Online Journal of Animal and Feed Research

Volume 2, Issue 2: 162-165 (2012) *ISSN 2228-7701*

VITAMIN D_3 INDUCED HYPERCALCEMIC RESPONSE IN THREATENED BRONZE FEATHER BACK (*Notopterus notopterus,* PALLAS)

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ABSTRACT: *Vitamin D_3 (0.0 IU.100 g body weight (BW)$^{-1}$.day^{-1}, 100 IU.100 g BW^{-1}.day^{-1}, 500 IU.100 g BW^{-1}.day^{-1} and 1000 IU.100 g BW^{-1}.day^{-1}) was administered intra-peritoneally (ip) to the freshwater threatened Bronze Featherback, Notopterus notopterus kept in freshwater for 9 days. Analyses of serum calcium levels were performed at 0, 6 hr. and 1, 2, 3, 5 and 9 days (four grow-out Notopterus notopterus from each group of ip doses at each interval). Administration of vitamin D_3 elevated the maximum serum calcium elevation occurred at day 2 freshwater in 500 IU.100 g BW^{-1}.day^{-1} (11.2±0.92 mg.dL^{-1}) and in 1000 IU.100 g BW^{-1}.day^{-1} (12.0±0.46 mg.dL^{-1}) of the fish maintained in the fresh water. There was gradual decrease in calcium levels from day 3 and became normocalcemia on day 9. Out of the three concentrations of ip Vitamin D_3 (100 IU.100 g BW^{-1}.day^{-1}, 500 IU.100 g BW^{-1}.day^{-1} and 1000 IU.100 g BW^{-1}.day^{-1}) the sharp elevation of serum calcium recorded in both 500 IU.100 g BW^{-1}.day^{-1} and 1000 IU.100 g BW^{-1}.day^{-1}. The control (0.0 IU.100 g BW^{-1}.day^{-1}) fish serum calcium behaves like normocalcemia (8.25±0.21 mg.dL^{-1}) in every sampling up to day 2. Results demonstrated that ip Vitamin D_3 exerted a dose-dependent and pronounced hypercalcemic effect in freshwater threatened Bronze Featherback, Notopterus notopterus.*

ORIGINAL ARTICLE

Key words: *Notopterus notopterus*, *Threatened fish, Vitamin D_3, Hypercalcemia*

INTRODUCTION

The physiological and cellular impact of vitamin D_3 and its metabolites have been recently under studies in lower vertebrates including fish. Numerous studies were made to study the physiological role of vitamin D_3 in teleostean fishes (Avioli et al., 1981; Marcocci et al., 1982; Hayes et al., 1986; Takeuchi et al., 1986, 1987; Rao and Raghuramulu, 1995) and changes in the blood calcium and phosphate contents of fish after administration of vitamin D_3 and/or its metabolites (Swarup and Srivastav, 1982; Srivastav, 1983; Swarup et al., 1984; Fenwick et al., 1984; Srivastav and Srivastav, 1988; Srivastav and Singh, 1992; Sundell et al., 1993; Fenwick et al., 1994). Teleost bone may or may not contain osteocytes and has been considered by some investigators to be metabolically inert and unable to contribute to calcium homeostasis. On this basis, we designed the present experiment to determine whether vitamin D_3 affects the serum calcium concentration of the featherback, *Notopterus notopterus,* when the external sources of calcium (environmental and dietary) are eliminated. For comparison, the effect of vitamin D_3 was also tested in this fish in control and experimental conditions.

MATERIALS AND METHODS

A total of 112 adult specimens of *Notopterus notopterus* of both sexes weighing 180-230 g were collected locally during the resting phase and acclimated to the laboratory under conditions of natural photoperiod and temperature (25± 2°C) for two weeks in plastic pools (300L). The fish were fed live feed during acclimatization. For the experiments, the fishes were kept in identical plastic pool. After acclimatization, the fishes were divided into four groups of 24 animals each and submitted to the following treatments:

Group 1: Injected *ip* with vehicle (0.1 ml Arachis oil 100 g BW^{-1} day^{-1}) and kept in freshwater;
Group 2: Injected *ip* with 100 IU of vitamin D_3 100 g BW^{-1} day^{-1} and kept freshwater;
Group 3: Injected *ip* with 500 IU of vitamin D_3 100 g BW^{-1} day^{-1}) and kept in freshwater;
Group 4: Injected *ip* with 1000 IU of vitamin D_3 100 g BW-I day-I and kept in freshwater.

To cite this paper: Srivastava SM, Srivastava PP, Dayal R, Chowdhary S, Lakra WS, Singh SP, Pandey AK. 2012. Vitamin d_3 induced hypercalcemic response in threatened bronze feather back (*Notopterus notopterus,* Pallas). *Online J. Anim. Feed Res.*, 2(2): 162-165.
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Vitamin D_3 (Arachitol, duphar - Interfran), administered to groups 2, 3 and 4 was dissolved in Arachis oil. The fish were not fed 24 h before and during the experiment. Blood samples were taken by sectioning the caudal peduncle 4 h after the injection on days 0, 6 hr, 1, 2, 3, 5 and 9 after treatment. The sera were separated and analyzed for calcium level according to the method of Trinder (1960). Data are reported for four specimens and the DMRT was used to determine statistical significance.

RESULTS

Group -1

The serum calcium levels exhibited almost no change throughout the experiment (Table 1, Figure 1). No change was observed in serum calcium level on day 0.0, 6 hr, day 1 following vitamin D_3 treatment. After 3rd day the insignificant increase was recorded on day 5 and 9 ($P>0.05$).

Group -2

The serum calcium levels of vitamin D_3 (100 IU of vitamin D_3 100 g BW^{-1} . day^{-1}) decreased progressively from day 1 to day 2 (Table 1, Figure 1), and decrease thereafter from day 2 to the end of the experiment. The serum calcium level decrease progressively from day 2 until day 9 ($P>0.05$).

Group -3

The serum calcium level of vitamin D_3 (500 IU of vitamin D_3 100 g . BW^{-1} day^{-1}) was moderately increased on day 1, and progressively increases till day 2 (Figure 1). The level decreased progressively from day 2 to day 9. The levels exhibited a significant increase from the control and remain above than the control until day 9 (Table 1, Figure 1).

Group -4

The serum calcium level of vitamin D_3 (1000 IU of vitamin D_3 100 g BW^{-1} . day^{-1}) was highly elevated on day 2 increased on day 1, and progressively increase till day 2 (Figure 1). The level decreased progressively from day 2 to day 9. The levels exhibited a significant increase from the 100 IU and 500 IU treatment and remain above than the control until day 9 (Table 1, Figure 1).

Table 1 - Serum calcium level in *Notopterus notopterus*

Concentrations of calcium	Time					
	6hr	1 day	2 day	3 day	5 day	9 day
Control	8.1±0.11	8.2±0.21	8.2±0.21^a	8.4±0.18^b	8.4±0.43^b	8.6±0.22^b
100 IU	9.3±0.08	9.6±0.23	10±0.14^b	9.8±0.17^b	9.3±0.19^b	8.6±0.10^a
500 IU	9.3±0.07	9.7±0.21	11.2±0.92^b	10.1±0.56^b	9.8±0.72^b	9.1±0.09^a
1000 IU	9.4±0.41	9.7±0.27	12±0.46^b	10.3±0.13^a	9.8±0.17^a	9.6±0.23^a

Means in a row having the same letter superscript are not significantly different at ($p<0.05$) by ANOVA and Duncan multiple range test

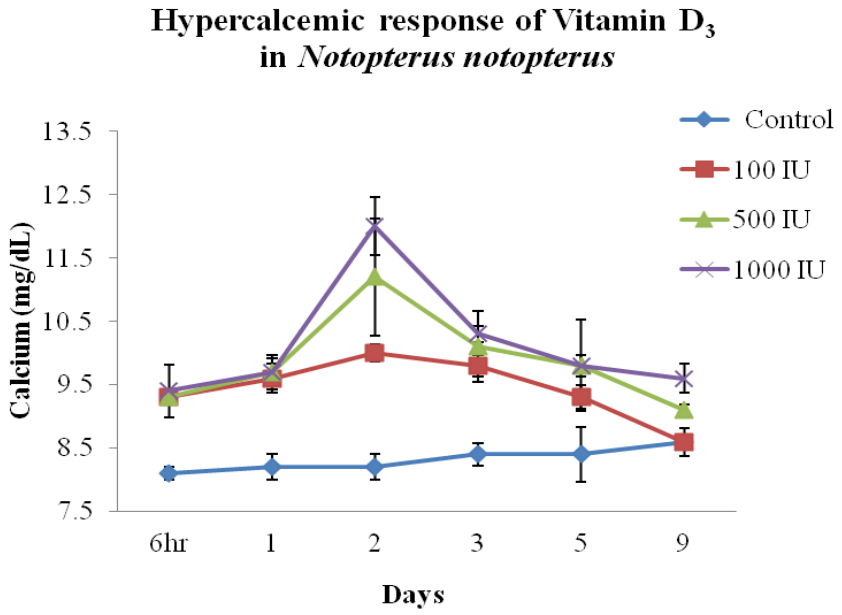

Figure 1 - Vitamin D_3 induced Hypercalcemic response in *Notopterus notopterus*

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DISCUSSION

In *N. notopterus* vitamin D_3 acted as an inducer of hypercalcemia when the fish were kept in freshwater. Earlier investigators working on sharks, rays and cyclostomes (Urist, 1962) and on lungfish (Urist et al., 1972) have reported that administration of vitamin D_3 fails to affect blood calcium contents. Lopez et al. (1977) injected 1,25 $(OH)_2D_3$ into *Anguilla anguilla* and found that the plasma calcium concentrations were not affected by the administration of the metabolite. Mac Intyre et al. (1976) noticed among eels treated with 1,25 $(OH)_2D_3$ but no change in calcium levels. The observed hypercalcemic effects of vitamin D_3 in *N. notopterus* are in good agreement with earlier reports of similar responses after vitamin D and/or maintenance of the fish in a freshwater environment (Swarup and Srivastav, 1982; Srivastav, 1983; Swarup et al., 1984; Fenwick et al., 1984; Srivastav and Srivastav, 1988; Srivastav and Singh, 1992). The present study also agrees with the reports of other investigators who have noticed hypercalcemia (Swarup et al., 1984; Fenwick et al., 1984; Srivastav and Srivastav, 1988) after administration of 1,25 $(OH)_2D_3$. A pronounced hypercalcemia has also been recorded after injecting the American eel *Anguilla rostrata* with calcium chloride solution (Fenwick et al., 1991). These studies support the hypercalcemia observed here in *N. notopterus* maintained in freshwater. In the present study vitamin D_3 treatment resulted in hypercalcemia a fact possibly explained by increased resorption of bone and/or mobilization of calcium from soft tissues.

There was a decline in the serum calcium level of *N. notopterus* maintained in freshwater. Wendelaar Bonga et al. (1984) also noticed significant hypocalcemia in tilapia after 5 days of transfer to a low-calcium environment, which they attributed to the increased efflux of this ion through the gill. The hypocalcemia observed in *N. notopterus* maintained in freshwater also confirms data reported by Wendelaar Bonga and van der Meij (1981) who noticed increased permeability at low-ambient Ca^{2+}. The low-ambient Ca^{2+} the increased water uptake may increase urine production which leads to extra Ca^{2+} loss from the body (Fenwick, 1981).

In D_3 injected *N. notopterus* kept freshwater, the serum calcium level was increased up to day 2 and was slightly reducing up to last day. This restoration of plasma calcium is most probably mediated by an enhanced production of prolactin, as previously suggested by Wendelaar Bonga et al. (1984). Flik et al. (1986) reported, prolactin stimulates Ca^{2+} uptake from the water in tilapia. In the present study, there was calcium available to the featherback from the surrounding medium; therefore, the restoration of calcium can be attributed to water source, bone demineralization and increased mobilization from soft tissues.

ACKNOWLEDGEMENTS

Authors are grateful to the Director, NBFGR, Lucknow and Principal, Government Post-Graduate College (Autonomous), Satna, Madhya Pradesh, India for providing working facilities.

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