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Effect of Vitamin D_3 Supplementary Shrimp Diet on Cuticle Formation of the Pacific White Shrimp (*Litopenaeus vannamei*)

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Abstract

Background and Objectives : The requirement of vit D_3 in form of 1,25-Dihydroxy-Vitamin D_3 (Calcitriol) for white shrimp (*Litopenaeus vannamei*) is still rather lacking in term of cuticular formation and mineralization due to the direct and indirect affect the success of molting, and subsequently affects the growth opportunity and survival rate. The study on effectiveness of vitamin D_3 in form of calcitriol to promote the shrimp exoskeleton in terms of ions content in cuticle and cuticular structure of *L. vannamei* were investigated to further decide the optimal level of vit D_3 in a practical diet be part of effectiveness to increase the shrimp production of Thailand.

Methodology : The three treatments followed by the concentrations of vitamin D_3 (AcD) at 100 mg (AcD100), 150 mg (AcD150) and 200 mg (AcD200) vit D_3 kg^{-1} diet and without AcD (Control) were designed with three replications using completely randomized design (CRD). Three replications were used in the study. The healthy *L. vannamei* juvenile aged 45 days with 12.50 $\pm$ 0.65 g in body weight and 9.90 $\pm$ 0.50 cm in total length from intensive pond of private sector were previously acclimated during 10 days in laboratory before sampling to the 500L experimental circular fiberglass tank with 1.05 m in diameter. The experimental shrimps were held in 25 ppt medium at 70 ind./m^2. Water was exchanged with debris every week and exchanged by 50% of cleaned water. Experimental shrimps were fed the experimental diets which was formulated to satisfy the nutritional requirement of *L. vannamei* with the isonitrogenous (38.15% crude protein) and isolipidic (7.6% crude lipid which stored in individual bag at 4°C in refrigerator for the whole trial duration. The ration of 5% in total wet weight and four times daily (08.00 am, 01.00 pm, 05.00 pm and 09.00 pm) were operated for 30 cultured days. Water quality parameters as salinity, temperature, dissolved oxygen (DO), alkalinity, nitrite and ammonia were monitored every week at 09.00 am and 03.00 pm. To controlled sunlight and temperature, blue coloured plastic covered the tank. At termination, the experimental shrimps at B (Postmolt) and Do (Early Premolt) stages (10 samples/replication) were individually sampled for examining ions and cuticular structure as cuticle thickness and number of layers within cuticle using carapace near the posterior rostral ridge using scanning electron microscopy (SEM) coupled with energy dispersive x-ray spectroscopy. The combination of SEM and EDS allows for the analysis of both the quantity of X-rays emitted and their energy, enabling the identification and quantification of chemical elements present at detectable concentrations in percentage by weight. Analysis of

variance with one-way ANOVA and Duncan's New multiple range test using SPSS software were used for statistically different the results among treatments. The level of significant difference was set at $p< 0.05$.

Main Results : The results found that cuticle thickness and number of layers of shrimp cuticle at B (45.2 ± 2.9 μm and 25.5 ± 1.9 layers) and Do stage (97.5 ± 8.4 μm and 69.4 ± 4.9 layers) of AcD200 group were the most significant highest ($p<0.05$), followed by AcD150 group with non-significant difference ($p>0.05$) for Do stage, which corresponded with higher percentage of Ca (11.23 ± 0.42%), Mg (0.65 ± 0.09%), Mn (0.71 ± 0.13%) and P (5.32 ± 0.87%) in cuticle at B molt stage ($p<0.05$) and Ca (13.23 ± 0.31%), C (56.13 ± 4.35%) and O (34.34 ± 2.15%) in cuticle at Do molt stage ($p<0.05$). The thickness and number of layers of cuticle at B and Do stages of AcD 150 and AcD100 groups were not significant different ($p>0.05$) but their values of both groups were significantly higher than that of control ($p<0.05$). The percentages of Cu, K, Na, O, C and Cl of shrimp cuticle at B molt stage and the percentages of Cu, K, Na, Mg, Mn and Cl of shrimp cuticle at Do molt stage were not significantly different ($p>0.05$) among groups respectively. All parameters of water quality as salinity, temperature, DO, alkalinity, nitrite and ammonia among the treatments were not significantly different ($p> 0.05$) and their values were acceptable for shrimp throughout the experimental period.

Conclusions : Finally, the research results demonstrate the supplementation of at levels of 150-200 mg kg^{-1} in diet for raising *L. vannamei* shrimp is the most suitable to contributes the higher mineralization rate of Ca, Mg and Mn in form of phosphate for post molt stage and carbonate form for intermolt stage to faster process of cuticle formation. It is high possibility that vit D_3 can improve the regulation of Ca and P metabolism and absorption to promote the early mineralization of postmolt cuticle led to the suprior nucleation of Ca and CO_3^- to better formation of the intermolt cuticle. These results highlight the benefits of supplementing the suitable concentration of vit D_3 in term of precision nutrition to support the cuticle formation in *L. vannamei* shrimp results in the higher growth performance and survival rate.

Keywords: vitamin D_3; white shrimp; *Litopenaeus*; cuticle; ions

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Introduction

Vitamin D (Vit D) analogs as ergocalciferol (vitamin D_2), cholecalciferol (vitamin D_3) and their metabolites (Anthony, 1979 ; Lawson, 1985), cholecalciferol is a fat-soluble steroid which animals are rarely or not synthesized and must be provided in the diet (Bhan, 2014). The main biological effects of vitamin D in animals are the regulation of calcium (Ca) and phosphorus (P) metabolism and improvement of absorption, homeostasis of blood Ca and P, and promoting calcification. Vitamin D_3 (Vit D_3) increased the plasma Ca and

P concentration by improving the intestinal absorption and transport of Ca and P, and promoting reabsorption of Ca and P in renal tubular epithelium (Deng, 2001; Lock *et al*., 2007). Vit D_3 can promote mineralization especially deposition of Ca and P with the increasing dietary vit D_3 levels, as Rainbow trout (*O. mykiss*) were fed diets containing different vit D_3 levels for 7 days, the concentration of phosphate in plasma in the group fed a high level of dietary vit D_3 increased significantly which proved that vit D_3 adjusted the phosphate concentration and absorption of P in the intestine (Enrique *et al*., 1999). However, abnormal bone mineralization may occur if the concentration of dietary vit D_3 is not suitable because of requirements markedly vary among species. Inadequate doses of vit D in fish may reduce growth, tetany, alteration of thyroid hormone levels, thin epidermis, muscle necrosis, hypocalcaemia, erosion of fins, and increased liver and muscle lipid deposition (Taveekijakarn *et al*., 1996; Halver, 2002; Lock *et al*., 2010). On the other hand, toxicity symptoms can reduce growth, hypercalcaemia, and elevated haematocrit levels (Fleming *et al*., 2005; Lock *et al*., 2010).

Optimum vit D dietary levels as different forms and units have been reported in many species of fishes e.g. 0.00004 mg vit D kg^{-1} diet for Atlantic salmon (*Salmo salar*) (Horvli *et al*., 1998), 0.005 mg vit D kg^{-1} diet for Wuchang bream (*Megalobrama amblycephala* (Ling-Hong *et al*., 2015), 0.00625 mg vit D kg^{-1} diet (Brown, 1988) compared to 500–2000 IU vit D kg^{-1} diet (Lovell & Li, 1978; Andrews *et al*., 1980) for channel catfish (*Ictalurus punctatus*) 0.00935 mg vit D kg^{-1} diet for hybrid tilapia (*Oreochromis niloticus* x *O.* aureus (Shiau & Hwang, 1993), 1,683–1,403 and 5,000 IU vit D kg^{-1} diet for Siberian sturgeon (*Acipenser baerii*) and rice field eel (*Monopterus albus*), respectively (Wang *et al*., 2017; Tan *et al*., 2007), 0.06 or < 0.2 mg vit D_3 kg^{-1} diet for Atlantic salmon (*S. salar*) (Woodward, 1994; Graff *et al*., 2002), 5,000 IU kg^{-1} for rice field eel (Tan *et al*., 2007), 1,683–1,403 IU vit D kg^{-1} for Siberian sturgeon (*A. baerii*) (Wang *et al*., 2017), 374 IU vit D kg^{-1} for juvenile hybrid tilapia (*O. niloticus* x *O. aureus*) (Shiau & Hwang, 1993), 0.05 mg vit D kg^{-1} for channel catfish (*Ictalurus punctatus*) (Brown & Robinson, 1992).

For crustaceans, it is well documented that vit D_3 could help the mineralization of the exoskeleton by supporting the transportation of absorbed Ca, even if marine crustaceans can absorb significant amounts of dissolved Ca^{2+} through the gills (He *et al*. 1992). For shrimp, rather limited research has been conducted to evaluate the vit D_3 requirements. Adequate dietary vit D_3 concentration for black tiger shrimp (*Penaeus monodon*) growth was 4,000 IU kg^{-1} in purified diets (Shiau & Hwang,1994) accord with Naik (1999) revealed that poor growth and food conversion efficiency were also found this species fed on vit D-deficient diets. Chen & Li (1995) also suggested that vit D_3 requirement of *Polistes chinensis* in purified feed was 8,000 IU kg^{-1}while 0.48 mg vit D_3 kg^{-1} for white shrimp (*L. vannamei*) achieved the best growth performance (Dai *et al*., 2022) which harmonized with Wen *et al*. (2015) demonstrated that Ca, Mg and P in *L. vannamei* body were significantly increased with the increasing of dietary vit D_3. Moreover, Growth performance and alkaline phosphatase activity

in hepatosomatic were not affected by the supplementation of vit D_3. Whole-body moisture and zinc content were significantly decreased with increasing vitamin D_3, and higher value was also observed on whole-body protein, ash content, Ca, P and Mg concentration with increasing vit D_3 and concluded that 6,366 IU vit D_3 kg^{-1} in diet was recommended for juvenile *L. vannamei* at low salinity rearing conditions. The optimal vit D_3 requirement for larval crabs (*Eriocheir sinensis*) was 0.12-0.15 mg kg^{-1}, and crabs fed 0.225 mg kg^{-1} showed the highest survival rate after 120-h salinity stress (Liu *et al*., 2021).

However, dietary vit D_3 requirement in practical diets for *L. vannamei* as the main worldwide culture speciesis still rather lacking in terms of ions in the cuticle and cuticular structure. In the meantime, *L. vannamei* is reared at various salinity in Thailand that effected directly to minerals concentration to created the problem on soft-shell from slow formation of cuticle after ecdysis to face cannibalism. Furthermore the other phenomenon is still thin and rough cuticle through the next molt. Hence, vit D_3 supplementation in diet should be one strategy to solved these problems.Therefore, the objective of the present study was to investigate the effect of different levels of dietary vit D_3 on mineralization of *L. vannamei* cuticle in terms of cuticular structure and its ions content to further decide the optimal level of vit D_3 in a practical diet.

Experimental Design

The three treatments as followed three doses of vit D_3 known as 1,25-dihydroxy-vitamin (Calcitriol) which were mixed in formulated diet at 100 mg kg^{-1} (AcD100), 150 mg kg^{-1} (AcD150), 200 mg kg^{-1} (AcD200) and diet without vit D_3 (Control) were designed using completely randomized design. Three replications were used in the study.

Shrimp preparation

L. vannamei shrimp juveniles aged 45 days purchased from intensive pond of private sector were previously acclimated during 10 days in laboratory before sampling to the experimental circular fiberglass tank with 1.05 m in diameter. The healthy *L. vannamei* juveniles with 12.50 $\pm$ 0.65 g in body weight and 9.90 $\pm$ 0.50 cm in total length were stocked at density of 70 ind./m^2.

Feed preparation

The isonitrogenous (38.15% crude protein) and isolipidic (7.6% crude lipid) experimental diets formulated to satisfy the nutritional requirement of *L. vannamei* were supplemented with 1,25-dihydroxy-vitamin (Cholecalciferol) in diet at 100 mg kg^{-1} (300 IU), 150 mg kg^{-1} (450 IU), and 200 mg kg^{-1} (600 IU) in comparison with a negative control diet. The dietary formulation and proximate composition of the experimental diets were shown in (Table 1). Diets were coated with squid liver oil sprayed at 20 g kg^{-1}. After an additional 24-h drying at room temperature, batches of diet were stored in individual bag and stored in 4°C refrigerator for the whole trial duration.

Methodology

The healthy *L. vannamei* juvenile were held at 70 ind/m^2, 60 shrimp per tank, using 500 L circular fiberglass tanks filled with 25 ppt water for 30 days. All the tanks were randomly placed in the same location. They were maintained under natural photoperiod and room temperature. At 50 % water was exchanged with debris every week and the cleaned water exchanged at 50% every week. Salinity was maintained at 25 ppt, water quality parameters as salinity, temperature, dissolved oxygen (DO), alkalinity, nitrite and ammonia were monitored every week at 09.00 am and 03.00 pm. To controlled sunlight and temperature, blue coloured plastic covered the tank. Experimental shrimps were fed the experimental diets with the ration of 5% in total wet weight and four times daily (08.00 am, 01.00 pm, 05.00 pm and 09.00 pm).

Table 1 Dietary formulation (% dry matter) and proximate composition of the experimental diets for *L. vannamei*

Ingredients	Experimental diets			
	Control	AcD100	AcD150	AcD200
Squid by product	3.45	3.45	3.45	3.45
Poultry meal 64% Protein	11.4	11.4	11.4	11.4
Fishmeal 60% Protein	15.8	15.8	15.8	15.8
Shrimp by Product	4.8	4.8	4.8	4.8
Soybean meal	19.5	19.5	19.5	19.5
Fermented soy bean	3.43	3.43	3.43	3.43
Wheat gluten	1.14	1.14	1.14	1.14
Corn protein concentrate	1.37	1.37	1.37	1.37
Wheat flour	24.810	24.8050	24.795	24.7850
Rice bran	6.6	6.6	6.6	6.6
Squid liver paste	1.7	1.7	1.7	1.7
Lecithin	2	2	2	2
Fish hydrolysate	2	2	2	2
Squid liver oil	2	2	2	2
AcD	0	0.00100	0.0150	0.0200
Total	100	100	100	100
Chemical composition				
%Crude Protein	38.12	38.16	38.22	38.10
%Crude Fat	7.65	7.70	7.66	7.75
%Crude Fiber	2.75	2.67	2.64	2.58
%Crude Ash	5.26	5.28	5.25	5.35
%Humidity	8.50	8.65	8.64	8.73

After termination, the portion of carapace near the posterior rostral ridge of experimental shrimp (4 mm x 6 min in size) at B (Postmolt) and Do (Early Premolt) stages (10 samples/molt stage/replication) were individually collected and dried in oven at 45 °C for 24 h then placed carapace sample in lateral to the stub on the carbon tape and thereafter coating with gold then kept the samples in desiccator until use. Evaluating the cuticular thickness and number of cuticle layer by checking the relative thickness from the scale bar and counting number of cuticle layer by eyes with higher magnification using scanning electron microscopy (SEM). SEM analysis performed in low vacuum conditions, using Joystick to focus the desired locating sample using electron gun with producing electrons direct to the sample surface and convert to visual signal. Ions as Ca, Mg, Cu, Na, K, Mn, Cl, P, S, C and O were examined by energy x-ray dispersive system (EDS). The EDS data was presented as a graph with KeV on the x-axis and peak intensity on the y-axis. The combination of SEM and EDS allows for the analysis of both the quantity of X-rays emitted and their energy, enabling the identification and quantification of chemical elements present at detectable concentrations in percentage by weight

Statistical Analysis

Analysis of variance with one-way ANOVA and Duncan's New multiple range test using SPSS software were used for statistically different among treatments. The level of significant difference was set at $p< 0.05$.

Results

1. Thickness and number of layers of cuticle at B molt stage

The thickness (Fig. 1a) and number of layer (Fig. 1b) of shrimp cuticle at B stage of AcD200 group were significantly higher than that of the others ($p<0.05$) while the thickness and number of layers of AcD 150 and AcD100 groups were not significant different ($p>0.05$) but their values of both groups were significantly higher than that of control ($p<0.05$) (Fig. 2).

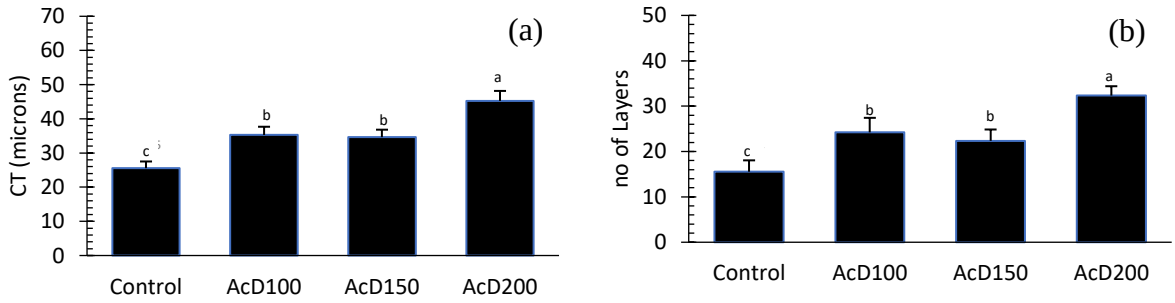

Figure 1 The thickness (a) and number of layer (b) of shrimp *L. vannamei* cuticle at B stage. Values were means with standard deviations represented by vertical bars. Mean values with different letter were significantly different ($p< 0.05$).

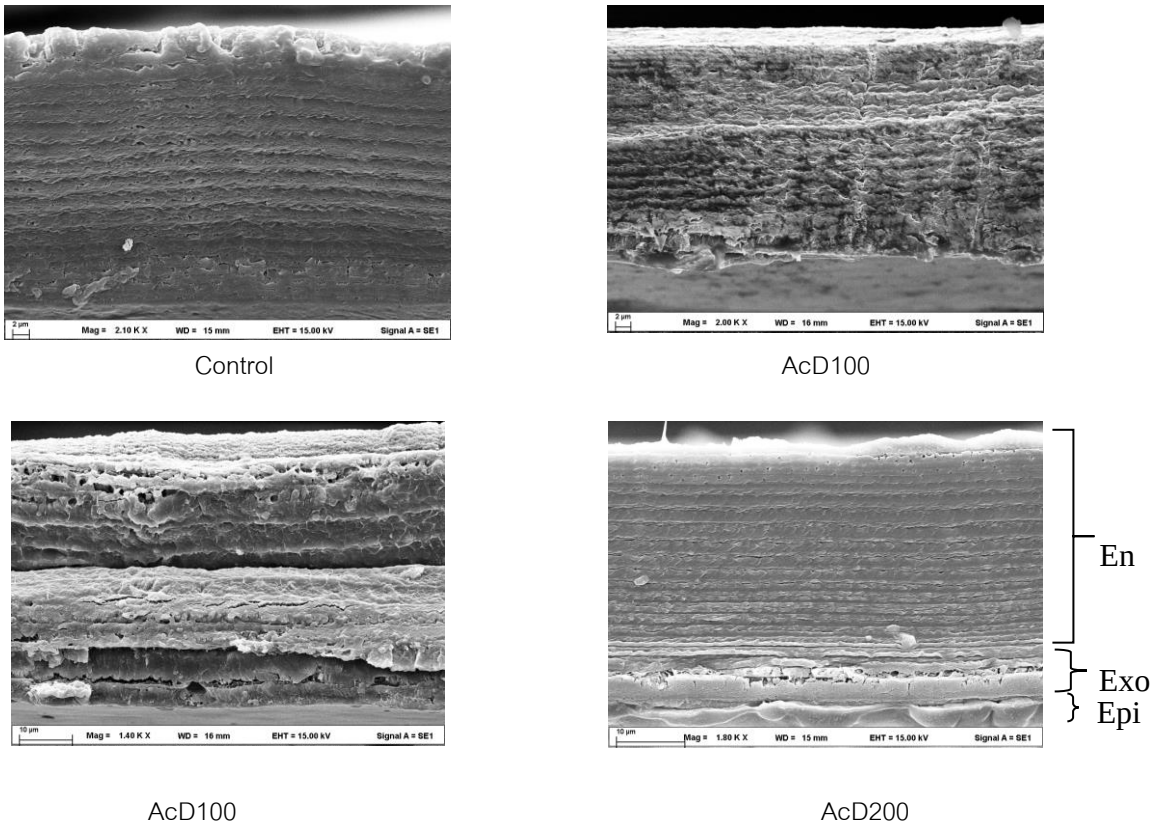

Figure 2 The cuticle architecture of treatments and control at B molt stage. En= Endocuticle, Ex=Exocuticle, Epi=Epicuticle

2. Thickness and number of layers of cuticle at Do molt stage

The thickness (Fig. 3a) and number of layers (Fig. 3b) of shrimp cuticle at Do stage of AcD200 group was significantly higher than those of control and AcD100 groups ($p<0.05$) but their values were not significantly different ($p>0.05$) with AcD150 group (Fig. 4).

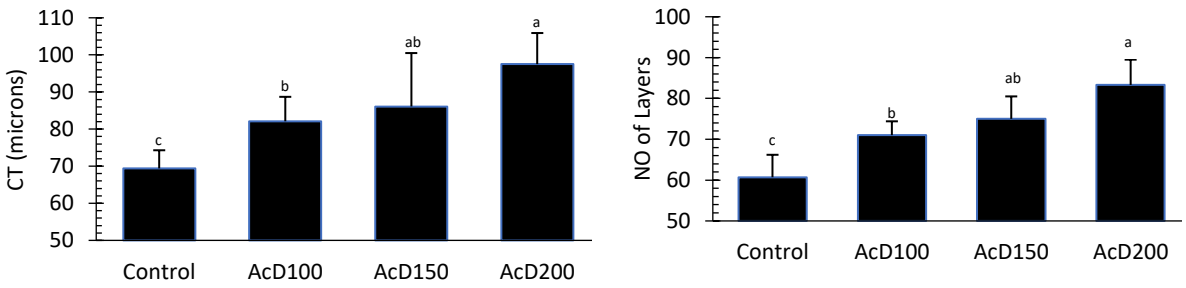

Figure 3 The thickness (a) and number of layer (b) of shrimp *L. vannamei* cuticle at Do stage. Values were means with standard deviations represented by vertical bars. Mean values with different letter were significantly different ($p< 0.05$).

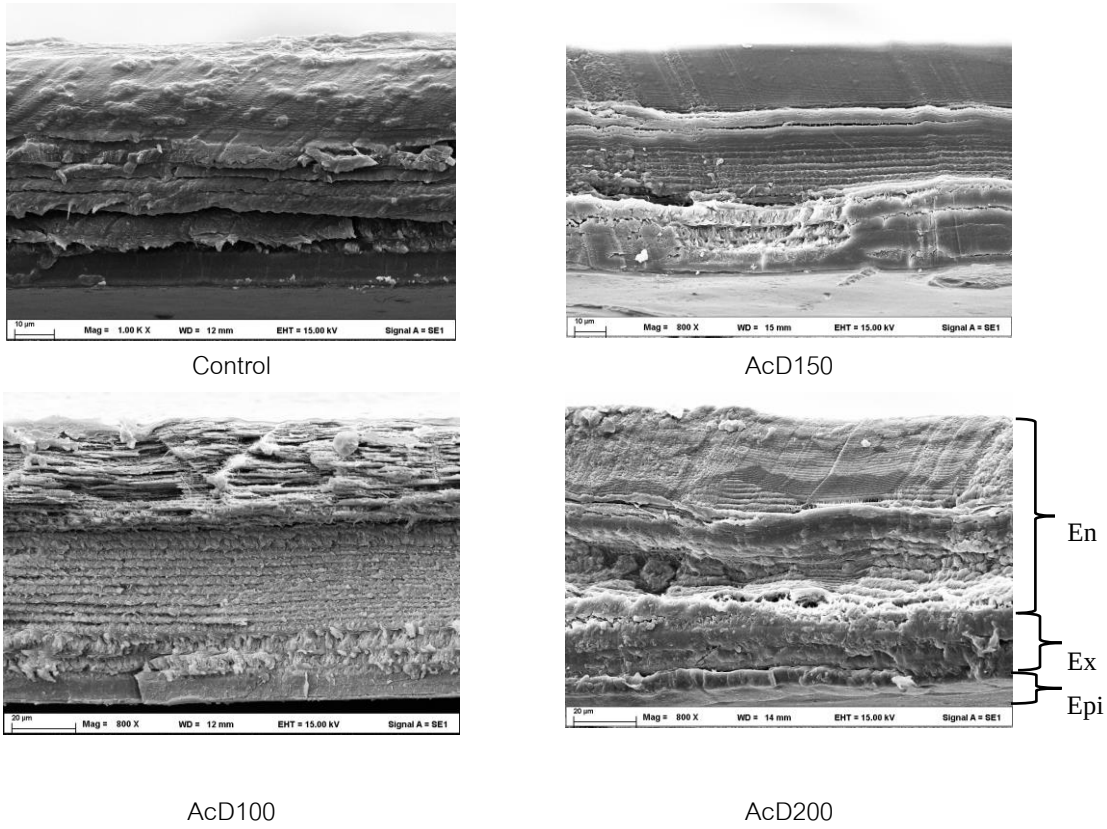

Figure 4 The cuticle architecture of treatments and control at Do molt stage. En= Endocuticle, Ex=Exocuticle, Epi=Epicuticle

3. The percentage of ions in the Cuticle at B Molt Stage.

The percentage of Ca (Fig. 5a) and Mg (Fig. 5b) of shrimp cuticle at B molt stage of AcD200 group were significantly higher than those of the others (p<0.05) while values of Mn (Fig. 5c) and P (Fig. 5d) were significantly higher than those of control (p<0.05) but their values were not significantly higher than those of AcD150 and AcD100 groups (p>0.05). The other ions as Cu (Fig. 5e), K (Fig. 5f) Na (Fig. 5g), O (Fig. 5h), C (Fig. 5i) and Cl (Fig. 5j) were not significant different among groups (p>0.05).

4. The percentage of ions in the Cuticle at Do Molt Stage.

The percentage of Ca (Fig. 6a) of shrimp cuticle at Do molt stage of AcD200 group was significantly higher than those of control and AcD100 groups (p<0.05). The percentage of P (Fig. 6a) of control group was significantly higher than those of AcD200 and AcD150 groups (p<0.05) but it was not significantly higher than that of AcD100 group (p>0.05). The percentage of O (Fig. 6h) was significantly higher than those of control and AcD100 group (p<0.05) while %C (Fig. 6i) of AcD200 group was significantly higher than that of control but it was not significantly different (p>0.05) with AcD100 and AcD150 groups. The percentage of Mg (Fig. 6b), Mn

(Fig. 6c), Cu (Fig. 6e), K (Fig. 6f), Na (Fig. 6g), Cl (Fig. 6j) of shrimp cuticle at Do molt stage were not significantly different (p>0.05) among groups.

5. Water Quality

All parameters of water quality among the treatments were not significantly different (p> 0.05) and their values were acceptable for shrimp throughout the experimental period (Table 2).

Table 2 Water quality throughout the experimental period. Values were means with standard deviations. Mean values in the same column with different letter were significantly different (p< 0.05).

Treatment	pH	Temperature (^{O}C)	Alkalinity (mg/l)	NO^{-}_{2} (mg/l)	NH_3-N (mg/l)	DO (mg/l)
Control	8.12 (0.06)	30.5 (0.10)	124 (3.1)	0.33 (0.02)	0.66 (0.05)	6.57 (0.10)
AcD100	8.15 (0.06)	30.6 (0.13)	126 (4.7)	0.41 (0.01)	0.56 (0.05)	6.63 (0.07)
AcD150	8.25 (0.08)	29.9 (0.11)	119 (4.2)	0.40 (0.01)	0.60 (0.04)	6.28 (0.07)
AcD200	8.09 (0.05)	29.8 (0.14)	128 (3.1)	0.34 (0.02)	0.68 (0.07)	6.45 (0.09)

Discussion

The thickness and number of cuticle layers of Do cuticle when shrimps fed on 200 mg vit D_3 kg^{-1} which is significantly higher than those of control and 100 mg vit D_3 kg^{-1}, but its value is not significantly different with shrimps fed on 150 mg vit D_3 kg^{-1}. This indicates that the supplementation of vit D_3 contributes the higher rate of precipitation of Ca in form of carbonate crystals to construct the cuticular layers in terms of thickness and number of cuticular layer especially in the endocuticle layer. This phenomenon complies with higher percentage of Ca, C and O found in shrimp fed on 200 mg vit D_3 kg^{-1}, however at 150 mg vit D_3 kg^{-1} is also promoted well the cuticle formation as the same reason. This indicates that the supplementation of vit D_3 contributes the higher rate of precipitation of Ca in form of carbonate crystals to accomplish the cuticular layers and thickness especially in the endocuticle layer.The phenomenon had described by Pratoomchat *et al.* (2002a) indicated that calcite $CaCO_3$ and hydromagnesite, $Mg_5(CO_3)_4(OH)_2$ $4H_2O$ were the main component in *Scylla serrata* cuticle afterward post-molt stage in normal condition. By this reason, the cuticle thickness and number of layers at Do stage of control was lower than those of shrimps fed with vit D_3 supplementary diet. In the same manner, Wen *et al*. (2015) stated that higher values of whole-body protein, ash content, Ca, P and Mg concentration with increasing vit D_3 which indicated that vit D_3 from ingredients could meet the growth requirement but not for whole-body mineral deposition and recommended 6,366 IU D_3kg^{-1} for juvenile *L. vannamei* at low salinity rearing conditions although

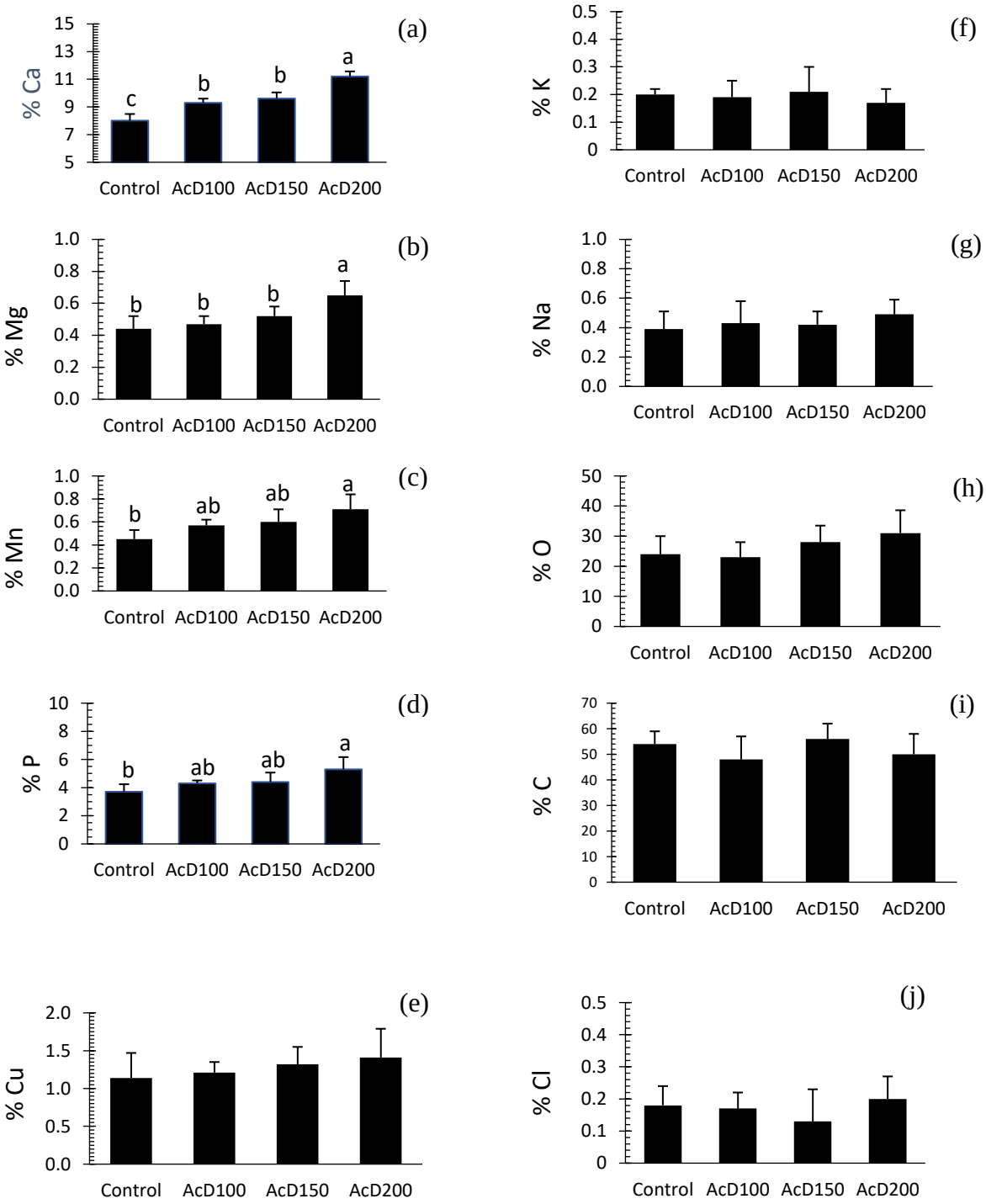

Figure 5 The percentage of Ca (a), Mg (b), Mn (c), P (d), Cu (e), K (f) Na (g), O (h), C (i) and Cl (j) *L. vannamei* cuticle at B molt stage. Values were means with standard deviations represented by vertical bars. Mean values with different letter were significantly different ($p < 0.05$).

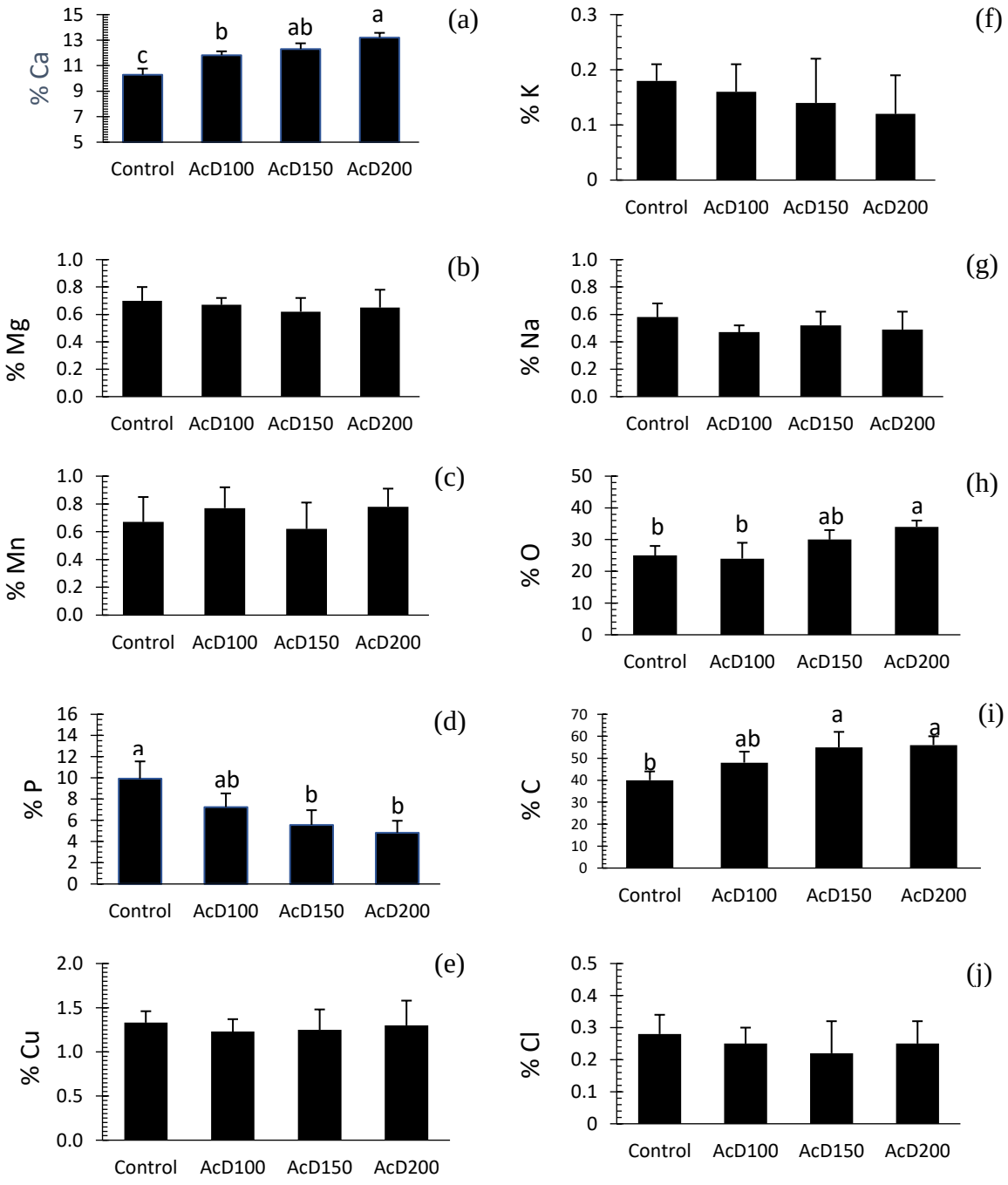

Figure 6 The percentage of Ca (a), Mg (b), Mn (c), P (d), Cu (e), K (f) Na (g), O (h), C (i) and Cl (j) *L. vannamei* cuticle at Do molt stage. Values were means with standard deviations represented by vertical bars. Mean values with different letter were significantly different ($p < 0.05$).

when compared to 600 IU D_3 kg^{-1} for juvenile *L. vannamei* at optmum salinity of this study. Our study is supported by the previous studies which explained that the primary biological function of vit D_3 was to maintain normal Ca and P homoeostasis by enhancing the absorption ability from the intestine or in the intestinal epithelium (Darias *et al*., 2011; Fleet, 2017) and maintain Ca-P homeostasis by acting in synergy with calcitonin and parathyroid hormone (Darias *et al*., 2011; Chen & Li, 1995). Higher percentage of P in the Do cuticle of shrimp fed control diet, it is possible to explains that the cuticle formation of control group is rather slower precipitating in forms of calcite and magnesite due to the exiting of P which is representative ions for initiative structure of cuticle. Li *et al.* (2021) indicated that one of the important nutrients for shrimp cuticle formation is chitin to form chitin-protein fiber complex to seizes the minerals for constructing the cuticle and the basic nutrient to build chitin is carbohydrates.

So it is possible that vit D_3 could affect glucose metabolism by regulating glycolysis, gluconeogenesis and pentose phosphate pathway including triggered the insulin signaling pathway, and further influence glucose transport and glycogen synthesis, which could promote the utilization of carbohydrate as studied in abalone *Haliotis discus hannai* by Vit D_3 improved the intracellular Ca^{2+} level, and promoted the secretion of insulin in rainbow trout (*Oncorhynchus mykiss*) (Enrique *et al*., 1999). Hence vit D_3 is possibly contribute the constructing *L. vannamei* cuticle via glucose metabolism and transport, however, there is no scientific information on the interaction between glucose and vit D_3 on glucose metabolism or insulin signaling pathway in shrimp. Similar to the study of Ling-Hong *et al.* (2015) pointed that diet containing 3.7515 IU vit D_3 kg^{-1} diet for Wuchang bream (*Megalobrama amblycephala*) promoted the deposition of minerals in muscles and increased the digestibility of Ca and P, plasma concentration of Ca and P increased and bone mineralization was further improved. Whether deficient or excessive dietary vit D_3 inhibited mineral accumulation. In the same manner, the increasing the level of vit D_3 (4,000 IU kg^{-1} diet) significantly increased whole body Na, K, P, Ca, Mg and Zn retention compared to the level of 2,000 IU vit D_3 kg^{-1} diet , while no effect on vertebrae mineral deposition was observed at the higher vit D_3 level (Zhu *et al*., 2015).

In addition to higher percentage of Ca, Mg, Mn and P together with higher thickness and number of cuticle layers at post-molt stage (B stage) when shrimps fed on 200 mg vit D_3 kg^{-1}, it is possible to explains that fast precipitating in forms of calcium, magnesium and manganese in form of phosphate due to the exiting of P is representative ions for initiative structure of cuticle as described by Pratoomchat *et al.* (2002b) who revealed that the presence of a crystalline solid solution of calcium and magnesium hydrated phosphate and carbonate. In effect, the identification of the three strongest peaks corresponds to octacalcium phosphate, ($Ca_8H_2(PO_4)_65H_2O$) and dicalcium phosphate dihydrate ($Ca_2HPO_42H_2O$) from X- ray diffraction patterns. This confirms that vit D_3 supplementary diet especially at 200 mg vit D_3 kg^{-1} can facilitate well the formation of the early post-molt cuticle in terms of number of layer and thickness. This event is very important for survival of

shrimp because they can prevention from cannibalism, return to create good osmotic balance after absorption lots of water during ecdysis and higher rate of reducing pore canal around new cuticle to decreasing the opportunistic diseases due to the opened circulatory system. These findings concurs with Liu *et al*. (2022) found that Ca and P concentration in the muscle and hemolymph and hydroxyproline (Hyp) concentration in the muscle of crab (*E. sinensis*) were significantly affected by the interactive effect between the optimal dietary Ca and vit D_3 level but excessive vit D_3 can lead to decoupling oxidative phosphorylation (Jones *et al*., 2014). Dietary vit D_3 and Ca synergistically increased sarcous-Ca and sarcous-P deposition and the concentration of Ca-P in the hemolymph (Liu *et al*., 2022). The present study is also in agreement with Wen *et al*. (2015) stated that vit-D_3 increases Mg absorption and deposition. In contrast with Shiau & Hwang (1993) indicated that Ca and P concentrations in shrimp *P. monodon* body were not affected by vit D supplementation although highest of weight gain, Protein efficiency ratio and survival rate was found in shrimp fed the diet with 0.1 mg D_3 kg^{-1}.

The majority of the white shrimp (*L. vannamei*) in the Thailand is reared at low salinity conditions due to lots of reasons specifically diseases avoidance. As we known that the concentration of mineral was direct play varied by salinity, especially for Ca, Mg, Na, K and Cl. From aforemention study, it is possible that shrimp need more vit D_3 for biomineralization when shrimp reared in a lower iso-osmotic point. From our study concluded that the most suitable dosage of vit D_3 in diet is 150-200 (450-600 IU) vit D_3 kg^{-1} diet for *L. vannamei* under the optimal salinity. By the reason, the mineralization efficiency will be related to the environmental conditions especially the salinity level specically Ca, Mg, HCO_3^- levels in medium and digestible P in diet, nutrition of diet and feeding management. The supplementing vit D_3 at 200 mg vit D_3 kg^{-1} diet recommends to the early period of culture (Day 0-45 days of grow-out culture) due to high molting frequency. Thereafter, It is possible to use at 150 mg vit D_3 kg^{-1} to prepare and increasing rate of precipitating to accomplish the cuticle formation under the optimal conditions of the environment. The application of vit D_3 at 200 mg vit D_3 kg^{-1} diet is also possible to use to solve the problem of lower rate of cuticle formation under the unusual environmental conditions especially the salinity level. However, for the perfect operation, the shrimp culturist should be intensive care the concentrations levels of Ca, Mg, HCO_3^- in medium and digestible P in diet, including quality of diet must be appropriate to efficiently support the cuticle formation as well as must be equilibrate the culturing medium especially DO., pH, nitrite and ammonia.

Conclusions

The supplementation of vit D_3 at levels of 150-200 mg kg^{-1} in diet for raising *L. vannamei* shrimp is the most suitable in terms of cuticular thickness, number of cuticle layers and percentage of ions in cuticle.

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