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# Plasma Calcium, Phosphate and Magnesium Levels of the Freshwater Male Stinging Catfish, *Heteropneustes fossilis* in Response to Hypophysectomy and Replacement Therapy with Prolactin

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**Abstract:** Hypophysectomy was performed on freshwater male stinging catfish, *Heteropneustes fossilis*. Prolactin (0.1 mg/100 g bw/day) was administered to the hypophysectomized fish for 10 days. The plasma calcium, phosphate and magnesium levels were determined on day 1, 3, 5 and 10. Hypophysectomy provoked hypocalcemia and hypophosphatemia whereas the plasma magnesium levels were not significantly altered. Administration of prolactin to hypophyesctomized fish elevated the levels of these electrolytes . These results indicate that prolactin may be involved in the regulation of calcium, phosphate and magnesium in fish.

**Keywords:** Hypophysectomy, Plasma calcium, Plasma phosphate, Plasma magnesium, Catfish

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## Introduction

Fish do not possess parathyroid glands and they depend on the pituitary hormone (s) as a source of hypercalcemic factor. Prolactin (PRL) is secreted by anterior pituitary hormone which exerts a broad range of functions in many vertebrates (Shu *et al.*, 2016). Pang *et al*. (1971) found that removal of pituitary gland in killifish (*Fundulus heteroclitus*) adapted to artificial calcium-deficient seawater elicited pronounced hypocalcemia and titanic seizures, while levels of other electrolytes remained unaffected. These symptoms were overcome by administration of pituitary homogenates or ovine prolactin, which led Pang *et al.* (1973) and Pang (1981) to suggest prolactin as the pituitary factor involved in fish calcium regulation. Further evidence regarding the involvement of prolactin in teleost calcium homeostasis has been provided by studies

which describe – (i) an inverse relationship between prolactin cells and plasma/ambient calcium concentration (Wendelaar Bonga and Van Der Meij, 1980; Wendelaar Bonga *et al.,* 1985; Srivastav, 1989; Srivastav and Singh, 1989), (ii) enhanced calcium uptake by chloride cells with altered high-affinity $Ca^{2+}$-ATPase enzyme activity (Flik *et al*., 1984, 1986) and (iii) hypercalcemic effects of administered prolactin in teleosts (Pang, 1981; Flik *et al.*, 1986, 1994; Hasegawa *et al*., 1986; Fargher and McKeown, 1989; Chakraborti and Mukherjee, 1995).

The available literature concerning the effects of hypophysectomy (HYPX) and prolactin (PRL) on phosphate and magnesium regulation in freshwater teleost is meager (Chan and Woo, 1978; Bjornsson and Hansson, 1983). Hence, the present study was undertaken to determine whether HYPX of a freshwater stinging catfish, *Heteropneustes fossilis* is effective to produce any changes on plasma phosphate and magnesium levels. Herein we also report the effect of administration of prolactin to HYPX *H. fossilis* on these electrolytes.

**Materials and Methods**

Adult male *Heteropneustes fossilis* (bw 32-45 g) were procured locally and acclimatized to the laboratory conditions (temp. 25-27 C, photoperiod 11.58-12.38) for a week in a plastic pool containing tap water ($Ca^{++}$ 0.2 mM). The water was changed daily and fish were fed on dried freshwater shrimp. Prior to the start of the experiment, an initial sampling of blood (zero hour) was taken from 5 specimens. Pituitaries were removed (HYPX) after anesthetizing the fish with MS 222. HYPX was performed by the method of Singh (1969). In sham-operated fish, all procedures were followed as for HYPX except that the pituitary was not removed. After HYPX and sham-operation, the fish were allowed to recover for 4 days. To assess the specific effects of HYPX the recovery period seems to be necessary to clear the endogenous hormone levels and also to overcome the shock of surgical procedure. During the recovery period (within 2 days) 14% of the HYPX fish showed erratic swimming movements. The other fish surviving the operation remained in healthy condition and were used for experiment.

After the recovery period four groups (A—D) were formed and were subjected to the following treatments for 10 days:

Group A: Sham-operated fish, served as controls for group B.

Groupo B: HYPX fish.

Group C: HYPX fish injected intraperitoneally (ip) daily with 0.1 ml/100 g bw of vehicle (0.6% NaCl) for 10 days. This group was employed as controls for group D.

Group D: HYPX fish injected ip daily with oPRL (0.1 mg/100 g bw; dissolved in 0.6% NaCl).

Fish were anesthetized with MS 222 and blood samples were collected from the caudal vein with heparinized syringes on day 1, 3, 5 and 10 after the start of the experiment (five fish from each group at every interval). Blood samples were obtained at the same time of the day to avoid any circadian variation. Plasma were separated by centrifugation and calcium (Calcium kit, Sigma), phosphate (Fiske and Subbarow, 1925) and magnesium(Phosphate kit, Sigma) levels were determined.

Completeness of HYPX was assessed in two ways –(a) by examining the pituitary at

the time of removal to check for intactness, and (b) by examining the subhypothalamic area at the time of sacrifice for pituitary remnants. Fish were not fed during the post-operative period. Student's t test was used for determining the statistical significance (group

B was compared with group A, and group D with group C). The data were also analyzed by ANOVA (two-way).

**Results**

In sham-operated (group A) fish the plasma calcium (Fig. 1), phosphate (Fig. 2) and magnesium (Fig. 3) levels remained unaltered throughout the experiment.

In HYPX (group B) fish the plasma calcium levels remained unaffected on day 1. From day 3 to day 5 the levels decreased progressively. Thereafter on day 10 it reached to almost normal level (Fig. 1). The plasma phosphate levels exhibited a significant decrease only on day 5 (Fig. 2). The plasma magnesium levels exhibited no significant change throughout the experiment (Fig. 3).

In group C (HYPX+ vehicle), the plasma calcium (Fig. 1) phosphate (Fig. 2) and magnesium (Fig. 3) levels exhibited almost the same pattern as observed for the fish from group B.

Administration of PRL to HYPX fish (group D) significantly elevated the plasma calcium (on day 5; Fig. 1), phosphate (on day 5 and day 10; Fig. 2) and magnesium (on day 5 and day 10; Fig. 3) levels as compared with group C (HYPX + vehicle).

Comparing (two-way ANOVA) plasma calcium, phosphate and magnesium levels of *H. fossilis* of different groups (A –D), it has been observed that these electrolytes differed significantly between the treatments (for calcium F=6.354, P<0.02; for phosphate F= 9.043, P< 0.01; and for magnesium F=4.263, P<0.05) whereas between the exposure period the differences were not significant (for calcium F=2.873, not significant; for phosphate F-1.933, not significant and for magnesium=1.48, not significant).

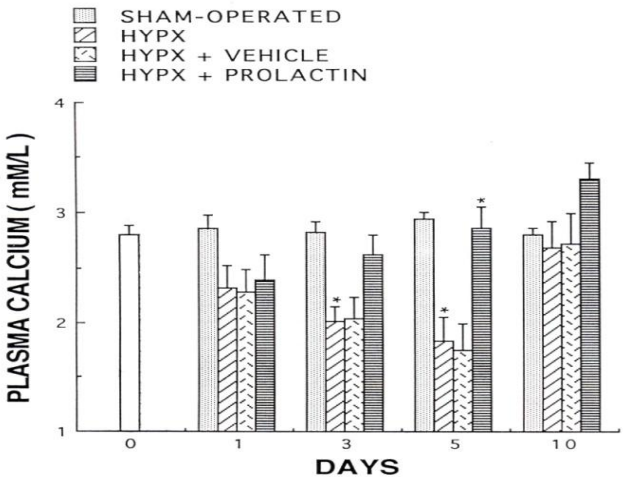


Fig. 1: Plasma calcium levels of sham-operated (group A), hypophysectomized (group B) or hypophysectomized *H. fossilis* treated with either vehicle (group C) or prolactin (group D). Each value represents mean ± SE of five specimens. Asterisks indicate significant differences (P<0.05) from controls (group B vs A and group D vs C).

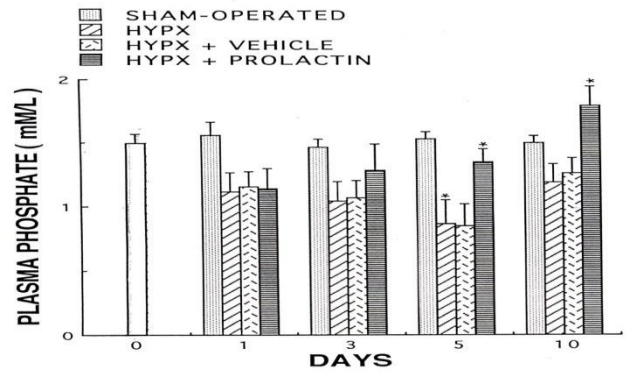


Fig. 2: Plasma phosphate levels of sham-operated (group A), hypophysectomized (group B) or hypophysectomized *H. fossilis* treated with either vehicle (group C) or prolactin (group D). Each value represents mean ± SE of five specimens. Asterisks indicate significant differences (P<0.05) from controls (group B vs A and group D vs C).

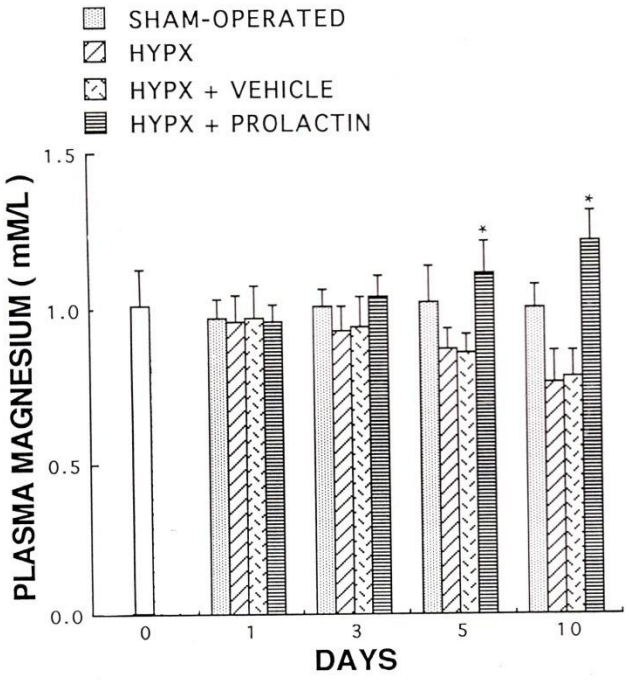


Fig. 3: Plasma magnesium levels of sham-operated (group A), hypophysectomized (group B) or hypophysectomized *H. fossilis* treated with either vehicle (group C) or prolactin (group D). Each value represents mean ± SE of five specimens. Asterisks indicate significant differences (P<0.05) from controls (group B vs A and group D vs C).

## Discussion

The present study indicates that HYPX of *H. fossilis* caused hypocalcemia which was completely overcome by the administration of PRL. This observation corroborates the findings of the earlier workers (Pang *et al*., 1978; Pang, 1981; Mugiya and Odawara, 1988). The hypercalcemic action of PRL has been confirmed for a variety of teleosts (Flik *et al.*, 1984, 1986, 1994; Hasegawa *et al.*, 1986; Fargher and McKeown, 1989; Chakraborti and Mukherjee, 1995) and non-piscine vertebrates (Srivastav and Rani, 1990, 1991; Srivastav and Yadav, 2008; Srivastav *et al*., 2009; Wongdee and Charoenphandhu, 2013). The observed hypocalcemia after HYPX of *H. fossilis* could be attributed to the possible loss of $Ca^{2+}$ via both the gills and the kidney. The restoration of plasma calcium levels in HYPX fish after PRL administration may be due to the utilization of environmental calcium by this fish and/or increased reabsorption of $Ca^{2+}$ by the kidney. Flik *et al.* (1994) have reported that exogenous PRL stimulates calcium uptake from the environment.

There exists no study regarding the role of PRL on phosphate homeostasis of fishes. Bjornsson and Hansson (1983) have reported that the pituitary gland plays a minimal role in regulation of plasma phosphate in *Salmo gairdneri*. Contrary to this, in the present study HYPX induced hypophosphatemia in *H. fossilis* which was corrected by administration of PRL, thus suggesting a role of pituitary/PRL in regulation of phosphate in this species. The observed hypophosphatemia in HYPX fish derives support from the studies of Srivastav and Rani (1990, 1991) who have reported hyperphosphatemia after PRL administration to frog and snake. Srivastav and Yadav (2008) have also noticed hyperphosphatemia after prolactin administration to pigeon. Flik *et al.* (1985) have reported that phosphate is absorbed exclusively via the gut. In the present study, the fish were not fed during the experiment, hence, the elevation of phosphate levels in HYPX fish after PRL administration could not be attributed to increased absorption of phosphate by gut or gill. The restoration of phosphate level after PRL treatment could be linked to the increased reabsorption of phosphate by the kidney and/or mobilization of phosphate from soft tissues.

To our knowledge the effect of HYPX on plasma magnesium level is restricted to one other study by Chan and Woo (1978). They have reported hypomagnesemia after HYPX in Japanese eel, *Anguilla japonica.* In contrast, no significant decrease in plasma magnesium

levels has been observed in *H. fossilis* after HYPX. However, an increased magnesium level has been noticed after PRL administration to HYPX *H. fossilis*, which could be linked possibly to the increased branchial and/or renal absorption of magnesium by PRL.

## References


Bjornsson B Th and Hansson T. (1983) effects of hypophysectomy on the plasma ionic and osmotic balance in rainbow trout, *Salmo gairdneri.* Gen. Comp. Endocrinol. 49: 240-247.

Chakrabarti P and Mukherjee D. (1995) Effects of prolactin and fish pituitary extract on plasma calcium levels in common carp, *Cyrinus carpio.* Gen. Comp. Endocrinol. 97: 320-326.

Chan DKO and Woo NYS. (1978) Effect of hypophysectomy on the chemical composition and intermediary metabolism on the Japanese eel, *Anguilla japonica*. Gen. Comp. Endocrinol. 35: 169-178.

Fargher RC and McKeown BA. (1989) The effect of prolactin on calcium homeostasis in coho salmon (*Oncorhynchus kisutch*). Gen. Comp. Endocrinol. 73: 298-303.

Fiske CH and Subbarow Y. (1925) The colorimetric determination of phosphorus. J. Biol. Chem. 66:375-400.

Flik G, Wendelaar Bonga SE and Fenwick JC. (1984) Calcium dependent phosphatase and calcium dependent ATPase activities in plasma membranes of the eel gill epithelium. III. Stimulation of branchial high affinity calcium ATPase activity during prolactin induced hypercalcemia in American eels. Comp. Bioche. Physiol. 79B: 521-524.

Flik G, Wendelaar Bonga SE, Kolar Z, Mayer-Gostan N and Fenwick JC. (1984) Effects of ovine prolactin on calcium uptake and distribution in *Oreochromis mossambicus*. Am. J. Physiol. 250: 161-166.

Flik G, Rentier-Delrue F and Wendelar Bonga SE. (1994) Calcitropic effects of recombinant prolactins in *Oreochromis mossambicus*. Am. J. Physiol. 266: 1302-1308.

Hasegawa S, Hirano T and Kawauchi H. (1986) Sodium-retaining activity of chum salmon prolactin in some euryhaline teleosts. Gen. Comp. Endocrinol. 63: 309-317.

MugiyaY and Odawara F (1988) effects of hypophysectomy and replacement therapy with ovine prolactin on serum calcium levels, and calcification in otoliths and scales in goldfish. Nippon Suisan Gakkaishi 54: 2079-2083.

Pang PKT. (1981) Hypercalcemic effects of ovine prolactin on intact killifish, *Fundulus heteroclitus* subjected to different environmental calcium challenges. Gen. Comp. Endocrinol. 42: 252-255.

Pang PKT, Griffith RW and Pickford GE. (1971) Hypocalcemia and titanic seizures in hypophysectomized killifish *Fundulus heteroclitus*. Proc. Soc. Exp. Biol. Med. 136: 85-87.

Pang PKT, Schreibman MP and Griffith RW. (1973) Pituitary regulation of serum calcium levels in killifish, *Fundulus heteroclitus* L. Gen. Comp. Endocrinol. 21: 536-542.

Pang PKT, Schreibman MP, Balbontin F and Pang RK. (1978) prolactin and pituitary control of calcium regulation in the killifish, *Fundulus heteroclitus*. Gen. Comp. Endocrinol. 36: 306-316.

Singh TP. (1969) Observations on the effects of gonadal and adrenal cortical steroids upon thyroid gland in hypophysectomized catfish, *Mystus vittatus* (Bloch). Gen. Comp. Endocrinol. 12: 550-560.

Shu Y, Qiyong L, Ziru D, Xiangyan D, Jiangyan H, Wei H and Zhan Y. (2016) The basal function of teleost prolactin as a key regulator on ion uptake identified with zebrafish knockout models. Sci. Rep. 6: 18597.
Srivastav AK. (1989) Effect of 1,25-dihydroxycholecalciferol administration on prolactin cells of the freshwater catfish, *Clarias batrachus*. Zool. Jb. Physiol. 93: 241-244.

Srivastav AK and Singh Purnima (1989) Responses of prolactin cells of the freshwater mud eel, *Amphipnous cuchia* to vitamin $D_3$ adminstration. Zool. Jb. Physiol. 93: 235-239.

Srivastav AK and Rani L. (1990) Prolactin induced hypercalcemia and hyperphosphatemia in the freshwater snake, *Natrix piscator*. Can. J. Zool. 68: 2702-2704.

Srivastav AK and Rani L. (1991) Response of ultimobranchial body, parathyroid gland, serum calcium and serum phosphate in the frog, *Rana tigrina* after prolactin administration. Eur. Arch. Biol. (Bruxelles) 102: 159-163.

Srivastav AK and Yadav Seema (2008) Prolactin effects on ultimobranchial gland and parathyroid gland in pigeon. North-Western Journal Zoology 4: 300-310.

Srivastav AK, Srivastava B, Mishra D, Srivastav SK and Suzuki N. (2009) Alterations in the ultimobranchial and parathyroid gland of the garden lizard, *Calotes versicolor* after prolactin administration, J. Biol. Res. Thessaloniki 12: 187–192.

Wendelaar Bonga SE and Van Der Meij MCA. (1980) The effects of ambient calcium on prolactin cell activity and plasma electrolytes *Sarotherodon mossambicus*. Gen. Comp. Endocrinol. 40: 391-401.

Wongdee K and Charoenphandhu N. (2013) Regulation of epithelial calcium transport by prolactin: From fish to mammals. Gen. Comp. Endocrinol. 181: 235-240.