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Dietary vitamin D_3 requirement for Pacific white shrimp, *Penaeus vannamei*

Gunho Eom[1,#], Yein Lee[1,#], Sehee Jo[1], Kyeong-Jun Lee[1,2,*]

[1] *Department of Marine Life Science, Jeju National University, Jeju 63243, Korea*
[2] *Marine Life Research Institute, Jeju National University, Jeju 63333, Korea*

Abstract

Vitamin D_3 (cholecalciferol), a fat-soluble vitamin and prohormone, is an important micronutrient in aquafeeds. This study aimed to determine the optimal dietary vitamin D_3 requirement for Pacific white shrimp, *Penaeus vannamei*. An eight-week feeding trial was conducted using 400 shrimp (0.07 ± 0.00 g), randomly assigned into 20 tanks with four replicates per treatment. Five diets were prepared by adding 0, 45, 90, 135, and 270 μg/kg of vitamin D_3 (designated as VD0, VD45, VD90, VD135, and VD270, respectively) and fed to the shrimp. The determined vitamin D_3 levels of experimental diets were 12, 35, 101, 152, and 294 μg/kg, respectively. Growth and feed utilization improved with increasing dietary vitamin D_3 levels, peaking at VD135 group ($p < 0.05$). Shrimp survival was not significantly different among all the groups ($p > 0.05$). Protein, lipid and dry matter digestibility of the shrimp were significantly increased with increasing dietary vitamin D_3 levels up to VD135 group but decreased again in VD270 group ($p < 0.05$). Shrimp fed VD270 diet showed significantly higher lysozyme and nitro-blue tetrazolium activities than VD0 group ($p < 0.05$). Hemolymph cholesterol level was significantly lower in VD270 group than that of VD45, VD90, and VD135 groups ($p < 0.05$). All vitamin D_3 supplemented groups showed significantly higher hemolymph triglyceride level than that of VD0 group ($p < 0.05$). Blasenzellen cell count and diameter in hepatopancreatic tubule were significantly increased with increasing dietary vitamin D_3 levels ($p < 0.05$). Vitamin D_3 supplementation promoted lipid droplet accumulation in hepatopancreatic cells, while excessive levels (VD270) impaired lipid metabolism ($p < 0.05$). The gene expression levels of insulin-like growth factor binding protein and prophenoloxidase were significantly upregulated in VD90 group in comparison to the VD0 group ($p < 0.05$). The estimated optimum vitamin D_3 level for maximum growth for Pacific white shrimp is likely to be 100.9–113.34 μg/kg, based on broken line regression with insulin-like growth factor binding protein gene expression level and final body weight.

Keywords: Dietary requirement, Pacific white shrimp, Vitamin D_3

Introduction

Vitamin D_3 is one of the essential fat-soluble vitamins in aquafeeds, known to regulate calcium and phosphorus homeostasis by promoting their intestinal absorption and to support lipid metabolism and immune responses in shrimp and fish (Liu et

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[#]These authors contributed equally to this work.
***Corresponding author:** Kyeong-Jun Lee
Department of Marine Life Science, Jeju National University, Jeju 63243, Korea
Tel: +82-64-754-3423, **Fax:** +82-64-756-3493, **E-mail:** kjlee@jejunu.ac.kr

al., 2021; Shao et al., 2022; Sivagurunathan et al., 2022; Wen et al., 2015). Vitamin D_3 enhances immune function by promoting the production of anti-bacterial peptides, stimulating anti-inflammatory cytokine secretion and activating macrophages (Liu et al., 2021; Shao et al., 2022). Both deficiency and excess of dietary vitamin D_3 are associated with impaired growth, reduced feed efficiency, and clinical signs such as lethargy, hypermelanosis and skeletal anomalies in fish (Knuth et al., 2020; Ling-hong et al., 2015; Shao et al., 2022; Zhu et al., 2015).

Recently, the replacement of fish meal and fish oil with plant-based ingredients in aquafeeds has increased substantially due to sustainability and cost considerations (Hossain et al., 2024). However, plant-based ingredients generally contain little or no vitamin D_3 compared to animal-derived ingredients, potentially leading to nutritional deficiencies in aquatic animals (EFSA FEEDAP Panel, 2017; Hansen et al., 2015). Prabhu et al. (2019) suggested that the optimum vitamin D_3 level for Atlantic salmon (*Salmo salar*) is 60–90 μg/kg (2,400–3,600 IU/kg), whereas higher levels may be needed under fish meal- and fish oil-reduced diets. The EFSA FEEDAP Panel (2017) reported that the increasing use of plant-based ingredients in aquafeeds can reduce vitamin D_3 content to about 25%–33% of that in traditional fish-based formulations. Therefore, determining the optimum dietary vitamin D_3 level under such alternative feeding strategies is essential to maintain growth performance, mineral balance, and overall health in aquaculture species. Shrimp and fish have limited capacity to synthesize vitamin D_3 endogenously; therefore, their dietary requirement must be met through formulated feeds (Hamre et al., 2010; Lambert, 2007). Previous studies suggested optimum vitamin D_3 levels in aquafeeds based on growth (Lock et al., 2010). However, studies on dietary vitamin D_3 requirements for crustaceans remain limited. In crustaceans, vitamin D_3 is important for growth and immune responses against pathogenic bacteria (Liu et al., 2021). Shiau & Hwang (1994) reported an optimal dietary vitamin D_3 requirement of 100 μg/kg (4,000 IU/kg) for grass shrimp (*Penaeus monodon*). The optimal dietary vitamin D_3 level for Chinese mitten crab (*Eriocheir sinensis*) was suggested as 120–150 μg/kg (4,825–5,918 IU/kg) (Liu et al., 2021). Wen et al. (2015) reported that the 160 μg/kg (6,366 IU/kg) diet is an optimum level for Pacific white shrimp (*Penaeus vannamei*) under low salinity condition (10–15 psu). In condition of 21.6–23.5 psu salinity, the optimum level of vitamin D_3 in diets for Pacific white shrimp was suggested at 480 μg/kg (19,200 IU/kg) (Dai et al., 2022). However, the optimum dietary vitamin D_3 levels for Pacific white shrimp have not yet been estimated in seawater condition. Thus, this study aims to determine the optimal dietary vitamin D_3 requirements for *P. vannamei* cultured under seawater conditions. The evaluation is based on growth performance, immune response, Ca and P balance, lipid metabolism and histopathological changes.

Materials and Methods

Experimental diets

A basal diet (VD0) with 35% crude protein and 9% crude lipid was prepared without vitamin D_3 supplementation. Four other diets were prepared by supplementing 45, 90, 135, and 270 μg vitamin D_3/kg diet (designated as VD45, VD90, VD135, and VD270, respectively). Vitamin D_3 (0.25%, DSM Nutritional Products, Kaiseraugst, The Netherlands) was mixed with α-cellulose as an inert carrier to obtain the graded target concentrations. The amounts of vitamin D_3 and cellulose were adjusted to ensure that the total weight and proximate composition of the diets remained balanced among treatments (Table 1). All the ingredients were kneaded with fish oil (4% w/w) and water (12% w/w). The mixed dough was pelletized with a diameter of 1.5 mm (SP-50, Kum-Kang Engineering, Daegu, Korea) and then, dried (24℃–25℃, 8 h) and stored (–20℃). The vitamin D_3 levels measured by high-performance liquid chromatography (HPLC) were 12, 35, 101, 132, and 294 μg/kg, respectively, following the analytical procedure described in the HPLC analysis section.

Experimental design

The shrimp were purchased from a local hatchery (Daedong susan, Muan-gun, Korea) and acclimated for 10 days. A total of 400 shrimp (20 shrimp per tank) (initial weight: 0.07 ± 0.00 g) were randomly distributed into 20 tanks (120 L) with four replicates per treatment, in a completely randomized design. Experimental diets were fed to the shrimp six times a day (08:00, 10:00, 12:00, 14:00, 16:00, and 18:00 h) for 8 weeks. Shrimp biomass in each tank was measured biweekly to adjust feeding rates (4%–10% of biomass), using a precision digital balance (Pioneer PX, PX2202KR; Ohaus, Parsippany, NJ, USA). Water was exchanged every three days (approximately 70% of total volume) using preheated seawater (28℃–30℃).Water temperature, salinity, dissolved oxygen, and pH were measured daily, whereas ammonia concentration was monitored once per week. The recorded tank water quality was temperature 29.3 ± 0.7℃,

Table 1. Formulation of the basal diet for Pacific white shrimp (*Penaeus vannamei*)

Ingredients (g/kg)	VD0	VD45	VD90	VD135	VD270
Fish meal (sardine)[1]	70	70	70	70	70
Fish meal (tuna)[2]	70	70	70	70	70
Soybean meal	380	380	380	380	380
Squid liver meal	50	50	50	50	50
Wheat flour	110	110	110	110	110
Vitamin D_3 premix[3]	0	0.018	0.036	0.054	0.108
α-cellulose	8	7.982	7.964	7.946	7.892
Starch	200	200	200	200	200
Fish oil	40	40	40	40	40
Mineral mix[4]	20	20	20	20	20
Vitamin mix (vitamin D_3 free)[5]	10	10	10	10	10
Cholesterol	2	2	2	2	2
Lecithin	10	10	10	10	10
Mono-calcium phosphate	30	30	30	30	30
Proximate composition (dry matter)					
Crude protein	345	353	355	354	353
Crude lipid	86	86	88	86	89
Ash	96	98	97	98	98

[1] Orizon S.A., Santiago, Chile.
[2] Wooginfeed Industry, Incheon, Korea.
[3] Mixture of vitamin D_3 (0.25%, DSM Nutritional Products) and cellulose to obtain graded levels of vitamin D_3. The determined dietary vitamin D_3 levels were 12, 35, 101, 132, and 294 μg/kg for VD0, VD45, VD90, VD135, and VD270, respectively.
[4] Each kg of premix contains 12.50 g $C_4H_2FeO_4$, 12.00 g $MnSO_4$, 20.00 g $FeSO_4$, 6.00 g $CuSO_4$, 0.75 g $CoSO_4$, 25.00 g $ZnSO_4$, 0.75 g $Ca(IO_3)_2$, 80.20 g $MgSO_4$, and 0.75 g Al_2O_3. The mixture was prepared to 1 kg using cellulose.
[5] Each kg of premix contains 1.65 g retinol, 20.0 g α-tocopherol, 5.0 g menadione, 20.0 g ascorbic acid, 2.0 g thiamin hydrochloride, 20.0 g riboflavin, 40.0 g nicotinic acid, 54.0 g myo-inositol, 18.0 g Ca-pantothenate, 15.0 g pyridoxine hydrochloride, 0.3 g biotin, 4.0 g folic acid and 0.01 g cobalamin. The mixture was prepared to 1 kg using cellulose.

salinity 33.5 ± 0.5 ppt, dissolve oxygen 5.84 ± 0.71 mg/L, pH 7.08 ± 0.15 and ammonia 0.05 ± 0.02 ppm.

Sampling and analyses

All the shrimp were counted and individually weighed after the 8 weeks of feeding trial. Growth performance (initial body weight [IBW]; final body weight [FBW]; weight gain [WG]; specific growth rate [SGR]) and feed utilization (feed conversion ratio [FCR]; protein efficiency ratio [PER]) and survival were calculated. Five shrimp per tank (20 shrimp per dietary group) were randomly captured and anesthetized with ice-cold water. After anesthetization, the hemolymph and hepatopancreas were collected. The collected hemolymph was kept at −80℃ until used for non-specific immunity analyses, cholesterol and triglyceride analyses. Four of the five collected hepatopancreas were immediately frozen with liquid nitrogen and kept at −80℃ until used to quantitative polymerase chain reaction (qPCR) assay (three shrimp per tank) and vitamin D_3 analysis (one shrimp per tank). The other hepatopancreas was immediately immersed in Davidson's solution for histomorphological analysis. The carapace was separated from one of the remained carcasses from each tank and was stored at −20℃ until used to analyses. Other shrimp carcass was used to proximate composition analysis. Proximate composition of experimental diets and whole-body sample was analyzed by the method described by AOAC (2005). Hemolymph cholesterol and triglyceride levels were analyzed by a biochemistry analyzer (SLIM, SEAC, Florence, Italy).

Histomorphological analysis

The hepatopancreas of shrimp were fixed for 24 h in Davidson's solution. Following fixation, the hepatopancreas was dehydrated with gradual concentrations of ethanol (70%–100%). Samples were cleaned with xylene and embedded in paraffin. 6 μm-thick sections were obtained using microtome and then, stained with hematoxylin and eosin solutions. The sections were observed using an optical microscope (DM750, Leica, Bensheim, Germany). The number and diameter of B cells, as well as the number

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of lipid droplets (LD), were determined in randomly selected hepatopancreas tubules from each treatment.

High-performance liquid chromatography analysis

A pyrogallol ethanol solution was prepared by dissolving pyrogallol (10 g, Sigma-Aldrich, St. Louis, MO, USA) in 100 mL of ethanol. The homogenized sample was aliquoted into 50 mL tube covered with aluminum foil. Pyrogallol ethanol solution (4 mL) and 90% potassium hydroxide solution (1 mL) were added into the tube. Samples were saponified at 30℃ for 16 h under gentle vortexing. 1 mL water and 3 mL n-hexane were added into saponified samples and mixture was centrifuged (1,000×g, 60 sec). Supernatants were collected and 3 mL n-hexane was added into samples and mixture was centrifuged (1,000×g, 60 sec) again. Supernatants were combined and washed with 2 mL water, centrifuged (1,000×g, 60 sec). Hexane layer containing vitamin D_3 was collected. 1 mL iso-propanol was added into combined samples and samples were dried with nitrogen. Dried samples were dissolved in 100 μL n-hexane. Arc 2998 HPLC (Waters, Milford, MA, USA) system equipped with a C18 (Waters, 4 μm, 4.6 × 250 mm) column and photodiode array detector (PDA) detector was used to detect vitamin D_3 in samples. The mobile phase (chloroform:methanol:acetonitrile = 6:12:82) was eluted 25 min (0.5 mL/min). The column temperature during the analysis was 30℃ and the absorbance was detected at 265 nm.

Quantitative polymerase chain reaction analysis

Hepatopancreas was homogenized using a tissue grinder (Kimble Chase, Vineland, NJ, USA) and RNA was extracted from the tissue suspension. The quantity and quality of total RNA were measured using a Nano drop 2000 (Thermo Scientific, Wilmington, DE, USA). The 260/280 nm ratio for all samples was between 1.85 and 1.96, and cDNA was synthesized using the PrimeScript™ RT reagent kit (Takara, Shiga, Japan). *β-actin* was used as a reference gene. A thermal cycler dice (Real time system III, Takara) was used for insulin-like growth factor binding protein (*IGF-BP*) and prophenoloxidase (*proPO*) expression analysis. The reaction was performed in a 20 μL sample containing 10 μL of TB green premix (Takara), 2 μL of cDNA and 0.4 μL of each primer pair, and all reactions were run in quadruplicate. The PCR was performed for one cycle at 95℃ for 30 sec, followed by 40 cycles at 95℃ for 10 sec, 55℃ for 30 sec. The results were evaluated by the 2-ΔΔCT method (Pfaffl, 2001). The analyzed gene primer sequences are provided in Table 2.

Table 2. Primer sequence of gene expression for Pacific white shrimp *Penaeus vannamei*

Primers[1)]	Sequences	GenBank reference
IGF-BP		
Forward	5'-GTGGGCAGGGACCAAATC-3'	KP1420228.1
Reverse	5'-TCAGTTACCACCAGCGATT-3'	
proPO		
Forward	5'-TCCATTCCGTCCGTCTG-3'	AY723296.1
Reverse	5'-GGCTTCGCTCTGGTTAGG-3'	
β-actin		
Forward	5'-CGTCACCAACTGGGACGACATGGA-3'	AF300705.2
Reverse	5'-GGGCCACGCGGAGCTCGTTGT-3'	

[1)] Primers are abbreviated as: insulin-like growth factor binding protein (*IGF-BP*), prophenoloxidase (*proPO*) and *β-actin*.

Digestibility test

Digestibility test diets were prepared by supplementing 1% chromium oxide (Cr_2O_3, DaeJung Chemical & Metals, Siheung, Korea) into the diets as an internal indicator to estimate apparent digestibility coefficient (ADC). After feeding trial, all the shrimp in same treatments were pooled and redistributed 12 shrimp per tank (48 shrimp per treatment) into 24 tanks (240 L) in four replicates. After 3 days of acclimation, shrimp were fed the experimental diets (3% biomass) two times daily (08:30 and 15:30 h). Uneaten feeds were removed every 30 minutes after feeding (09:00 and 16:00 h). Feces were collected and separated using a paper filter two times daily (11:00 and 18:00 h). Collected feces were freeze-dried and used for nutrients digestibility analysis. Fecal samples were freeze-dried for 24 h and stored at –20℃ until analyzed. The Cr_2O_3 level of the diets and fecal samples were measured by the method of Divakaran et al. (2002). Following formulas were used to calculate ADCs of diets.

$$\text{ADC of dry matter (\%)} = 100 - (100 \times \%\ Cr_2O_3 \text{ in diet} / \%\ Cr_2O_3 \text{ in feces})$$

$$\text{ADC of nutrients (\%)} = 100 \times (1 - [\%\ Cr_2O_3 \text{ in diet} / \%\ Cr_2O_3 \text{ in feces}]) \times (\%\ \text{nutrient in feces} / \%\ \text{nutrient in diet})$$

Non-specific immune response analysis

The method from Hernández-López et al. (1996) was applied to determine phenoloxidase (PO) activity. A cacodylate buffer was prepared by mixing 10 mM sodium cacodylate and 10 mM calcium chloride to a pH of 7.0. Trypsin (5 mg) was then dissolved in 50 mL of cacodylate buffer. A L-DOPA solution was prepared by dissolving L-DOPA (50 mg) in 5 mL distilled

water. The sample (50 μL) was aliquoted into a 96-well plate. After adding 50 μL trypsin solution, the mixture was incubated (25℃, 30 min). Then, L-DOPA solution (50 μL) was added and incubated again (25℃, 10 min). A wavelength of 492 nm was used to determine the optical density. The method from Paglia & Valentine (1967) was applied to determine lysozyme activity. A 0.1 M phosphate buffer was prepared by dissolving potassium phosphate dibasic (0.518 g) and potassium phosphate monobasic (0.956 g) in distilled water and adjusted to a final volume of 100 mL and pH 6.4. A bacterial suspension (*Micrococcus lysodeikticus*) was prepared by dissolving bacteria (15 mg) in 20 mL of phosphate buffer. The sample (20 μL) was aliquoted into a 96-well plate and 200 μL bacterial suspension was added. The mixture was incubated at 25℃ for 30 min. A wavelength of 570 nm was used to determine the optical density. A zymosan solution (0.1%) was prepared by dissolving zymosan (10 mg) in 10 mL of Hank's balanced salt solution (Sigma-Aldrich). A nitro-blue tetrazolium (NBT) solution (0.3%) was prepared by dissolving three NBT tablet (Sigama-Aldrich) in 10 mL of distilled water. The sample (50 μL) was aliquoted into a 96-well plate and 200 μL Hank's balanced salt solution was added. The mixture was incubated (37℃, 30 min). After incubation, the zymosan solution (100 μL) was added into the mixture and incubated again (37℃, 2 h). NBT solution (100 μL) was added into the mixture and incubated again (37℃, 2 h). After incubation, methanol (600 μL) was added into the mixture and centrifuged (4,800×g, 10 min). After discarding the supernatant, the remaining pellet was rinsed three times with 100 μL of 70% methanol. The pellet was dissolved with 700 μL of 2M potassium hydroxide solution and 800 μL of dimethyl sulfoxide. A wavelength of 620 nm was used to determine the optical density.

Calcium and phosphorus analysis

Before analysis, the 0.5 g of carapace was dissolved into 20 mL of 12M HCl. Then, the sample pH was adjusted to 7 using 8M NaOH. Distilled water was added to adjust the sample volume to be 100 mL. P level in the shrimp carapace was measured by colorimetric method. The molybdo vanadate solution was prepared by mixing 25 g of ammonium molybdate, 1.25 g of ammonium metavanadate and 700 mL of distilled water. 250 mL of nitric acid was added into mixture and then, distilled water was added to adjust that total volume to be 1 L. P standard (500 ppm) solution was prepared by dissolving 2.195 g of potassium phosphate monobasic with 1 L of distilled water and diluted for preparing standard curve. The molybdo vanadate (2.5 mL) was added into the sample (1 mL) and then, the volume was adjusted to 25 mL by adding distilled water. The samples were incubated 15 min in room temperature. A wavelength of 470 nm was used to determine the optical density. Ca level in the shrimp carapace was measured by ethylenediaminetetraacetic acid (EDTA) titration. Distilled water (40 mL) and 8 mL of 8M NaOH were added into the sample (10 mL). Then, 10 mg of Patton-Reeder was added into sample and titrated with 0.025M EDTA untill a persistent blue endpoint was observed. The Ca level was calculated by following formula.

$$\text{Ca level (\%)} = 100 \times [\text{EDTA titration (mL)} \times (0.025 / 1000)] \times 40.08 \times 5.8 / \text{sample weight (g)}$$

Statistical analysis

A completely randomized design was used to assign the experimental tanks. Percentage data were converted to arcsine values before statistical analysis, and all data were plotted as mean ± SD. Normality was assessed with the Shapiro–Wilk test, and homogeneity of variances with Levene's test. To determine differences between treatments, a one-way analysis of variance (ANOVA) was performed. Significant differences among treatments were compared using Tukey's HSD post-hoc test ($p < 0.05$). To determine whether the effect of the diet was linear or quadratic, an orthogonal polynomial contrast was performed. SPSS 24.0 (International Business Machines, Armonk, NY, USA) was used to perform statistical analysis. The optimum dietary vitamin D_3 requirement was estimated by broken-line regression analysis, using FBW and relative *IGF-BP* expression as response variables. Regression analyses were conducted with SigmaPlot version 14.0 (Systat Software, San Jose, CA, USA).

Results

Growth and feed utilization

FBW and WG in VD135 group was significantly higher than that in VD0 group (Table 3). SGR in VD90 and VD135 groups were significantly higher than that in VD0 group ($p < 0.05$). FCR was significantly lower in VD90, VD135, and VD270 groups than that in VD0 group ($p < 0.05$). Shrimp fed VD135 and VD270 diets showed significantly higher PER than shrimp fed VD0 diet ($p < 0.05$). Survival rate was not significantly different among all the treatments. FBW, SGR, and FCR exhibited both significant linear and quadratic trends with increasing

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Table 3. Growth performance, feed utilization and survival of Pacific white shrimp (*Penaeus vannamei*) fed the experimental diets for 8 weeks

Diets	IBW[1]	FBW[2]	WG[3]	SGR[4]	FCR[5]	PER[6]	Survival (%)
VD0	1.41 ± 0.01	5.19 ± 0.31^{b}	7,280 ± 459^{b}	7.41 ± 0.11^{b}	2.03 ± 0.05^{a}	1.43 ± 0.03^{b}	95.0 ± 5.0
VD45	1.39 ± 0.01	5.83 ± 0.39^{ab}	8,280 ± 501^{ab}	7.63 ± 0.10^{ab}	1.73 ± 0.13^{ab}	1.65 ± 0.13^{ab}	100.0 ± 0.0
VD90	1.41 ± 0.01	6.09 ± 0.38^{ab}	8,562 ± 606^{ab}	7.69 ± 0.12^{a}	1.69 ± 0.09^{b}	1.68 ± 0.09^{ab}	100.0 ± 0.0
VD135	1.40 ± 0.00	6.20 ± 0.30^{a}	8,762 ± 424^{a}	7.73 ± 0.08^{a}	1.60 ± 0.12^{b}	1.78 ± 0.14^{a}	100.0 ± 0.0
VD270	1.40 ± 0.00	5.83 ± 0.29^{ab}	8,244 ± 414^{ab}	7.63 ± 0.08^{ab}	1.62 ± 0.12^{b}	1.77 ± 0.12^{a}	96.7 ± 2.9
Pr > F[7]							
ANOVA	0.109	0.032	0.031	0.027	0.016	0.054	0.109
Linear	0.489	0.030	0.029	0.024	0.002	0.008	0.496
Quadratic	0.960	0.010	0.011	0.010	0.046	0.174	0.014

Values are mean of quadruplicates (n = 4) and presented as mean ± SD. Different superscripts in each column indicate significant differences ($p < 0.05$). The determined dietary vitamin D_3 levels were 12, 35, 101, 132, and 294 μg/kg for VD0, VD45, VD90, VD135, and VD270, respectively.
[1] Initial body weight (g).
[2] Final body weight (g).
[3] Weight gain (%) = (final body weight – initial body weight) / (initial body weight) × 100
[4] Specific growth rate (%/day) = [($\log_e$ final body weight – $\log_e$ initial body weight) / days] × 100
[5] Feed conversion ratio = feed intake (g) / wet weight gain (g)
[6] Protein efficiency ratio = wet weight gain (g) / total protein given (g)
[7] Significance probability associate with F-statistic.
IBW, initial body weight; FBW, final body weight; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio; ANOVA, one-way analysis of variance.

dietary VD levels ($p < 0.05$). PER showed only a significant linear trend ($p < 0.05$). Survival of the shrimp showed significant quadratic trend with increment of vitamin D_3 level in diets ($p < 0.05$). In the broken-line regression based on FBW, the slope before the breakpoint was significant ($p = 0.001$), whereas the slope after the breakpoint was not ($p = 0.196$), indicating a plateau response. Based on this model, the optimum dietary vitamin D_3 requirement for Pacific white shrimp was estimated at 113.34 μg/kg (Fig. 1).

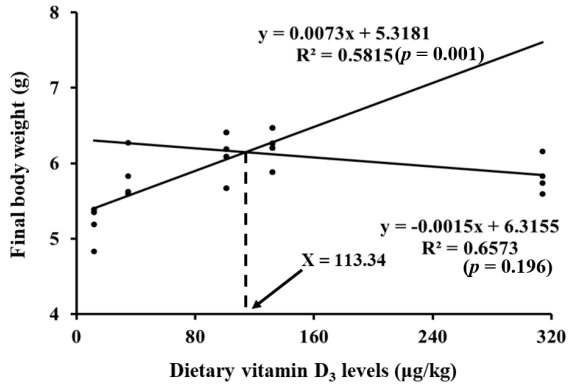

Fig. 1. Dietary vitamin D_3 requirements of the Pacific white shrimp estimated by broken line regression analysis with final body weight. The X value indicate the optimum vitamin D_3 level ($R^2 = 0.5815$, $p = 0.001$; $R^2 = 0.6573$, $p = 0.196$).

Hepatopancreas vitamin D levels and whole-body proximate composition

The hepatopancreatic vitamin D_3 levels, as well as whole-body protein, lipid, ash, and moisture, were not significantly affected by dietary vitamin D_3 levels ($p > 0.05$; Table 4). Hepatopancreatic vitamin D_3 level of the shrimp was not significantly linear or quadratic with an increase in dietary vitamin D_3 level ($p < 0.05$). The whole-body protein, lipid and ash were not significantly linear or quadratic with increment of vitamin D_3 level in diets ($p > 0.05$).

Nutrient digestibility

The ADCs of dietary protein, lipid and dry matter were significantly increased with increasing dietary vitamin D_3 levels up to 132 μg/kg (Table 5). ADCs of protein, lipid, and dry matter were significantly higher in VD45, VD90, and VD135 groups compared to VD0 and VD270 groups. Results of protein ADC was both linear and quadratic with increasing dietary vitamin D_3 level ($p < 0.05$). Results of ADCs of lipid and dry matter showed significantly quadratic trends with increment of dietary vitamin D_3 ($p < 0.05$).

Non-specific immune responses

All the groups showed no significant difference in PO activity ($p > 0.05$) (Table 6). Lysozyme and NBT activities in VD270 group were significantly higher than those in VD0 group. PO activity

Table 4. Hepatopancreas vitamin D_3 (μg/kg) levels and whole-body proximate composition (%, wet weight basis) of Pacific white shrimp (*Penaeus vannamei*) fed the experimental diets for 8 weeks

Diets	Vitamin D_3[1)]	Moisture	Crude protein	Crude lipid	Ash
VD0	2.44 ± 0.39	75.6 ± 0.7	19.9 ± 0.7	1.36 ± 0.13	3.26 ± 0.12
VD45	3.03 ± 0.92	75.5 ± 1.1	19.7 ± 0.1	1.46 ± 0.10	3.16 ± 0.16
VD90	3.69 ± 0.49	74.6 ± 1.0	20.1 ± 0.9	1.64 ± 0.25	2.98 ± 0.66
VD135	3.34 ± 0.07	74.3 ± 0.7	20.4 ± 0.8	1.50 ± 0.17	3.07 ± 0.47
VD270	3.79 ± 0.52	75.4 ± 1.5	20.2 ± 0.2	1.43 ± 0.17	2.87 ± 0.46
Pr > F[2)]					
ANOVA	0.230	0.300	0.745	0.272	0.740
Linear	0.057	0.360	0.280	0.534	0.220
Quadratic	0.412	0.133	0.956	0.060	0.930

Values are mean of quadruplicate groups and presented as mean ± SD. Values with different superscripts in the same column indicate significantly different ($p < 0.05$). The determined dietary vitamin D_3 levels were 12, 35, 101, 132, and 294 μg/kg for VD0, VD45, VD90, VD135, and VD270, respectively. The lack of superscript letter indicates no significant difference among the treatments.

[1)] Vitamin D_3 level (mg/kg).

[2)] Significance probability associate with F-statistic.

ANOVA, one-way analysis of variance.

Table 5. Apparent digestibility coefficients (ADCs, %) of nutrients in experimental diets for Pacific white shrimp (*Penaeus vannamei*)

Diets	Protein ADC[1)]	Lipid ADC[2)]	DM ADC[3)]
VD0	82.3 ± 1.0^{c}	74.1 ± 1.4^{c}	49.2 ± 0.2^{c}
VD45	85.1 ± 0.5^{b}	77.7 ± 0.7^{b}	52.9 ± 0.9^{b}
VD90	86.6 ± 0.6^{ab}	78.8 ± 0.9^{ab}	55.9 ± 0.8^{a}
VD135	87.1 ± 0.9^{a}	80.8 ± 1.3^{a}	57.1 ± 1.9^{a}
VD270	82.8 ± 0.6^{c}	71.6 ± 1.1^{c}	46.1 ± 0.9^{d}
Pr > F[4)]			
ANOVA	0.000	0.000	0.000
Linear	0.044	0.379	0.381
Quadratic	0.000	0.000	0.000

Values are mean of quadruplicate (n = 4) and presented as mean ± SD. Different superscripts in each column indicate significant differences ($p < 0.05$). The determined dietary vitamin D_3 levels were 12, 35, 101, 132, and 294 μg/kg for VD0, VD45, VD90, VD135, and VD270, respectively.

[1)] Protein apparent digestibility coefficient (%).

[2)] Lipid apparent digestibility coefficient (%).

[3)] Dry matter apparent digestibility coefficient (%).

[4)] Significance probability associate with F-statistic.

ANOVA, one-way analysis of variance.

Table 6. Non-specific immunity in hemolymph of Pacific white shrimp (*Penaeus vannamei*) fed the experimental diets for 8 weeks

Diets	PO[1)]	Lysozyme[2)]	NBT[3)]
VD0	0.27 ± 0.06	13.06 ± 1.43^{bc}	1.40 ± 0.32^{bc}
VD45	0.31 ± 0.03	11.26 ± 0.78^{c}	1.18 ± 0.10^{c}
VD90	0.38 ± 0.11	13.50 ± 0.90^{ab}	1.74 ± 0.34^{bc}
VD135	0.29 ± 0.06	13.96 ± 0.62^{ab}	2.07 ± 0.40^{b}
VD270	0.27 ± 0.05	15.24 ± 1.31^{a}	2.90 ± 0.69^{a}
Pr > F[4)]			
ANOVA	0.077	0.000	0.000
Linear	0.852	0.000	0.000
Quadratic	0.019	0.024	0.007

Values are mean of quadruplicate (n = 4) and presented as mean ± SD. Different superscripts in each column indicate significant differences ($p < 0.05$). The determined dietary vitamin D_3 levels were 12, 35, 101, 132, and 294 μg/kg for VD0, VD45, VD90, VD135, and VD270, respectively.

[1)] Phenoloxidase activity (absorbance).

[2)] Lysozyme activity (μg/mL).

[3)] Nitro-blue tetrazolium activity (absorbance).

[4)] Significance probability associate with F-statistic.

PO, phenoloxidase; NBT, nitro-blue tetrazolium; ANOVA, one-way analysis of variance.

showed no linear or quadratic trends with increment of vitamin D_3 level in diets ($p > 0.05$). Lysozyme and NBT activities exhibited both significant linear and quadratic responses with increasing dietary vitamin D_3 levels ($p < 0.05$).

Hemolymph triglyceride and cholesterol levels

Hemolymph cholesterol levels in VD45, VD90, and VD135 groups were significantly higher than that in VD270 group (Table 7). All vitamin D_3 supplemented groups showed significantly higher hemolymph triglyceride level than that in VD0 group . Cholesterol levels showed a significant quadratic trend with increasing dietary vitamin D_3 ($p < 0.05$). Triglyceride levels exhibited both linear and quadratic trends ($p < 0.05$).

Hepatopancreas histomorphology

Representative histological images of shrimp hepatopancreas

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Table 7. Cholesterol and triglyceride levels in hemolymph of Pacific white shrimp (*Penaeus vannamei*) fed the experimental diets for 8 weeks

Diets	Cholesterol[1)]	Triglyceride[2)]
VD0	7.92 ± 1.99[ab]	10.49 ± 0.77[b]
VD45	12.01 ± 2.42[a]	21.63 ± 1.99[a]
VD90	14.11 ± 5.53[a]	18.06 ± 2.96[a]
VD135	13.89 ± 5.88[a]	19.10 ± 3.24[a]
VD270	3.39 ± 0.96[b]	18.29 ± 1.53[a]
Pr > F[3)]		
ANOVA	0.000	0.000
Linear	0.183	0.000
Quadratic	0.000	0.000

Values are mean of quadruplicate (n = 4) and presented as mean ± SD. Different superscripts in each column indicate significant differences ($p < 0.05$). The determined dietary vitamin D_3 levels were 12, 35, 101, 132, and 294 μg/kg for VD0, VD45, VD90, VD135, and VD270, respectively.
[1)] Cholesterol level (mg/dL).
[2)] Triglyceride level (mg/dL).
[3)] Significance probability associate with F-statistic.
ANOVA, one-way analysis of variance.

Table 8. Hepatopancreas histomorphology of Pacific white shrimp (*Penaeus vannamei*) fed the experimental diets for 8 weeks

Diets	B cell counts[1)]	B cell diameter[2)]	LD[3)]
VD0	3.73 ± 0.45[d]	27.4 ± 1.10[d]	19.1 ± 1.41[c]
VD45	4.43 ± 0.50[c]	28.0 ± 1.80[d]	52.3 ± 3.27[a]
VD90	5.57 ± 0.50[b]	35.1 ± 2.00[b]	41.9 ± 5.94[b]
VD135	5.80 ± 0.41[b]	32.0 ± 1.21[c]	42.1 ± 3.10[b]
VD270	7.50 ± 1.01[a]	39.2 ± 2.86[a]	21.4 ± 2.34[c]
Pr > F[4)]			
ANOVA	0.000	0.000	0.000
Linear	0.000	0.000	0.005
Quadratic	0.010	0.025	0.000

Values are mean of quadruplicate (n = 4) and presented as mean ± SD. Different superscripts in each column indicate significant differences ($p < 0.05$). The determined dietary vitamin D_3 levels were 12, 35, 101, 132, and 294 μg/kg for VD0, VD45, VD90, VD135, and VD270, respectively.
[1)] B cell counts (number/tubule).
[2)] B cell diameter (μm).
[3)] Lipid droplet (number/tubule).
[4)] Significance probability associate with F-statistic.
LD, lipid droplet; ANOVA, one-way analysis of variance.

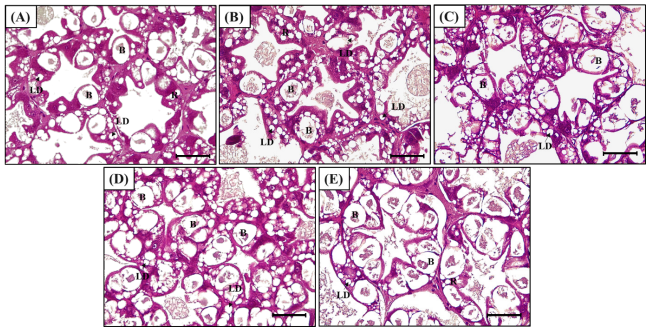

Fig. 2. Histomorphological images of the hepatopancreas of Pacific white shrimp (*Penaeus vannamei*) fed the experimental diets for 8 weeks. The determined dietary vitamin D_3 concentrations were 12, 35, 101, 132, and 294 μg/kg for VD0 (A), VD45 (B), VD90 (C), VD135 (D), and VD270 (E), respectively. Hematoxylin & eosin stained. Magnification scale, 400×; scale bar = 50 μm. LD, lipid droplet; B, blasenzellen cell.

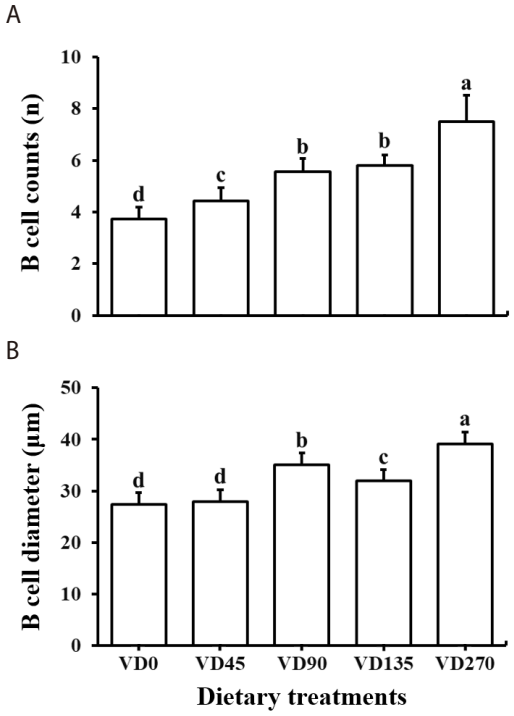

Fig. 3. The number (A) and diameter (B) of B cells in hepatopancreas of Pacific white shrimp (*Penaeus vannamei*) fed the experimental diets for 8 weeks. The determined dietary vitamin D_3 levels were 12, 35, 101, 132, and 294 μg/kg for VD0, VD45, VD90, VD135, and VD270, respectively. Values are mean of quadruplicate (n = 4) and presented as mean ± SD. Different superscripts indicate significant differences ($p < 0.05$).

were presented in Fig. 2. The hepatopancreatic blasenzellen cell (B cell) count was significantly increased with the increment of vitamin D_3 levels in diets (Table 8, Fig. 3). All the vitamin D_3-supplemented groups showed significantly higher B cell counts than that in VD0 group ($p < 0.05$). Shrimp fed VD90, VD135, and VD270 diets showed significantly higher B cell diameter than shrimp fed VD0 and VD45 diets ($p < 0.05$). Both B cell counts and diameters increased in a linear and quadratic manner with increasing dietary vitamin D_3 levels ($p < 0.05$). LD

in hepatopancreatic cells was significantly higher in all vitamin D_3-supplemented groups compared to VD0 but markedly reduced in VD270 group. LD counts increased in both linear and quadratic trends with dietary vitamin D_3 level ($p < 0.05$).

Ca and P levels in carapace

Ca and P levels in the shrimp carapace were not significantly different ($p > 0.05$) among all the treatments (Table 9). Ca and P levels in the shrimp carapace was not significantly linear or quadratic ($p > 0.05$).

qPCR assays

IGF-BP gene expression in VD90 group was significantly up-regulated than that in VD0 group (Fig. 4). VD90 and VD135 groups showed significantly higher *proPO* gene expression levels than that of VD0 group. *IGF-BP* and *proPO* gene expressions showed significant quadratic trends with increasing dietary vitamin D_3 level ($p < 0.05$). In the broken-line regression based on *IGF-BP* expression, the slope before the breakpoint was significant and positive ($p = 0.003$), whereas the slope after the breakpoint was significant and negative ($p = 0.024$), suggesting that *IGF-BP* expression increased up to the breakpoint and then declined. According to this model, the optimum dietary vitamin D_3 requirement for Pacific white shrimp was estimated at approximately 100.9 μg/kg (Fig. 5).

Table 9. Carapace calcium and phosphorus levels in Pacific white shrimp (*Penaeus vannamei*) fed the experimental diets for 8 weeks

Diets	Calcium[1)]	Phosphorus[2)]
VD0	3.92 ± 0.14	1.34 ± 0.03
VD45	4.15 ± 0.11	1.26 ± 0.05
VD90	4.42 ± 0.53	1.18 ± 0.03
VD135	3.61 ± 0.47	1.43 ± 0.10
VD270	4.10 ± 0.26	1.35 ± 0.09
Pr > F[3)]		
ANOVA	0.139	0.028
Linear	0.758	0.247
Quadratic	0.468	0.095

Values are mean of quadruplicate (n = 4) and presented as mean ± SD. Different superscripts in each column indicate significant differences ($p < 0.05$). The determined dietary vitamin D_3 levels were 12, 35, 101, 132, and 294 μg/kg for VD0, VD45, VD90, VD135, and VD270, respectively.
[1)] Calcium levels (%).
[2)] Phosphorus levels (%).
[3)] Significance probability associate with F-statistic.
ANOVA, one-way analysis of variance.

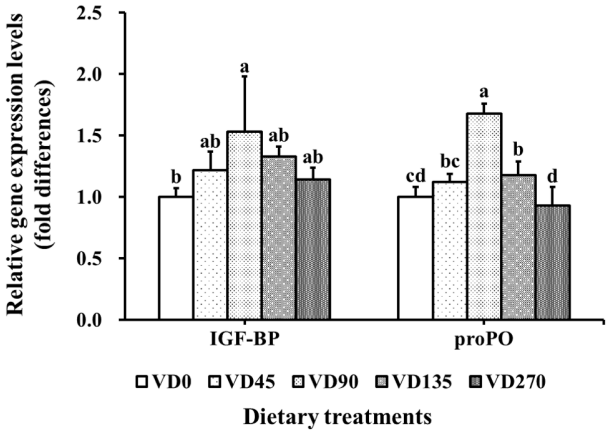

Fig. 4. Relative gene expression in hepatopancreas of Pacific white shrimp (*Penaeus vannamei*) fed the experimental diets for 8 weeks. The expression of genes was normalized to *β-actin* and expressed relative to VD0 group. Values are mean of quadruplicate (n = 4) and presented as mean ± SD. Different superscripts indicate significant differences ($p < 0.05$). The determined dietary vitamin D_3 levels were 12, 35, 101, 132, and 294 μg/kg for VD0, VD45, VD90, VD135, and VD270, respectively. *IGF-BP*, insulin-like growth factor binding protein; *proPO*, prophenoloxidase.

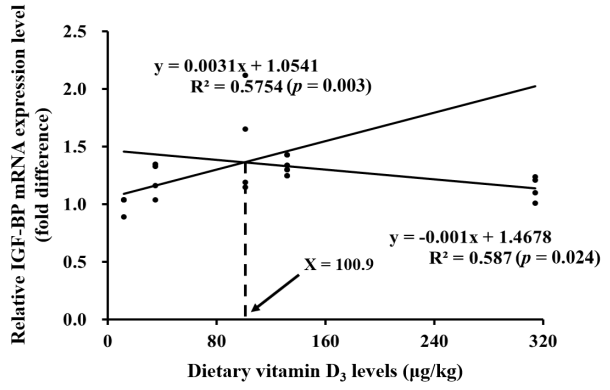

Fig. 5. Dietary vitamin D_3 requirements of the Pacific white shrimp estimated by broken line regression analysis with relative gene expression levels of insulin-like growth factor binding-protein. The X value indicate the optimum vitamin D_3 level ($R^2 = 0.5754$, $p = 0.003$; $R^2 = 0.5870$, $p = 0.024$).

Discussion

Our findings suggest that, based on broken-line regression analyses of growth performance and relative *IGF-BP* expression, the optimal dietary vitamin D_3 range for juvenile Pacific white shrimp is comparable to that reported for other marine crusta-

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ceans. Liu et al. (2021) suggested 120–150 μg/kg (4,825–5,918 IU/kg) of vitamin D_3 as an optimum level in diets for maximum growth of Chinese mitten crab, and Shiau & Hwang (1994) suggested 100 μg/kg (4,000 IU/kg) as the optimum dietary vitamin D_3 level for grass shrimp. Lee & Lee (2025) estimated a higher optimum of 186.03 μg/kg (7,441 IU/kg) for post-larvae, likely reflecting the elevated metabolic rate, accelerated tissue differentiation and greater nutritional demands during early ontogeny compared to juveniles.

In this study, nutrient digestibility of the shrimp was improved by dietary vitamin D_3 supplementation up to 132 μg/kg (5,280 IU/kg), consistent with the growth results. Although the specific mechanism is unclear, it is plausible that optimum levels of dietary vitamin D_3 enhance digestive enzyme activities in shrimp. Lee & Lee (2025) reported that optimal vitamin D_3 supplementation enhanced digestive enzyme activity and digestive tissue morphology in post-larvae, suggesting that optimal vitamin D_3 can promote growth by stimulating digestive capacity and nutrient assimilation.

In addition, gene expression level of *IGF-BP*, a regulator associated with cell growth and development (Ipsa et al., 2019), was also upregulated in VD90 and VD135 groups, suggesting a molecular basis for the observed growth improvement. These growth-promoting effects may not be solely due to improved nutrient assimilation but may also linked to endocrine regulation. In crustaceans, vitamin D_3 regulates the endocrine systems associated with the molting cycle (Chen et al., 2012). In this study, the cholesterol level of hemolymph in shrimp fed VD45, VD90, and VD135 diets were higher than that in shrimp fed VD0 and VD270 diets. Cholesterol is an important precursor of various endocrine hormones, including molting hormones in shrimp (Mykles, 2011), suggesting that dietary vitamin D_3 supplementation may stimulate endocrine cycles associated with molting and growth.

Vitamin D_3 is closely associated with lipid metabolism in the body (Botella-Carretero et al., 2007), and similar effects have been reported in aquatic species. In Wuchang bream (*Megalobrama amblycephala*), chronic stress such as abnormal lipid metabolism and intestinal structure was observed in fish fed diet with excessive levels of vitamin D_3 (Miao et al., 2015) and lipid accumulation in the fillet of fish fed low vitamin D_3 diet (Linghong et al., 2015). In shrimp, dietary vitamin D_3 level significantly affected the expression of lipid synthesis and lipolysis genes in Pacific white shrimp (Dai et al., 2022). In addition, the hepatosomatic index of Pacific white shrimp increased with increment of dietary vitamin D_3 from 17 to 189 μg/kg (685–7,550 IU/kg) (Wen et al., 2015). Similarly, in this study, hemolymph triglyceride level in all the shrimp fed vitamin D_3-supplemented diets was higher than that in VD0 group. In addition, the number of LD in shrimp hepatopancreatic cells was greater in all vitamin D_3 supplemented groups than that in VD0 group, indicating that the lipid metabolism of shrimp was significantly upregulated. Lee & Lee (2025) similarly observed that optimal vitamin D_3 supplementation in post-larvae enhanced lipid metabolic efficiency, likely by promoting lipid utilization pathways and balancing lipid synthesis and oxidation. However, the hepatopancreas of shrimp fed VD270 diet exhibited enlarged B cells and drastically reduced LD counts, suggesting that 294 μg/kg (11,760 IU/kg) may impair lipid metabolic processes. The histopathological observation in this study suggest that 101–132 μg/kg (4,040–5,280 IU/kg) of dietary vitamin D_3 may be the optimum range for lipid metabolism in shrimp, whereas excessive dietary vitamin D_3 at 294 μg/kg (11,760 IU/kg) is likely to reduce lipid metabolic processes.

Malnutrition or dysregulation of vitamin D_3 has been associated with hypercalcemia, chronic stress and high mortality of fish (Lock et al., 2010). Gilthead seabream (*Sparus aurata*) fed excessive dietary levels of vitamin D_3 showed high cortisol levels, an indicator of stress (Abbink et al., 2004). This stress response aligns with the B cell development and cell structural changes observed in shrimp hepatopancreas in this study. Notably, the number and diameter of B cells in the shrimp hepatopancreas enlarged with increasing dietary vitamin D_3 level, resembling the increase in B cell size of hepatopancreatic cells in shrimp exposed to oxidative stress (Xie et al., 2018). The simultaneous increase in B cell counts and the pronounced vacuolization of cells in VD270 may have collectively contributed to the structural collapse of hepatopancreatic integrity, suggesting that excessive dietary vitamin D_3 higher than 294 μg/kg (11,760 IU/kg) can lead to chronic stress and disrupted cellular integrity in the shrimp hepatopancreas.

Vitamin D_3 plays a critical role in calcium and phosphorus homeostasis, both of which are essential for normal skeletal formation in crustaceans, as calcium and phosphorus are the key components of the exoskeleton (Muralisankar et al., 2022). In Pacific white shrimp, Lee & Lee (2025) reported that dietary vitamin D_3 supplementation significantly increased calcium and phosphorus levels in the carapace, indicating that vitamin D_3 directly influences mineral absorption and exoskeleton development. Wen et al. (2015) demonstrated that the increasing of dietary vitamin D_3 from 17–189 μg/kg (685–7,550 IU/kg)

enhanced whole-body ash in Pacific white shrimp reared under low salinity condition (21.6–23.5 psu). In addition, Dai et al. (2022) described that the Ca and P levels in the carapace of Pacific white shrimp were increased by supplementation 480–980 μg/kg (19,200–39,200 IU/kg) of dietary vitamin D_3 under low salinity condition (10–15 psu). However, in the present study, dietary vitamin D_3 levels did not significantly affect carapace calcium and phosphorus or whole-body ash level in shrimp. This outcome may be attributed to the high salinity (32.5 ± 0.5 psu) of the rearing water. Marine crustaceans can readily absorb minerals, such as calcium and phosphorus, directly from seawater to support exoskeleton formation (Luquet, 2012). Therefore, under seawater conditions, shrimp may not require more than 12 μg/kg (480 IU/kg) of dietary vitamin D_3 to achieve adequate mineralization of the exoskeleton.

Liver is one of the major organs of nutrient storage including vitamin D_3 (Fraser, 2018). Liver vitamin D_3 level in Atlantic salmon was significantly increased by the increment of vitamin D_3 level in feeds (Graff et al., 2002). Wang et al. (2017) reported that hepatic $1,25(OH)_2D_3$ in Siberian sturgeon (*Acipenser baerii*) increased with vitamin D_3. Whole-body vitamin D_3 level in Chinese mitten crab also increased by the increment of vitamin D_3 in diets (Liu et al., 2021). However, hepatopancreatic vitamin D_3 level in the shrimp was not significantly different among all the groups in this study. Thus, dietary vitamin D_3 levels within 12–314 μg/kg (486–12,553 IU/kg) may not affect vitamin D_3 accumulation in the shrimp hepatopancreas.

Recently, the immunostimulants effects of vitamin D_3 in aquafeeds have actively been reported. Yang et al. (2019) suggested that 25 μg/kg (1,000 IU/kg) of dietary vitamin D_3 as an optimum level for improving non-specific immune responses including the activities of phosphatase and lysozyme in pearl oyster (*Pinctada fucata*). Liu et al. (2021) reported that Chinese mitten crab fed the diets with 148 μg/kg (5,940 IU/kg) of vitamin D_3 exerted enhanced activities of antioxidant enzyme activity and total antioxidant capacity. Dai et al. (2022) reported that supplementing 480 μg/kg (19,200 IU/kg) of vitamin D_3 to Pacific white shrimp upregulated the gene expression of superoxide dismutase, catalase, glutathione and lysozyme. In our study, same phenomenon was observed in the activities of lysozyme and NBT of shrimp increased with increasing dietary vitamin D_3 levels. In addition, shrimp fed VD90 and VD135 diets showed significantly higher gene expression level of *proPO*, which has a role in promotion of phenoloxidase activity, one of the most important defense mechanisms in shrimp (Amparyup et al., 2013).

In conclusion, the optimal dietary vitamin D_3 level for Pacific white shrimp is estimated at 100.9–113.34 μg/kg (4,036–4,534 IU/kg), as determined by broken-line regression analyses of growth performance and *IGF-BP* expression. Dietary vitamin D_3 supplementation significantly improved feed utilization, nutrient digestibility, lipid metabolism and non-specific immune responses of the shrimp. Histological observations further supported this conclusion, as shrimp fed diets within this optimum range exhibited healthier hepatopancreatic structures, whereas excessive vitamin D_3 supplementation (294 μg/kg) led to enlarged B cells, reduced LD, and impaired tissue integrity. These findings demonstrate that adequate—but not excessive—vitamin D_3 supplementation is crucial for promoting growth, metabolic balance and tissue health in Pacific white shrimp.

Competing interests

No potential conflict of interest relevant to this article was reported.

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Not applicable.

Availability of data and materials

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Ethics approval and consent to participate

All the experimental procedures utilized in this study were authorized by the Animal Care and Use Committee of Jeju National University (protocol number, 2022-0044). The experimental protocols complied with the guidelines of animal ethical treatment of Jeju National University for the management and use of experimental animals.

ORCID

Gunho Eom https://orcid.org/0000-0003-4224-5885
Yein Lee https://orcid.org/0000-0001-5565-5002
Sehee Jo https://orcid.org/0000-0002-3898-5541
Kyeong-Jun Lee https://orcid.org/0000-0003-0268-578X

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References

Abbink W, Bevelander GS, Rotllant J, Canario AVM, Flik G. Calcium handling in *Sparus auratus*: effects of water and dietary calcium levels on mineral composition, cortisol and PTHrP levels. J Exp Biol. 2004;207:4077-84.

Amparyup P, Charoensapsri W, Tassanakajon A. Prophenoloxidase system and its role in shrimp immune responses against major pathogens. Fish Shellfish Immunol. 2013;34:990-1001.

Association of Official Analytical Chemists International (AOAC). Official methods of analysis. Arlington, VA: Association of Official Analytical Chemists International; 2005.

Botella-Carretero JI, Alvarez-Blasco F, Villafruela JJ, Balsa JA, Vázquez C, Escobar-Morreale HF. Vitamin D deficiency is associated with the metabolic syndrome in morbid obesity. Clin Nutr. 2007;26:573-80.

Chen HY, Dillaman RM, Roer RD, Watson RD. Stage-specific changes in calcium concentration in crustacean (*Callinectes sapidus*) Y-organs during a natural molting cycle, and their relation to the hemolymphatic ecdysteroid titer. Comp Biochem Physiol A Mol Integr Physiol. 2012;163:170-3.

Dai T, Jiao L, Tao X, Lu J, Jin M, Sun P, et al. Effects of dietary vitamin D_3 supplementation on the growth performance, tissue Ca and P concentrations, antioxidant capacity, immune response and lipid metabolism in *Litopenaeus vannamei* larvae. Br J Nutr. 2022;128:793-801.

Divakaran S, Obaldo LG, Forster IP. Note on the methods for determination of chromic oxide in shrimp feeds. J Agric Food Chem. 2002;50:464-7.

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, et al. Safety of vitamin D_3 addition to feedingstuffs for fish. EFSA J. 2017;15:e04713.

Fraser DR. Evolutionary biology: mysteries of vitamin D in fish. In: Feldman D, editor. Vitamin D. 4th ed. Biochemistry, physiology and diagnostics. London: Academic Press; 2018. p. 13-27.

Graff IE, Høie S, Totland GK, Lie Ø. Three different levels of dietary vitamin D_3 fed to first-feeding fry of Atlantic salmon (*Salmo salar* L.): effect on growth, mortality, calcium content and bone formation. Aquac Nutr. 2002;8:103-11.

Hamre K, Krossøy C, Lock EJ, Moren M. Roles of lipid-soluble vitamins during ontogeny of marine fish larvae. Aquac Res. 2010;41:745-50.

Hansen AC, Waagbø R, Hemre GI. New B vitamin recommendations in fish when fed plant-based diets. Aquac Nutr. 2015;21:507-25.

Hernández-López J, Gollas-Galván T, Vargas-Albores F. Activation of the prophenoloxidase system of the brown shrimp *Penaeus californiensis* Holmes. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol. 1996;113:61-6.

Hossain MS, Small BC, Kumar V, Hardy R. Utilization of functional feed additives to produce cost-effective, ecofriendly aquafeeds high in plant-based ingredients. Rev Aquac. 2024;16:121-53.

Ipsa E, Cruzat VF, Kagize JN, Yovich JL, Keane KN. Growth hormone and insulin-like growth factor action in reproductive tissues. Front Endocrinol. 2019;10:777.

Knuth MM, Mahapatra D, Jima D, Wan D, Hammock BD, Law M, et al. Vitamin D deficiency serves as a precursor to stunted growth and central adiposity in zebrafish. Sci Rep. 2020;10:16032.

Lambert J. Vitamin D deficiency. Br J Gen Pract. 2007;57:669.

Lee Y, Lee KJ. Vitamin D_3 improves growth performance, digestive enzyme activity, antioxidant capacity, lipid metabolism, histomorphology, Ca and P homeostasis and ammonia stress resistance in Pacific white shrimp (*Penaeus vannamei*). Mar Biotechnol. 2025;27:118.

Ling-hong M, Xian-ping G, Jun X, Bo L, Ke-bao W, Jian Z, et al. Dietary vitamin D_3 requirement of Wuchang bream (*Megalobrama amblycephala*). Aquaculture. 2015;436:104-9.

Liu S, Wang X, Bu X, Zhang C, Qiao F, Qin C, et al. Influences of dietary vitamin D_3 on growth, antioxidant capacity, immunity and molting of Chinese mitten crab (*Eriocheir sinensis*) larvae. J Steroid Biochem Mol Biol. 2021;210:105862.

Lock EJ, Waagbø R, Wendelaar Bonga S, Flik G. The significance of vitamin D for fish: a review. Aquac Nutr. 2010;16:100-16.

Luquet G. Biomineralizations: insights and prospects from crustaceans. Zookeys. 2012;176:103-21.

Miao LH, Xie J, Ge XP, Wang KB, Zhu J, Liu B, et al. Chronic stress effects of high doses of vitamin D_3 on *Megalobrama amblycephala*. Fish Shellfish Immunol. 2015;47:205-13.

Muralisankar T, Mohan K, Udhayakumar V, Balamuralikrishnan B. Potential role of dietary minerals in fish and crustaceans. In: Balasubramanian B, Liu WC, Sattanathan G, editors. Aquaculture science and engineering. Singapore: Springer; 2022. p. 431-61.

Mykles DL. Ecdysteroid metabolism in crustaceans. J Steroid Biochem Mol Biol. 2011;127:196-203.

Paglia DE, Valentine WN. Studies on the quantitative and qual-

itative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967;70:158-69.

Pfaffl MW. A new mathematical model for relative quantification in real-time RT–PCR. Nucleic Acids Res. 2001;29:e45.

Prabhu PAJ, Lock EJ, Hemre GI, Hamre K, Espe M, Olsvik PA, et al. Recommendations for dietary level of micro-minerals and vitamin D_3 to Atlantic salmon (*Salmo salar*) parr and post-smolt when fed low fish meal diets. PeerJ. 2019;7:e6996.

Shao R, Liu J, Lan Y, Liao X, Zhang J, Xu W, et al. Vitamin D impacts on the intestinal health, immune status and metabolism in turbot (*Scophthalmus maximus* L.). Br J Nutr. 2022;128:2083-96.

Shiau SY, Hwang JY. The dietary requirement of juvenile grass shrimp (*Penaeus monodon*) for vitamin D. J Nutr. 1994;124:2445-50.

Sivagurunathan U, Dominguez D, Tseng Y, Eryalçın KM, Roo J, Boglione C, et al. Effects of dietary vitamin D_3 levels on survival, mineralization, and skeletal development of gilthead seabream (*Sparus aurata*) larvae. Aquaculture. 2022;560:738505.

Wang L, Xu H, Wang Y, Wang C, Li J, Zhao Z, et al. Effects of the supplementation of vitamin D_3 on the growth and vitamin D metabolites in juvenile Siberian sturgeon (*Acipenser baerii*). Fish Physiol Biochem. 2017;43:901-9.

Wen M, Liu YJ, Tian LX, Wang S. Vitamin D_3 requirement in practical diet of white shrimp, *Litopenaeus vannamei*, at low salinity rearing conditions. J World Aquac Soc. 2015;46:531-8.

Xie S, Zheng L, Wan M, Niu J, Liu Y, Tian L. Effect of deoxynivalenol on growth performance, histological morphology, anti-oxidative ability and immune response of juvenile Pacific white shrimp, *Litopenaeus vannamei*. Fish shellfish Immunol. 2018;82:442-52.

Yang C, Du X, Hao R, Wang Q, Deng Y, Sun R. Effect of vitamin D_3 on immunity and antioxidant capacity of pearl oyster *Pinctada fucata* martensii after transplantation: insights from LC–MS-based metabolomics analysis. Fish Shellfish Immunol. 2019;94:271-9.

Zhu Y, Ding Q, Chan J, Chen P, Wang C. The effects of concurrent supplementation of dietary phytase, citric acid and vitamin D_3 on growth and mineral utilization in juvenile yellow catfish *Pelteobagrus fulvidraco*. Aquaculture. 2015;436:143-50.

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