</sup>), FCR feed conversion ratio

**Fig. 1** Relationship between weight gain and dietary vitamin D<sub>3</sub> levels for juvenile Siberian sturgeon. Values are mean  $\pm$  SE ( $n = 3$ )



## Discussion

Vitamin D is crucial to preserve calcium and phosphorus homeostasis and to protect the skeletal integrity. Besides, vitamin D also acts directly on osteoblasts, the resident bone-forming cells of the skeleton, to inhibit proliferation, modulate differentiation, and regulate mineralization of the extracellular matrix (Darias et al. 2011). Vitamin D is essential for the normal growth of fish, although the metabolic roles of vitamin D and its analogs in fish are not well characterized (Vandenberg et al. 2012). In the present study, all of the dietary treatments were adequate to develop the juvenile sturgeon in suitable conditions, as confirmed by the high percentage of weight gain (>739%). A vitamin D<sub>3</sub> level higher than 450 IU/kg is needed to maximize growth.

The weight gain data obtained in this experiment showed that vitamin D<sub>3</sub> is essential for the maximal growth of juvenile Siberian sturgeon. Similarly, Andrews et al. (1980) found that the average weight gain of channel catfish (6.0 g) in the control group was significantly lower than that of the groups fed supplemented with 2000 IU/kg vitamin D<sub>3</sub>. Lovell and Li (1978) found that a basal diet (0 IU/kg vitamin D<sub>3</sub>) resulted in significantly lower weight gain and body Ca and P of channel catfish (0.5 g) compared to those fed diets supplemented with 1000 IU/kg vitamin D<sub>3</sub>. Another study showed that inadequate levels of dietary vitamin D<sub>3</sub> negatively influenced the intestinal tract maturation of European sea bass larvae (Darias et al. 2010). The excessive intake of vitamin D<sub>3</sub> depressed the growth rate of channel catfish (6.0 g) (Andrews et al.

**Table 3** Effect of dietary vitamin D<sub>3</sub> on body composition of Siberian sturgeon (%)

Dietary vitamin D (IU/kg)	Crude protein	Crude lipid	Ash	Moisture
60	11.91 $\pm$ 0.21	5.44 $\pm$ 0.28 <sup>b</sup>	3.18 $\pm$ 0.10 <sup>b</sup>	79.19 $\pm$ 0.09
240	11.72 $\pm$ 0.12	6.10 $\pm$ 0.42 <sup>ab</sup>	3.45 $\pm$ 0.08 <sup>ab</sup>	78.75 $\pm$ 0.79
450	11.49 $\pm$ 0.60	6.49 $\pm$ 0.29 <sup>ab</sup>	3.43 $\pm$ 0.08 <sup>ab</sup>	79.93 $\pm$ 0.56
880	11.96 $\pm$ 0.70	7.41 $\pm$ 0.21 <sup>a</sup>	3.23 $\pm$ 0.03 <sup>ab</sup>	77.86 $\pm$ 0.96
1670	11.81 $\pm$ 0.36	7.39 $\pm$ 0.42 <sup>a</sup>	3.56 $\pm$ 0.09 <sup>a</sup>	79.89 $\pm$ 0.52
3300	11.85 $\pm$ 0.21	7.66 $\pm$ 0.64 <sup>a</sup>	3.56 $\pm$ 0.06 <sup>a</sup>	77.55 $\pm$ 0.53
1.0 $\times$ 10 <sup>5</sup>	11.85 $\pm$ 0.23	6.82 $\pm$ 0.57 <sup>ab</sup>	3.32 $\pm$ 0.07 <sup>ab</sup>	79.19 $\pm$ 0.09

Values are means from triplicate groups of juvenile fish where the means in each column with a different superscript are significantly different ( $P < 0.05$ ) by one-way ANOVA ( $n = 3$ ), means  $\pm$  SE

**Table 4** Concentrations of vitamin D metabolites in liver and serum fed diets supplemented various levels of vitamin D<sub>3</sub>

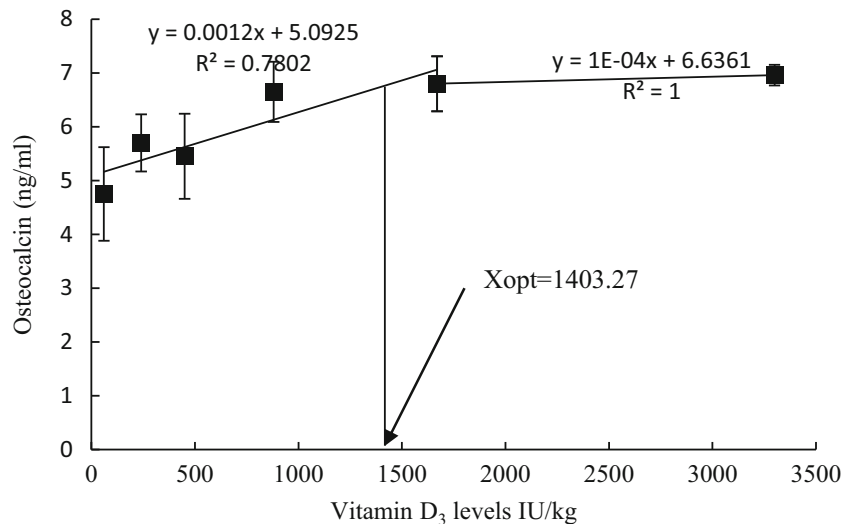
Dietary vitaminD (IU/kg)	25-OH-D <sub>3</sub> in serum (nmol/L)	1,25-(OH) <sub>2</sub> -D <sub>3</sub> in serum (nmol/L)	25-OH-D <sub>3</sub> in liver (nmol/L)	1,25-(OH) <sub>2</sub> -D <sub>3</sub> in liver (nmol/L)	Osteocalcin (ng/mL)
60	2.71 ± 0.25 <sup>b</sup>	1.56 ± 0.18 <sup>b</sup>	2.47 ± 0.29	2.61 ± 0.22 <sup>c</sup>	4.75 ± 0.87 <sup>b</sup>
240	3.01 ± 0.16 <sup>ab</sup>	1.65 ± 0.23 <sup>b</sup>	2.54 ± 0.22	3.09 ± 0.15 <sup>bc</sup>	5.70 ± 0.53 <sup>ab</sup>
450	3.10 ± 0.26 <sup>ab</sup>	1.84 ± 0.17 <sup>ab</sup>	2.04 ± 0.33	3.58 ± 0.05 <sup>ab</sup>	5.45 ± 0.79 <sup>ab</sup>
880	3.59 ± 0.17 <sup>a</sup>	2.19 ± 0.34 <sup>a</sup>	2.22 ± 0.28	4.33 ± 0.22 <sup>a</sup>	6.65 ± 0.56 <sup>a</sup>
1670	4.10 ± 0.20 <sup>a</sup>	2.70 ± 0.17 <sup>a</sup>	2.32 ± 0.30	4.50 ± 0.13 <sup>a</sup>	6.80 ± 0.51 <sup>a</sup>
3300	3.75 ± 0.19 <sup>a</sup>	1.92 ± 0.32 <sup>a</sup>	2.23 ± 0.27	4.45 ± 0.10 <sup>a</sup>	6.96 ± 0.19 <sup>a</sup>
1.0 × 10 <sup>5</sup>	3.35 ± 0.37 <sup>ab</sup>	1.54 ± 0.13 <sup>b</sup>	2.22 ± 0.13	3.23 ± 0.17 <sup>abc</sup>	6.80 ± 0.51 <sup>a</sup>

Values are means from triplicate groups of juvenile Siberian sturgeon where the means in each column with a different superscript are significantly different ( $P < 0.05$ ) by one-way ANOVA ( $n = 3$ ), means ± SE

1980) and rainbow trout (51.6 g) (Vielma et al. 1998) fed diets supplemented with 50,000 or 250,000 IU/kg vitamin D<sub>3</sub>, respectively. High levels of vitamin D (3750 IU vitamin D<sub>3</sub>/g diet) caused impaired growth, lethargy, and dark coloration in brook trout, *Salvelinus fontinalis* (Poston 1969). Our results, together with previous reports, also demonstrated that no effect of excess vitamin D<sub>3</sub> was detected in feed conversion, specific growth rate, and survival rate in our trial, and the results were the same as those of a vitamin D excess study on rainbow trout with a dose of up to  $1.004 \times 10^6$  (Hilton and Ferguson 1982). Yellow catfish that were fed a 2000 IU/kg diet of vitamin D<sub>3</sub> may have already met their growth requirements, and higher levels of vitamin D<sub>3</sub> are not necessary to improve growth performance in juvenile yellow catfish (Zhu et al. 2015).

Different vitamin D studies have been performed in adults and juveniles of several fish species and dietary requirements of this vitamin D have been recently reviewed by Lock et al. (2010). The NRC (2011) recommends 1600 IU vitamin D<sub>3</sub>/kg diet for an optimal performance of juvenile fish based on analyses made on young rainbow trout, which is the actual value of the reference in the diet formulation for fish. The minimum dietary requirement for vitamin D has been established in *Monopterus albus* (Tan and He 2007), *Salmo gairdneri* (Barnett et al. 1982), *Salmo salar* (Woodward 1994), *Ictalurus punctatus* (Andrews et al. 1980), *Oreochromis niloticus* × *Oreochromis aureus* (Shiau and Hwang 1993), and *Megalobrama amblycephala* (Miao et al. 2015) as 5000, 1600, 2400, 2000, 375, and 5430 IU/kg per diet, respectively. The

**Fig. 2** Relationship between osteocalcin and dietary vitamin D<sub>3</sub> levels for juvenile Siberian sturgeon. Values are mean ± SE ( $n = 3$ )



dietary vitamin D<sub>3</sub> requirement of juvenile Siberian sturgeon was estimated to be 1683.30 or 1403.27 IU/kg per diet by weight gain or osteocalcin, respectively. It might be worth to note that vitamin D<sub>3</sub> requirements at juvenile and larval stage are different; for instance, the optimal amount has been shown to be higher during the larval stage for European sea bass than that recommended by the NRC (1993) for juveniles.

A decrease in carcass ash was reported in channel catfish and Wuchang bream fed low levels of vitamin D<sub>3</sub> (Lovell and Li 1978; Miao et al. 2015). Consistent with the abovementioned studies, the fish fed with 1670 and 3300 IU/kg vitamin D<sub>3</sub> diets had higher crude lipid and ash levels than the fish fed the 60 IU/kg diet in the present study. This demonstrated that appropriate dietary vitamin D<sub>3</sub> promoted the deposition of minerals in fish, and deficient or excessive dietary vitamin D<sub>3</sub> inhibited mineral accumulation. However, there was little change in carcass ash between the vitamin D deficient and supplemented groups after 6 months of feeding for *Labeo rohita* (Rora) (Ashok et al. 1998).

Then, 25-OH-D<sub>3</sub> is processed into the most active molecule, 1,25-dihydroxy vitamin D, after two hydroxylation steps. The 1,25-(OH)<sub>2</sub>-D<sub>3</sub> compound is the most active metabolite of vitamin D<sub>3</sub> and regulates the calcium and phosphorus balance in mammals (Fraser 1980; De Luca and Schnose 1983). The salmon responded to the two high dietary levels of vitamin D<sub>3</sub> tested (88.4 and 1147.2 IU/g per diet) by accumulating the 1,25-(OH)<sub>2</sub>-D<sub>3</sub> in a dose-dependent manner, and similar results were obtained in the investigated serum and liver. This dietary dose-dependent accumulation of 1,25-(OH)<sub>2</sub>-D<sub>3</sub> was also found by Takeuchi et al. (1991) in the livers of the bastard halibut and carp, *Cyprinus carpio*. Similar to this study, the fish fed 880, 1670, or 3300 IU/kg vitamin D<sub>3</sub> diets had higher 1,25-(OH)<sub>2</sub>-D<sub>3</sub> levels than the fish fed the 60, 240, and  $1.0 \times 10^5$  IU/kg vitamin D<sub>3</sub> diets. One possible reason for the lower 1,25-(OH)<sub>2</sub>-D<sub>3</sub> level is that  $1.0 \times 10^5$  IU/kg vitamin D<sub>3</sub> dietary inhibited the kidney microsomes that metabolize either vitamin D<sub>3</sub> or 25-(OH)D<sub>3</sub> to 1,25-(OH)<sub>2</sub>-D<sub>3</sub> for juvenile Siberian sturgeon.

Vitamin D is also needed for bone growth and bone remodeling by osteoblasts and osteoclasts. Osteocalcin is one of the marker genes for the progression of osteoblastic differentiation and is associated with the mineralization of the extracellular matrix (Lian and Stein 1995). In human osteoblast cell culture, vitamin D, particularly 1,25-(OH)<sub>2</sub>-D<sub>3</sub>, stimulates the production

of osteocalcin (Price and Baukol 1980). Compared with the control group, the serum osteocalcin significantly increased in the D880 group, and the variation trend was similar to the 1,25-(OH)<sub>2</sub>-D<sub>3</sub> content in serum. Similarly, the supplementation of vitamin D<sub>3</sub> promoted the expression of the osteocalcin gene of sea bass larvae (Darias et al. 2010).

Some reports have shown that vitamin D had no marked effect on the utilization of dietary calcium for tissue mineralization (O'Connell and Gatlin 1994; Graff et al. 2002). Similarly, Zhu et al. (2015) reported that supplementation with vitamin D<sub>3</sub> did not significantly affect vertebrae and serum Ca or P contents. The present study also demonstrates the same results, i.e., that no significant differences were recorded in calcium and phosphorus both in the plasma and bone. This fact may indicate that calcium and phosphorus in the surrounding water in our trial were collected by the gills and satisfied the need of calcium and phosphorus to maintain homeostasis and support normal mineralization of bone for juvenile sturgeon. Furthermore, similar calcium and phosphorous levels in all dietary treatments are more alike due to the fact that juvenile sturgeon did not feed the vitamin D<sub>3</sub>-deficient diet.

In conclusion, the differences observed may indicate that supplementation of vitamin D<sub>3</sub> could enhance weight gain, crude lipid, and ash in the carcass, the concentration of 25-OH-D<sub>3</sub>, 1,25-(OH)<sub>2</sub>-D<sub>3</sub> and osteocalcin in serum, and the concentration of 1,25-(OH)<sub>2</sub>-D<sub>3</sub> in the liver. Based on the broken line method analysis of weight gain and osteocalcin, the dietary vitamin D<sub>3</sub> requirement of juvenile Siberian sturgeon was estimated to be between 1683.30 and 1403.27 IU/kg per diet.

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