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Effects of Sex Steroids (Testosterone and Estradiol) on Serum Calcium and Phosphate Levels of a Freshwater Male Teleost, *Heteropneustes fossilis*

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Abstract: Adult male specimens of *Heteropneustes foissilis* were injected daily intraperitoneally with vehicle (peanut oil) or testosterone (10µg/g body wt) or estradiol (10µg/g body wt) for 10 days. Fish were sacrificed on day 1, 3, 5 and 10 after initiation of the experiment and serum calcium and inorganic phosphate levels were analyzed. The serum calcium level of estradiol treated fish exhibits no change on day 1. The level increases progressively from day 3 to day 5. However, on day 10 the level slightly decreases as compared to day 5. Testosterone treatment caused no significant change in serum calcium level throughout the experiment. The serum phosphate level shows a significant increase following estradiol treatment from day 1 to day 5. On day 10 the level decreases slightly. Testosterone treated fish exhibits no change in serum phosphate level on day 1 and 3. On day 5 and 10 the level significantly increases.

Keywords: Testosterone, Estradiol, Calcium, Phosphate, *Heteropneustes fossilis*

Introduction

In fishes, ovarian maturation which is associated with an increase in blood calcium level has been worked out by many investigators (Hess *et al*., 1928; Pora, 1935, 1936; Fleming *et al*., 1964; Oguri and Takada, 1967; Woodhead, 1968; Fontaine *et al*., 1969; Scott *et al*., 1980; Bromage *et al*., 1982; Swarup *et al*., 1986; Singh and Srivastav, 1990). Some workers (Fleming *et al*., 1964; Oguri and Takada, 1966, 1967; Mugiya and Watabe, 1977; Pang and Balbontin, 1978) have reported that administration of estrogen to either female or male fish affects calcium regulation. In fish, mobilization of calcium from internal stores such as scales during sexual maturation has been reported (Pinto *et al.* 2008). Pinto *et al.* (2008) have suggested that the calcium mobilizing action of 17β-estradiol on fish scales is through its direct action on estrogen receptors in osteoclasts.

Rotlland *et al.* (2005) have reported that fish scales act as calcium stores and 1-34 PTHrP is involved in calcium mobilization from their scales. In male fish the relation between testicular maturation and plasma calcium is not very clear. Woodhead and Woodhead (1964) and Woodhead (1968) have reported a positive correlation, but in other studies (Fleming *et al*., 1964; Oguri and Takada, 1967; Singh and Srivastav, 1990) such a correlation is not evident. There is meager work concerning the effect of androgen injections on plasma calcium level. Balbontin *et al.* (1978) and Pang and Balbontin (1978) have suggested that androgen has no effect on plasma calcium level. However, Peterson and Shehadeh (1971) suggested that methyl testosterone increases plasma calcium level in male *Mugil cephalus*. Singh and Srivastav (1990) have reported that serum phosphate level increases corresponding to increased GSI in both male and female *Heteropneustes fossilis*.

The aim of the present work was to determine whether the sex steroids (testosterone and estradiol) have any effect on serum calcium and inorganic phosphate regulation of the freshwater male catfish, *Heteropneustes fossilis*.

Materials and Methods

130 adult live (male) specimens of freshwater catfish, *Heteropneustes fossilis* (body wt 60-80 g) were collected and acclimatized for two weeks under laboratory conditions. After acclimation the fish were divided into three groups (A, B, and C) and treated as follow:

Group A: Fish from this group served as control and were injected intraperitoneally (ip) with vehicle (peanut oil) daily for 10 days.

Group B: In these fish testosterone (10µg/g body wt) were ip administered daily for 10 days.

Group C: These fish were daily ip injected with estradiol (10µg/g body wt) for 10 days.

Blood sample from 10 specimens was taken prior to start of the experiment (zero hour). 10 fish from each group were anaesthetized with MS 222 and blood samples were taken by sectioning of the caudal peduncle 8h after last injection on day 1, 3, 5 and 10 following the treatment. The sera were separated and analyzed for calcium and inorganic phosphate levels according to Trinder (1960) and Fiske and Subbarow (1925) methods, respectively.

Fish were not fed during the experiment. Student's t-test was used to determine the statistical significance. The experimental group was compared with its specific time control group.

Results

The serum calcium level of estradiol treated specimens exhibit no change on day 1. The level increases significantly from day 3 to day 5 following the treatment. However, on day 10 the level slightly decreases as compared to day 5. Testosterone treatment caused no significant change in the serum calcium level throughout the experiment (Fig. 1). The serum phosphate level shows a significant increase after estradiol treatment from day 1 to day 5. On day 10 the phosphate level tends to decrease. Serum phosphate level of testosterone treated fish remains unaffected on day 1 and day 3. However, the level increases significantly on day 5 and day 10 (Fig. 2).

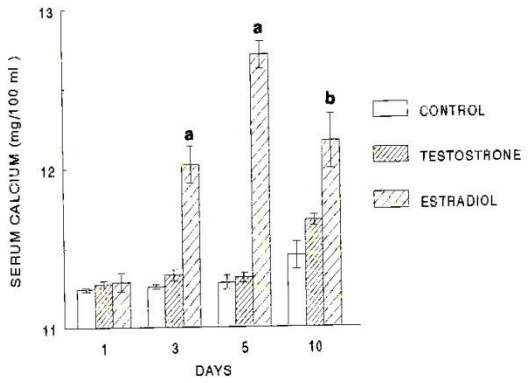

Fig. 1. Changes in serum calcium level of *Heteropneustes fossilis* treated either with testosterone or estradiol or vehicle. Each value represents mean ± SE of ten specimens. a and b represent significant responses: P<0.001 and P<0.005, respectively**.**

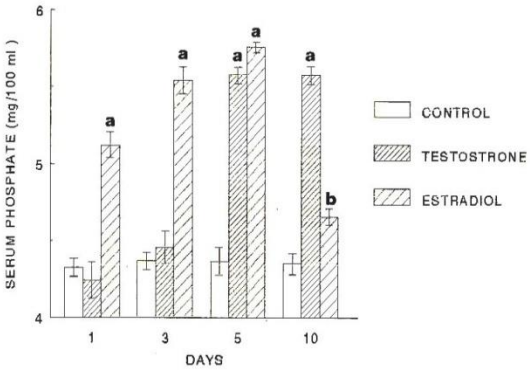

Fig. 2. Changes in serum phosphate level of *Heteropneustes fossilis* treated either with testosterone or estradiol or vehicle. Each value represents mean ± SE of ten specimens. a and b represent significant responses: P<0.001 and P<0.01, respectively.

Discussion

In the present investigation testosterone has no effect on serum calcium level of male *Heteropneustes fossilis*. This is in agreement with the earlier reports of Baily (1957) in male goldfish and Pang and Balbontin (1978) in killifish who have noticed no effect on blood calcium level after administration of testosterone propionate. Contrary to this, there exists a single report that androgen produces hypercalcemia in male *Mugil cephalus* (Peterson and Shehadeh, 1971). Woodhead and Woodhead (1964) and Woodhead (1968) have suggested a positive correlation between testicular maturation and blood calcium level. Such a correlation is not evident in other studies (Fleming *et al*., 1964; Oguri and Takada, 1967; Balbontin *et al*., 1978; Swarup *et al*., 1986; Singh and Srivastav, 1990). The serum phosphate level shows a distinct rise in the present study following testosterone treatment on day 5 and 10. In male *Heteropneustes fossilis* the serum phosphate level showed an increase in relatation to increased GSI (Singh and Srivastav, 1990). Balbontin *et al.* (1978) have also reported an increase in serum phosphate level of male killifish with increase in gonadal size.

In this study marked hypercalcemia has been noticed after estradiol treatment in male *H. fossilis*. This derives support from the observations of earlier investigators who have reported that injection of estradiol given to male fish - *Carassius auratus* (Bailey, 1957); *Fundulus* (Ho and Vanstone, 1961); *Gadus morhua* (Woodhead, 1969) and *Fundulus heteroclitus* (Pang and Balbontin, 1978) resulted in an increase in serum/plasma calcium level. Ovarian maturation is associated with the increase in serum/plsma calcium level (Woodhead and Woodhead, 1954; Fleming *et al*., 1964; Oguri and Takada, 1967; Woodhead, 1968; Fontaine *et al*., 1969; Balbontin *et al*., 1978; Bromage *et al*., 1982; Bjornsson *et al.,* 1986; Singh and Srivastav, 1990; Mugia and Hazama, 1994; Persson *et al*., 1994; Al-Jandal *et al.,* 2011; Srivastava, 2013; Srivastav *et al*., 2017)**.** AL-Jandal (2011) have reported that elevated level of circulating 17β-estradiol (E_2) may enhance Ca^{++} uptake via the gut. The increase

in serum calcium level in correlation with ovarian maturation can be attributed due to enhance estrogen secretion during sexual maturation of females. This derives support from other studies which describe that administration of estradiol increases the serum calcium level of female fish (Bailey, 1957; Ho and Vanstone, 1961; Fleming *et al*., 1964; Chan and Chester Jones, 1968; Woodhead, 1969). Bjornsson and Haux (1985) have reported that increase in total plasma calcium is due to vitellogenin which is calcium containing yolk precursor. According to them free plasma calcium level is not affected.

The observed hypercalcemia and hyperphosphatemia after estrogen treatment is in agreement with the suggestions of Pang and Balbontin (1978) who have correlated hypercalcemia in some sexually mature male fish with the presence of estrogen in them.

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