



Phosphocalcemic responses of vitamin D₃ administered intact or hypophysectomized *Heteropneustes fossilis* maintained either in artificial freshwater or calcium deficient freshwater

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ABSTRACT

The serum calcium level of vehicle injected fish (group-A) exhibits no alteration throughout the experiment. Vitamin D₃ administration to fish *Heteropneustes fossilis* exhibited hypercalcemia from day 3 to day 7 (group-B). However hypophysectomized and vehicle injected fish showed hypocalcemia from day 3 to day 7 (group-C). While hypophysectomized and vitamin D₃ injected fish exhibited hypercalcemia on day 3 till the end of experiment (group -D). The serum phosphate of group A fishes is unaltered throughout experiment. The phosphate level of vitamin D₃ treated fish (group-B) exhibits hyperphosphatemia from day 3 to day 7. In group C fishes exhibited a significant decrease in phosphate level throughout the experiment. While the group D fishes showed hyperphosphatemia from day 3 to day 7. In group E fishes the serum calcium level remains unaffected up to day 3 the level decreases on day 5 to day 7. In group F fishes showed hypercalcemia from day 3 to day 7. In group H fishes a progressive decrease in serum calcium level is noticed on day 3 to day 7. The group H fishes shows increase in the serum calcium level from day 3 to day 7. The serum phosphate level of group E fishes showed a decrease from day 3 to day 5. The phosphate level increases from day 3 to day 7 in group F fishes. The phosphate level exhibited a decrease from day 3 to day 7 (group G). The phosphate level increases from day 5 to day 7 of group H fishes.

Keywords: Hypophysectomy, vitamin D₃, serum calcium, serum phosphate, *Heteropneustes fossilis*.

INTRODUCTION: A large fraction of calcium within cell is sequestered in mitochondria and endoplasmic reticulum. The intracellular free calcium concentrations fluctuate greatly, from roughly 100 nm to greater than 1 μm, due to release from cellular stores or influx from extra-cellular fluid. These fluctuations are integral to calcium's role in intracellular signaling, enzyme activation and muscle contraction. Roughly half of the calcium in blood is bound to proteins. A vast majority of body's calcium resides in bone. Within bone 99% of calcium is tied up in the mineral phase, and the remaining 1% can be rapidly exchanged. In teleosts about 99% of total calcium pool is incorporated into the skeleton and dermal scales as calcium phosphate and to a little extent as calcium carbonate (Flik *et al.*, 1986). Numerous studies on fish calcium physiology (Bonga and Pang, 1991; Flik and Verbost, 1993; Riccardi, 1999) have established that calcium, particularly the ionic calcium is a crucial factor in a wide variety of processes, including neuronal excitability, membrane permeability, muscle contraction, cell division, adhesion of one cell to another, hormone release and mineralization of bone tissue. In contrast to terrestrial vertebrates, fish have access to unlimited supply of calcium through external environment. Moreover, the skeleton and dermal scales, important for shape, armor, structure and muscle attachment, serve a role as reservoirs for calcium as well. The external calcium in fish is taken up via gill and the intestinal tract. Extracellular calcium concentrations are thus maintained at a relatively constant level and many endocrine systems play an important role in calcium metabolism throughout the vertebrate classes. In fish this regulation is accomplished by calcium regulating hormones acting upon target organs, thus affecting uptake, excretion and turnover of internal calcium stores.

The main role of Vitamin D is to provide the mineral balance. It

regulates the metabolism and absorption of minerals and determines serum calcium and phosphorus levels. In vertebrates, the vitamin D endocrine system is a major regulator of calcium and phosphate homeostasis (Norman *et al.*, 2002). Liver and fat stores large quantity of vitamin D in fishes. That is why fish are an important dietary source of vitamin D. Vitamin D is not synthesized by fish and are fully dependent on dietary source (Lock *et al.*, 2010). Fish absorb vitamin D directly from their diet (Dusso *et al.*, 2011). Uptill 1970s it was considered that the fish accumulate vitamin D but not metabolize vitamin D. But now it is clear that the fish have a vitamin D endocrine system with similar function like mammals (Lock *et al.*, 2010). 1, 25 (OH)₂ D₃ (calcitriol), an active metabolite of vitamin D₃ exerts its hypercalcemic effects through high affinity vitamin D₃ receptor (Lock *et al.*, 2010). Calcitriol plays a role in fish calcium metabolism by stimulation of intestinal calcium absorption and is a key factor in bone formation (Haga *et al.*, 2004); the effects of calcitriol can be considered hypercalcaemic in mammals and fish alike.

OBJECTIVES: The objective of the present study is to investigate the Phosphocalcemic responses of vitamin D₃ administered intact or hypophysectomized *Heteropneustes fossilis* maintained either in artificial freshwater or calcium deficient freshwater.

MATERIALS AND METHODS: **Area of study:** Adult specimens of *Heteropneustes fossilis* (bw 27-36g) were procured locally and acclimatized to the laboratory conditions for one week (temp 22-26°C) An initial sampling of blood was collected (from 10 fish) before the start of the experiment (zero hour). Then they were divided into following groups and given following treatments:

Group A: This group received daily intraperitoneal injection of vehicle (0.1 mL of 96% ethanol /100g bw) and kept in artificial

freshwater media.

Group B: Fish from this group were given a daily intraperitoneal injection of vitamin D₃ (100 ng/100g bw) and kept in artificial freshwater.

Group C: In this group hypophysectomized (HYPX) fish was given daily intraperitoneal injection of vehicle (0.1 mL of 96% ethanol /100g of bw) and maintained in artificial freshwater.

Group D: In this group the HYPX fish were given daily intraperitoneal injection of vitamin D₃ (100 ng/100g bw) and kept in artificial freshwater.

Group E: This group received daily intraperitoneal injection of vehicle (0.1 mL of 96% ethanol/100g bw) and kept in calcium-deficient freshwater.

Group F: Fish from this group were given daily intraperitoneal injection of Vitamin D₃ (100ng/100g bw) and kept in calcium-deficient freshwater.

Group G: In this group HYPX fish were given daily intraperitoneal injection of vehicle (0.1 mL of 96% ethanol/100g bw) and maintained in calcium- deficient freshwater.

Group H: In this group HYPX fish were given daily intraperitoneal injection of vitamin D₃ (100ng/100g bw) and kept in calcium- deficient freshwater.

Vitamin D₃ was dissolved in 96% ethanol. From each of above mentioned groups 10 fishes were anaesthetized with MS222 and blood samples were taken after 1, 3, 5 and 7 days following initiation of the treatments.

Statistical analysis: All data were presented as the mean \pm S.E of six specimens and Student's t test was used to determine statistical significance. In all cases the experimental group was compared to its specific time control group.

RESULTS: Serum calcium: The serum calcium level of vehicle injected fish (group A) exhibit no alteration throughout the experiment (figure 1). After day 1 following vitamin D₃ treatment (Group B) the serum calcium level remain unchanged. An increase in the serum calcium level has been recorded on day 3 which progresses till day 7 (figure 1).

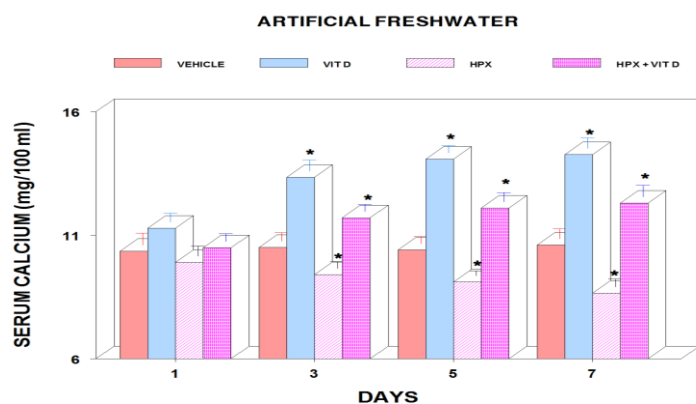


Figure 1: Changes in the serum calcium level of intact hypophysectomized *Heteropneustes fossilis* kept in artificial freshwater and treated with vehicle or Vitamin D. Each value represents mean \pm S.E of six specimens. Asterisk indicates significant differences (P<0.05) as compared with vehicle-injected specimen

In hypophysectomized and vehicle injected fish (group C) serum calcium level significantly decreases from day 3 to day 7

(figure 1). An increase in the serum calcium level of hypophysectomized and vitamin D₃ treated fish (group D) has been recorded on day 3 which persists till the end of the experiment (day 7) (figure 1).

Serum phosphate: Serum phosphate levels of fish injected with vehicle (group A) have not shown any change throughout the experiment (figure 2). The serum phosphate level of fish treated with vitamin D₃ (group B) remains unaltered on day1. The serum phosphate level fish of group B exhibits progressive increase from day 3 to 7 (figure 2). Hypophysectomized fish injected with vehicle (Group C) exhibit significant decrease in serum phosphate level from day 1 to day 7 (figure 2). In hypophysectomized fish treated with vitamin D₃ (group D) A significant increase has been recorded in serum phosphate level from day 3 to day 7 (as compared to group C) (figure 2).

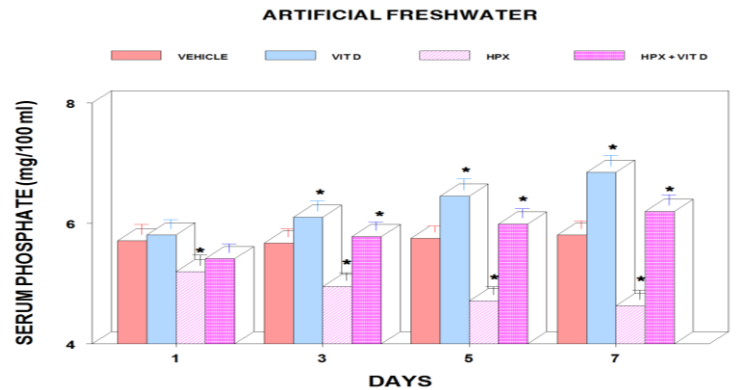


Figure 2: Changes in the serum phosphate level of intact hypophysectomized *Heteropneustes fossilis* kept in artificial freshwater and treated with vehicle or Vitamin D. Each value represents mean \pm S.E of six specimens. Asterisk indicates significant differences (P<0.05) as compared with vehicle-injected specimen.

Discussion: In the present study vitamin D₃ treatment provoked elevation in the serum calcium and phosphate levels of the fish kept in artificial freshwater. This study derives support from the reports of other investigators who have also noticed increased total calcium levels in fish treated with vitamin D₃/ vitamin D₃ metabolites (Lock *et al.*, 2010). Fenwick *et al.* (1984) injected *Pagothenia bernacchii* with 1, 25(OH)₂D₃ and reported reduced free plasma calcium, however, total plasma calcium remained unchanged. However, Sundell *et al.* (1993) treated Atlantic cod with 1, 25(OH)₂D₃ and recorded an increase in free calcium while total calcium levels were not affected. Increased total plasma calcium without any change in free calcium levels have been observed by Srivastav and Flik (1998) after injecting male Mozambique tilapia with 1,25(OH)₂D₃. In contrast, other investigators have reported that vitamin D₃ fails to affect blood calcium level in sharks, rays and cyclostomes and lungfish.

Srivastav *et al.* (1997) has suggested that the magnitude and duration of the increase of blood calcium in response to vitamin D₃/1,25(OH)₂D₃ treatment are dependent on the availability of calcium content in ambient water. Also, fish can supplement plasma calcium from internal sources if calcium is not sufficiently available from external sources (food/water). Physiological doses of vitamin D₃/1,25(OH)₂D₃ administered to unfed common carp provoked hypercalcemia and hyperphosphatemia which indicates that these electrolytes

must have been mobilized from internal sources. In contrast, vitamin D₃/1,25(OH)₂D₃ treatment to fed American eel (*Anguilla rostrata*) caused elevation in calcium and phosphate levels whereas in unfed eels this effect was absent (Fenwick *et al.*, 1984).

MacIntyre *et al.* (1976) observed hyperphosphatemia in eels treated with vitamin D₃ metabolite (1,25(OH)₂D₃) but no change in calcium levels. These authors have suggested that this metabolite mediates phosphate homeostasis in marine fish which live in the environment rich in calcium but poor in phosphorus. In calcium-deficient freshwater administration of vitamin D₃ to *H. fossilis* provoked hypercalcemia and hyperphosphatemia. The hypocalcemic response after vitamin D₃ treatment to the fish kept in calcium-deficient freshwater cannot be attributed to calcium absorption through intestine as the fish were not fed and surrounding medium lack calcium. In vehicle-injected fish *H. fossilis* kept in calcium-deficient freshwater the serum calcium and phosphate levels showed a decrease. This derives support from the studies of other investigators who have also reported hypocalcemia and/or hypophosphatemia in fish kept in calcium-free environment. Wendelaar Bonga *et al.* (1984) noticed a significant hypocalcemia in tilapia after 5 days of its transference to low-ambient calcium. This can be due to the increased efflux of these ions through gills. Flik *et al.* (1986) reported that calcium-free environment would allow diffusion of intracellular Ca⁺⁺ out of the fish. The observed hypocalcemia in vehicle-injected fish kept in calcium-deficient medium derives support from report of Wendelaar Bonga and Van der Meij (1981) who have reported increased integumental water permeability in low Ca⁺⁺ environment. Fenwick (1981) suggested that increased water uptake in low-calcium media may increase urine production which leads to calcium loss from the fish.

In the present study significant decrease in serum calcium and phosphate levels have been noticed in HYPX *H. fossilis*. Earlier, hypocalcemia has been reported after removal of pituitary. Prolactin in fish acts on the gills to stimulate the enzyme high affinity Ca⁺⁺ATPase (Flik *et al.*, 1986) which is considered to be the driving force for transmembrane calcium transport. In the foregoing study the observed hypocalcemia in hypophysectomized *H. fossilis* suggest the involvement of the pituitary in the uptake of calcium from the ambient water. This derives support from the studies of Flik *et al.* (1994) who have also reported that exogenous prolactin stimulates calcium uptake from the environment.

Conclusion: It has been concluded from the present study that vitamin D plays a major role in the regulation of serum calcium and phosphate levels in fish *Heteropneustes fossilis*.

CONFLICT OF INTEREST: Authors have no conflict of interest.

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