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EFFECTS OF PHYSIOLOGICAL DOSES OF VITAMIN D_3 (CHOLECALCIFEROL) ON INDUCED MOLTING AND GROWTH IN GIANT FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII* (de Man)

S. Y. Metar, P. P. Srivastava, K. K. Jain, S. M. Manush, S. Raizada and S. Ayyappan†

Central Institute of Fisheries Education, Fisheries University Road, Versova, Mumbai – 400 061

†Indian Council of Agricultural Research, Krishi Anusandhan Bhavan - II, Pusa, New Delhi –110 012

ABSTRACT

Effects of three different doses of vitamin D_3 on molting, growth, and calcium and phosphate composition of tissue and molt during the grow-out of the giant freshwater prawn *Macrobrachium rosenbergii* (average weight 10.56 ± 0.20 g), obtained from a grow-out pond, were studied. Intra-muscular doses of vitamin D_3 (100, 500 and 2000 IU/kg body weight) were given on the 1st, 3rd, 5th, 7th, 9th, 11th, 13th, 15th, 20th, 25th and 30th days. All the experimental animals were fed with a basal diet containing fish meal, shrimp meal, wheat flour, groundnut de-oiled cake, soybean meal and wheat bran at 3% of the body weight. The numbers of molts were recorded as 20±0.50, 29±1.16, 51±1.87, and 30±1.60 in control, 100, 500 and 2000 IU/kg body weight physiological doses, respectively. Maximum growth was recorded in prawns given 500 IU/kg dose. Survival was between 58.33 ± 9.13 and 77.77 ± 8.61%. The ash content and calcium level increased significantly ($p<0.05$) and recorded the highest values in 500 IU/kg physiological dose. However, the inorganic phosphate (P_i) content recorded the highest values in tissue in 2000 IU/kg dose ($p<0.05$, $F = 50.60613$). There is no significant difference in calcium contents ($p>0.05$) in both tissue and molt at 500 and 2000 IU/kg doses. It was found that a higher physiological dose (2000 IU/kg) of vitamin D_3 increased the rate of mortality. Results have shown that vitamin D_3 has a positive impact on the growth and survival of *M. rosenbergii* and it interferes with the metabolism of Ca and P_i in tissue, and alters molting frequency. Results on physiological dose suggest an alternative and effective dietory supplementation method of vitamin D_3 in the grow-out phase of *M. rosenbergii.*

Keywords: Vitamin D_3, growth, molting, calcium and phosphate, *Macrobrachium rosenbergii*

INTRODUCTION

Vitamin D plays a central role in bone growth and health (Fraser, 1995). Vitamin D_3 (cholecalciferol) is synthesized in the body by catalyzing the photolysis reaction using ultraviolet light. Vitamin D_2 (ergocalciferol) is the form mostly found in plant tissues. A unique property of vitamin D is that it functions very much like a hormone (calcitriol) when synthesized in kidney as 1,25 –$(OH)_2$ calciferol. Its target tissues in the body include the gills, kidneys, intestines and bones, where it acts to regulate calcium and phosphorus homeostasis and calcium metabolism (Swarup *et al.*, 1991a, b). Vitamin D_3 in the intestine stimulates the active transport of proteins that mediate the absorption of calcium. In bone tissue, vitamin D plays an active role in regulating calcium deposition (bone mineralization and mobilization).

Considerable work has been done on the role of vitamin D_3 in calcium homeostasis of vertebrates starting from pisces to mammals. It is reported that vitamin D when applied at a physiological dose, exerts hypercalcemia in vertebrates (Srivastav *et al.*, 1995). There is a report available on calcium regulation in crustaceans, where an important cyclic activity such as molting takes place (Taketomi *et al.*, 1992). An improved performance was reported when *Penaeus japonicus* larvae were fed with vitamin D (Kanazawa, 1989). Reduced growth (76%) and poor appetite were observed in shrimp (*P. vannamei*) fed with vitamin D_3-deficient diet (Haiqi *et al.*, 1992). The nature of calcified crustacean exoskeleton suggests that vitamin D may also function in the mineralization of exoskeleton in crustaceans (Deshimaru *et al.*, 1978). Shrimp (*P. monodon*) fed on vitamin D-deficient diet showed poor response to growth and food conversion ratio, and the relative percentage weight gain was 90% of that of the control (Reddy *et al.*, 1999) and weight gain reported in white shrimp (*P. indicus*).

Different levels of vitamin D affect the amount of some minerals absorbed and deposited by the shrimp *P. chinensis*. Higher vitamin D concentration helps the absorption of phosphorus and a lower concentration helps absorption of calcium; so proper vitamin content in the diets can help the shrimps to absorb calcium and phosphorus, and promote hardening of shrimp shells (Chen and Li, 1995). Thus, we investigated the effect of physiological administration of vitamin D_3 on growth performance, molting and mineral deposition (calcium and phosphorus) of *Macrobrachium rosenbergii*.

MATERIAL AND METHODS

Experimental Animals

M. rosenbergii (average weight 10.56 ± 0.20 g) cultured in grow-out ponds were brought from Green Earth Aqua Farm, Neral, Maharashtra. Prawns were acclimated for a period of 15 days in laboratory conditions before the start of the experiment, during which prawns were fed with a vitamin D_3-deficient diet at 3% of body weight.

Experimental Design

Twenty-four plastic pools (300 l capacity) were arranged in four treatments having six replicates and treated with potassium permanganate (at 10 mg/l for 2 h). A uniform volume of 200 l water was maintained in each pool throughout the experimental period. Round the clock aeration was provided in all the pools with an air blower. Six prawns of average weight (10.56 ± 0.20 g) were collected from the grow-out and stocked in each pool. Each pool was properly covered and polyvinyl chloride pipes were provided as hiding place to prevent cannibalism among the stocked prawns. The duration of the experiment was for a period of 30 days. The feeding was maintained at 3% of body weight and fed twice a day.

Experimental Diet

The experimental diet was formulated with approximate 32% protein and 10% fat. Dried and powdered ingredients (Table 1) were properly mixed with and passed through a twin-screw extruder having a die of 2-mm size (Basic Technology Pvt. Ltd, Kolkata). Pellets obtained were dried at 60°C in a hot air oven for 6 hours and stored in a cool (4°C), dry place.

Table 1: Composition of the basal diet used to feed *M. rosenbergii* during the 30- day experiment

Ingredient	Inclusion rate
Fish meal (%)	40.0
Acetes spp. (%)	10.0
Wheat flour (%)	10.0
Groundnut de-oiled cake (%)	10.0
Wheat bran (%)	10.0
Soybean meal (%)	9.5
Sunflower oil (%)	5.0
Gelatin (%)	2.0
Vitamin and mineral mix, deficient in vitamin D_3 (%)	2.0
Soylecithin (%)	1.0
Carboxy-methyl cellulose (%)	0.3
Nachini - ragi seed (%)	0.2
Feed Composition:	
Moisture (%)	4.7 ± 0.5
Lipid (%)	8.4 ± 0.1
Protein (% N x 6.25)	32.1 ± 0.2
Ash (%)	8.1 ± 0.3
Carbohydrate (%)	24.3 ± 0.4
Gross Energy (kcal/100 g)	327.53 ± 16.6

Vitamin and mineral mix composition (per gramme):

Vitamin A, IP - 10000 IU; Vitamin E, IP - 30 mg; Vitamin B_1, IP - 10 mg; Vitamin B_2, IP - 10 mg; Nicotinamide, IP - 30 mg; D-Panthenol, IP - 10 mg; Vitamin B_6, IP - 4 mg; Vitamin C, IP - 150 mg; Folic acid, IP - 2000 µg; Vitamin B_{12}, IP - 10 µg; Dibasic calcium phosphate, IP - 140 mg; Copper sulphate, BP - 0.2 mg; Manganese sulphate, BP - 0.02 mg; Zinc sulphate, USP - 28.7 mg; Potassium iodide, IP - 0.025 mg; Magnesium oxide, IP - 0.15 mg.

Tagging of Prawn

Six plastic tags (laboratory made) were prepared in different shapes and these tags were tied to the tail of the prawns in each pool. After molting, tags were collected along with the molt. Through this technique, we could easily identify the prawn that had molted and the tag had been tied again to the same prawn to know successive molting in each prawn.

Vitamin D_3 Injection

Vitamin D_3 (300,000 IU, Arachitol, Mfg. No. G/2191A, Batch No. W/1416, manufactured in August 2000, expiry date April 2003) was procured from Duphar-Interfran Ltd., Vapi. It was diluted with Arachis Oil (Duphar-Interfran Ltd., Vapi) to the desired concentrations, *i.e.*, 100, 500 and 2000 IU. Intra-muscular dose of vitamin D_3 was given to the prawns at the concentration of 100, 500 and 2000 IU/kg body weight on the 1^{st}, 3^{rd}, 5^{th}, 7^{th}, 9^{th}, 11^{th}, 13^{th}, 15^{th}, 20^{th}, 25^{th} and 30^{th} days. The injections were given on alternate days and/or after four days at later stage, to maintain the levels of vitamin D_3 in the body at the physiological levels so that the triggering of molting and growth, if occurs, should not pass through lag phases. The three concentrations of vitamin D_3 were selected to observe the dose-dependent effect on molting and growth.

Vitamin D_3 injection was carried out using insulin syringes (1 ml, B. Braun Medicals, Switzerland) with a maximum liquid quantity of 0.2 ml. The control animals were injected with 0.2 ml of the Arachis Oil to maintain similar conditions at par with the experimental ones except the dose of vitamin D_3. A separate syringe was used for each injection to avoid infection. The antibiotic, ciprofloxacin and the antifungal solution miconazole nitrate (Ranbaxy Laboratories Ltd, Mumbai) were applied in the water to prevent bacterial and fungal infections following standard protocols. Different doses of injections were administered to deliver vitamin D_3 to estimate the physiological doses enough to trigger the molting in the prawn and it was very clear that dietary dose also could activate the process. But, understanding the effect of physiological dose was the motive behind taking up this piece of work.

Sample Analysis

Tissue samples were taken in the beginning and end of the experiment. Molt samples were collected at regular intervals. Ash content was determined from dry weighed sample in a Thiasil crucible placed in muffle furnace at 600°C for six hours. Tissue and molt calcium level was estimated by the method of Trinder (1960), and

inorganic phosphate (P_i) by the method of Fiske and SubbaRow (1925).

Water Quality Parameters

Water quality parameters such as water temperature, pH, dissolved oxygen, free carbon dioxide and total alkalinity were recorded at 5-day intervals following AOAC (1984) and APHA (1985) methods.

Calculations

1. Per day increment, g/d: (final weight – initial weight)/experimental period
2. Percentage weight gain, %/d: (final weight – initial weight)/initial weight x 100
3. Specific growth rate (SGR), %/d: (final weight – initial weight)/experimental period x 100
4. Absolute growth, g: final weight – initial weight
5. Relative growth rate, %/d: absolute growth/initial weight (RGR) x 100
6. Feed conversion ratio (FCR): dry feed intake/wet weight gain

Statistical Analysis

Data were processed for the analysis of variance (ANOVA) as per Snedecor and Cochran (1961). The least significant difference was used to compare the individual treatment means.

RESULTS AND DISCUSSION

Results on the intra-muscular dose of vitamin D_3 on growth parameters (per day increment and SGR and of *M. rosenbergii* showed significant ($p<0.05$) increase in 500 IU dose. However, the growth parameters decreased at 2000 IU (Table 2).

Table 2: Results of feeding trial of prawns after injecting vitamin D_3 at four different doses

Parameter	Treatment			
	T_1	T_2	T_3	T_4
Increment (g/d)	$0.05 \pm 0.01^*$	$0.04 \pm 0.01^*$	0.07 ± 0.02^a	$0.04 \pm 0.01^*$
Weight gain (%)	$19.31 \pm 7.43^*$	$17.57 \pm 2.73^*$	27.00 ± 7.95^a	$17.40 \pm 4.09^*$
Absolute growth (g)	$1.67 \pm 0.34^*$	$1.47 \pm 0.31^*$	2.21 ± 0.52^a	$1.35 \pm 0.51^*$
RGR (%)	$19.42 \pm 7.49^*$	$17.57 \pm 2.73^*$	27.00 ± 7.96^a	$16.00 \pm 4.44^*$
SGR (g)	$0.54 \pm 0.21^*$	$0.55 \pm 0.08^*$	0.80 ± 0.21^a	$0.55 \pm 0.12^*$
FCR	$2.10 \pm 0.14^*$	$2.00 \pm 0.14^*$	$2.10 \pm 0.11^*$	$2.20 \pm 0.20^*$
Survival (%)	$72.21 \pm 8.61^*$	$74.99 \pm 8.61^*$	$77.77 \pm 8.61^*$	58.33 ± 9.13^b

T_1 - Control
T_2 - 100 IU kg^{-1} body weight
T_3 - 500 IU kg^{-1} body weight
T_4 - 2000 IU kg^{-1} body weight

* = Not significant at 5%
a = $p<0.05$
b = $p<0.01$

Superscripts in a row with different alphabets/asterisk indicate significance difference ($p<0.05$, $p<0.01$)

The ash (F = 3.1115, 131.5758 in tissue and molt), calcium (F = 46.0037, 113.8723 in carcass and molt) and P_i (F = 50.60613, 4127.5458 in carcass and molt) contents consistently increased with increasing vitamin D_3 concentration (Table 3). The number of molts in each experimental group in 30 days is also shown in Table 3. The maximum number of molts was found in T_3 (51.0 ± 1.87) treatment and the least in control (20.0 ± 0.5). There was no effect of Arachis oil on molting and even if there were any effects, those were in all the animals whether control or test. The water quality parameters are shown in Table 4.

Table 3: Calcium and inorganic phosphate content in tissue and molt, and the number of molts in each treatment*

Parameter	Treatment			
	T_1	T_2	T_3	T_4
Carcass ash (%)	1.48 ± 0.14	1.62 ± 0.17*	1.82 ± 0.12[a]	1.74 ± 0.31[a]
Molt ash (%)	6.49 ± 0.23	6.80 ± 0.41*	8.79 ± 0.08[a]	8.60 ± 0.17[a]
Tissue calcium (mg/g ash)	59.80 ± 2.96	73.88 ± 4.33[a]	82.37 ± 2.02[b]	73.35 ± 3.73[a]
Molt calcium (mg/g ash)	31.52 ± 0.81	41.85 ± 0.97[a]	45.38 ± 2.05[a]	43.77 ± 1.55[a]
Tissue inorganic phosphate (mg/g ash)	25.14 ± 0.65	32.14 ± 0.98[a]	36.35 ± 2.68[a]	41.07 ± 3.61[b]
Molt inorganic phosphate (mg/g ash)	12.05 ± 0.60	17.85 ± 0.65[a]	22.14 ± 1.36[b]	22.72 ± 1.40[b]
Total molts (no.)	20.00 ± 0.50	29.00 ± 1.16[a]	51.00 ± 1.87[b]	30.00 ± 1.60[a]

*Please refer Table 2 for legends.

Table 4: Average physico-chemical parameters of the water during the experiment

Day	Mean water temperature (°C)	Mean water pH	Mean dissolved oxygen (mg/l)	Mean free CO_2 (mg/l)	Mean total alkalinity (mg/l)
0	23.0	8.3	6.9	Nil	241
5	25.6	8.2	6.0	Nil	233
10	22.6	8.3	5.6	Nil	219
15	21.4	8.0	6.0	Nil	223
20	20.8	8.3	6.7	Nil	228
25	20.6	8.4	6.8	Nil	226
30	20.1	8.3	6.6	Nil	233

Vitamin D_3 at dose of 500 IU/kg body weight showed a consistent increase in per day increment and SGR in comparison to the control. The present investigation shows a similarity with the findings of Wheatly (1996). Lower survival was recorded in 2000 IU/kg dose, which may be due to the physiological stress associated with the overdose of vitamin D_3.

Calcium is important for growth, reproduction and many other physiological processes such as muscle contraction, nerve signal transduction, control of membrane permeability and cellular metabolism in vertebrates (Pang *et al.*, 1971). Invertebrates are faced with a number of dilemmas with respect to the regulation of calcium due to the presence of a calcium-rich exoskeleton (molt). They require supply of calcium to maintain calcified cell such as the shells of molluscs and the cuticle of crustaceans. Crustaceans undergo molting cycle in order to grow in size. This necessitates huge supplies of calcium. Moreover, there are scanty reports available on the tissue composition of crustaceans on the administration of physiological dose of vitamin D_3 (Chen and Li, 1995; Reddy *et al.*, 1999)

The target organs for calcium regulation are gills and carapace. Crustaceans have different calcium demands that change depending on the stage of molting. During intermolt, calcium concentration in the extra-cellular fluid is kept constant above 100 m*M* (Wheatly, 1996). The calcium and P_i content showed a dose-dependent increase up to 500 IU/kg treatment in carcass and molt. However, 2000 IU/kg dose showed either the same or nearer values of calcium, P_i and ash as those for 500 IU/kg dose. The values of calcium, P_i and ash in both tissue and molt were higher than those of control. In freshwater prawns, as in other crustaceans, calcium supply is largely from the mobilization of calcium from the exoskeleton (Wheatly and Gannon, 1995).

Wheatly (1996) reported that as the freshwater prawn approaches molt and enters the pre-molt stage, blood calcium level increases. This calcium is liberated from the old cuticle and can be either stored or excreted. The present study indicates that probably hypercalcemic and hyperphosphatemic effects of vitamin D_3 might have caused the increase in calcium and phosphate levels in carcass and molt. Thus, our present result shows similarity to that of Wheatly (1996).

In the case of marine crustaceans (where the availability of calcium is abundant), the animals are under less pressure of calcium scarcity. However, freshwater prawns are under pressure to conserve calcium, since their environment has a lower calcium level. Consequently, on applying the physiological dose, the growth of the animal also improved besides increases in the concentrations of calcium and P_i. Intra-muscular dose of vitamin D_3 increased the serum calcium and phosphate levels of freshwater catfish, *Heteropneustes fossilis* (Srivastav *et al.*, 1997). Avila *et al.* (1999) demonstrated that when fish are fed with phosphate-sufficient diet, dietary cholecalciferol increases the plasma P_i concentration, but decreases liver weight. The present investigation on the physiological dose of vitamin D_3 in *M.*

rosenbergii is a new mode of supplementation of vitamin D_3, especially in the case of grow-out and berried prawns, where individual care is a pre-requisite for reducing the cannibalistic behaviour. This is based on the hypothesis that the ratio and turnover of calcium and phosphorus are important during the molting phenomenon, as vitamin D_3 might have acted upon the epithelial cells of the intestine, which would have increased the absorption of calcium from gills and cuticle to synthesize molt. In the grow-out system, it is not practically feasible to inject vitamin D_3 and therefore, this piece of research may have indirect relation to boost aquaculture by adding vitamin D_3 through diet after understanding the action of the physiological dose on increasing molting cycle in *M. rosenbergii*. Similar practices can be adopted and followed for dietary addition of vitamin D_3 for penaeid shrimps. However, this method is not feasible to larval or post-larval stages. The present investigation indicated that vitamin D_3 affects the calcium and P_i system in tissue, molt composition, survival and growth. It also suggests a new direction of research dealing with calcium homeostasis/ endocrinological aspects with reference to cultivable species of crustaceans to increase aquaculture production.

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