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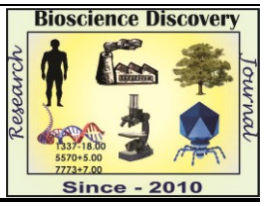

Influence of *β*- estradiol hormone and eyestalk ablation on Protein Metabolism in fresh water crab, *Barytelphusa cunicularis*

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Abstract

Crustacean reproduction is controlled by number of hormonal and neuronal factors. The present investigation has been undertaken to observe the influence of *β*- estradiol hormone and eyestalk ablation on the ovarian maturation in relation to protein changes in the ovary and hepatopancreas of fresh water crab, *Barytelphusa cunicularis.* Protein levels were estimated by Lowry *et al.,* (1951); Folin phenol reagent method and were calculated on dry weight basis and expressed as % mg. Base control crab ovaries showed (25.71%), experimental control (29.12 %), *β*- estradiol hormone injected (49.39 %) and eyestalk ablated crabs (53.58 %) protein levels. Whereas hepatopancreas showed (38.21 %), (36.11 %), (26.21 %) and (22.28 %) in base control, experimental control, *β*-estradiol hormone injected and eyestalk ablated crabs respectively. The studies showed that, administration of *β*- estradiol hormone indicated considerable changes in protein content in the ovary and hepatopancreas during ovarian maturation over base control and experimental control crabs. Moreover, Eyestalk ablated crabs showed significant increase in ovarian maturation over base control, experimental control and hormone treated crabs by elevating the mobilization of biochemical constituent(s) from hepatopancreas to the ovary indicating ovarian maturation in fresh water crab, *Barytelphusa cunicularis.* Such type of research will be helpful for understanding fundamentals of reproduction and enhancing crustacean aquaculture.

INTRODUCTION

Steroid hormones are biologically active in crustaceans present in the hepatopancreas, ovary and haemolymph which control vitellogenesis and are apparently necessary for both reproduction and moulting processes; the vital high energy demanding physiological process (Stevenson *et al*., 1979; Quackenbush, 2001; Lafont and Mathieu, 2007). Vitellogenesis is an important physiological process associated with female reproduction, because the primary source for the developing crustacean embryo is yolk protein and is a pivotal stage during crustacean reproduction. It is the synthesis of yolk proteins i.e. Vitellin (Vn) and Vitellogenin (Vg) which are the two main yolk proteins, that are important nutritive sources, which are necessary for the proper maturation and development of the oocytes (Tseng *et al*., 2001; Zapata *et al*., 2003).

In decapod crustaceans, hepatopancreas acts as center for storage and / or synthesis of biochemical material which is transferred to sites of gametogenesis for the purpose of growth, maintenance and reproduction (Adiyodi and Adiyodi, 1970). The mobilization and accumulation of protein, lipid and glycogen reserves in several tissues have been documented in several crustacean species (Khayat *et al.*, 1994; Harrison, 1997;

Palacios *et al.*, 2000; Tseng *et al.*, 2001; Thomas *et al*., 2005). Coordination of ovarian maturation and yolk biosynthesis is achieved through a complex interaction of many integrative factors mediated through the endocrine system. Role of some vertebrate-type steroids such as *β*- estradiol and progesterone in ovarian maturation have been reported by authors. Reddy *et al.* (2006) demonstrated that 17α- hydroxyprogesterone hormone induced ovarian growth and ovarian VTG synthesis in the fresh water crab, *Oziotelphusa senex senex*. Coccia *et al.* (2010) reported positive effect of estradiol & progesterone hormones on the reproduction of fresh water crayfish, *Cherax albidus*. Muhd-Farouk *et al.* (2014) studied effect of vertebrate steroid hormones on the ovarian maturation stages of orange mud crab, *Scylla olivacea* and found enhancement in the ovarian maturation. Sujathamma and Dayakar (2015) observed effect of estradiol and 17α-hydroxyprogesterone on ovarian development of fresh water paddy field crab, *Oziotelphusa senex senex* and found that both hormones were influencing the gonadal growth. Kale (2017) studied the effect of *β*- estradiol hormone and eyestalk ablation on ovarian maturation in fresh water crab, *B. cunicularis* and found significant ovarian maturation. The eyestalk seems to be a critical factor and has been known to control crustacean reproduction by a series of inhibitory neurosecretory factors which effectively targets the ovaries and hepatopancreas (Laufer *et al*., 1998; Aktas *et al*., 2003). Pervaiz and Sikdar (2014) studied the effect of bilateral eyestalk ablation on gonads in fresh water prawn, *Macrobrachium dayanum* and noticed eyestalk consists of inhibiting factor which on ablation enhances the gonadal development. Hussain *et al.* (2014) studied the effect of unilateral eyestalk ablation in fresh water prawn, *Macrobrachium lamarrei lamarrei* and noticed to induce gonadal development. Samyappan *et al.* (2015) studied impact of unilateral eyestalk ablation on lipid profiles in fresh water female crab, *Oziotelphusa senex senex* which showed a marked decrease in the hepatopancreas and a significant increase in ovarian tissue indicating ovarian maturation.

Hence in the present investigation comparative effect of *β*- estradiol hormone and eyestalk ablation was tested for the ovarian maturation in relation to synthesis and mobilization of protein from hepatopancreas to ovary.

MATERIALS AND METHODS

Barytelphusa cunicularis used in present investigation were collected from Godavari River near Kaigaon Toka, Newasa. Female crabs were selected and kept in the laboratory for acclimatization for 10 days in plastic troughs. Healthy well-adapted crabs of approximately same weights and size ranging between 35-40g body weights probably of intermoult stage were used in the experiments. Water in the troughs was changed daily and crabs fed by small pieces of earthworm and bivalve flesh on alternate days. Other parameters like temperature, pH, salinity, photoperiod etc. were maintained constant as far as possible.

The crabs were divided into four groups, as base control, experimental control, *β*- estradiol hormone injected and eyestalk ablated group, each containing 10 crabs. The final concentration of the hormone preparation was 1µl = 1µg. The hormone was injected into the abdominal musculature of arthropodial membrane through 3rd walking legs, receiving a dose of 20µl /crab with the help of hypodermic syringe having a 27-guaze needle.

Preparation of *β*-estradiol hormone injection:10mg of *β*-estradiol hormone (Sigma Chem., USA) was dissolved in 1ml of 1% ethanol and resulting solution diluted to 10ml by adding glass distill water. The final concentration of the hormone preparation was 1µl = 1µg. From this preparation hormone was injected to crab receiving a dose of 20µl hormone/crab.

Experimental design: Total 40 female crabs were selected and divided into four groups each containing 10 crabs. The first group was served as base control (normal) and crabs were sacrificed on o-day, second group as experimental injected by 1% ethanol, third group was injected by *β*-estradiol hormone with the help of hypodermic syringe having a 27-guaze-needle, hormone injections were administered through 3rd walking legs in the arthrodial membrane into the abdominal musculature female crabs receiving a dose of 20 µl /crab. Fourth group consisted of surgically eyestalk ablated crabs. The whole experiment was conducted for a period of 21 days and the crabs from second, third and fourth were sacrificed on 21st day of the experiment. Ovaries and hepatopancreas were dissected out for estimation of protein levels by Lowry *et al.,* (1951); Folin phenol reagent method. Protein levels were calculated on dry weight basis and expressed as % mg.

RESULTS AND DISCUSSION

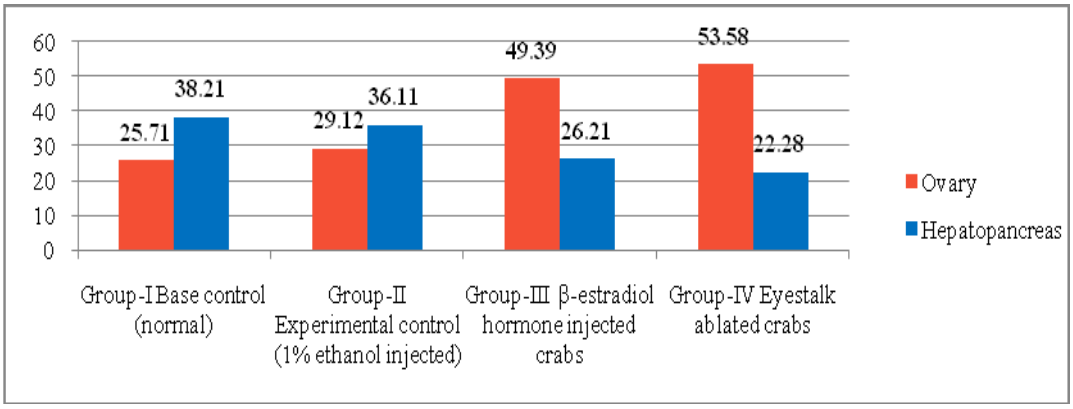
Biochemical studies are very essential from the nutritional point of view. The biochemical constituents in animals are known to vary with season, size of the animal, stage of maturity, temperature, and availability of food, and so forth; particularly crustacean shellfish are also good sources of various minerals and high quality protein (Sudhakar *et al*., 2009). Results shows that protein levels in ovary of *β*-estradiol hormone injected crabs was (49.39 ± 0.27)

Table-1: Showing levels of protein (% mg on dry weight basis) in ovary and hepatopancreas of different groups in fresh water crab, *B. cunicularis.*

Animal Category	Exp. Day	Tissue	Protein (%mg)
Group-I Base control (normal)	0-day	Ovary	25.71 ± 0.41
		Hepatopancreas	38.21 ± 0.12
Group-II Experimental control (1% ethanol injected)	21-day	Ovary	29.12 ± 0.14
		Hepatopancreas	36.11 ± 0.20
Group-III *β*-estradiol hormone injected crabs	21-day	Ovary	49.39 ± 0.27
		Hepatopancreas	26.21 ± 0.52
Group-IV Eyestalk ablated crabs	21-day	Ovary	53.58 ± 0.19
		Hepatopancreas	22.28 ± 0.45

± S. D.: Mean Standard Deviation

Fig.1: Comparative levels of protein (% mg on dry weight basis) in ovary and hepatopancreas of different groups in fresh water crab, *B. cunicularis.*



% mg which is more elevated than protein levels in ovary of base control – normal crabs (25.71 ± 0.41) % mg & experimental control - 1% ethanol injected crabs (29.12 ± 0.14) % mg. Whereas, eyestalk ablated crabs protein levels was found to be significantly increased (53.58 ± 0.19) % mg over all other groups crabs. On the other hand protein levels in the hepatopancreas of *β*-estradiol hormone injected crabs was (26.21 ± 0.52) % mg, protein levels in hepatopancreas of base control – normal crabs was (38.21 ± 0.12) % mg & experimental control - 1% ethanol injected crabs (36.11 ± 0.20) % mg. Whereas, eyestalk ablated crabs protein levels in hepatopancreas was found to be (22.28 ± 0.45) % mg (Table -1& Fig. -1). It indicates that *β*-estradiol hormone and eyestalk ablation caused more accumulation of proteins in ovary and decreased the levels of proteins in the hepatopancreas by mobilization. These findings are in accord with Rodriguez *et al. (*2002b) who noticed significant increase in the gonad somatic index and oocyte diameter in *Procambarus clarkii* by the administration of 17 *β*-estradiol and 17α-hydroxyprogesterone. Zapata *et al*. (2003) observed ovarian growth in the crab, *Chasmagnathus granulata* by the induction of 17α-hydroxyprogesterone and Juvenile hormone III. Reddy *et al.* (2006) demonstrated that 17α-hydroxyprogesterone hormone induced ovarian growth and ovarian VTG synthesis in the fresh water crab, *Oziotelphusa senex senex*.

Sujathamma and Dayakar (2015) noticed significant increase in total carbohydrates, glycogen and hemolymph sugar levels fresh water paddy field crab, *Oziotelphusa senex senex* by estradiol and 17α-hydroxyprogesterone hormones. suggesting ovarian development by stimulating the gonadal growth and reproduction. Muhd-Farouk *et al.* (2016) observed significant ovarian maturation in orange mud crab, *Scylla olivacea* by the administration of vertebrate steroid hormones.

Eyestalk ablation is expected to remove the source of vitellogenesis inhibiting hormone for the acceleration of ovarian maturation and regulation of molting. It also influence lipid metabolism, protein metabolism, carbohydrate metabolism, hydromineral regulation, gonad inhibition and limb growth (Charniaux-cotton and Payen, 1988; De Kleijn and Van Herp, 1995; Laufer *et al*., 1998; Tsukimura, 2001; Wilder *et al*., 2002; Longyant *et al*., 2003; Uawisetwathana *et al.,* 2011). In the present study decrease in protein levels were observed in hepatopancreas as an effect of eyestalk ablation. It does seem reasonable that decreases were a consequence of accelerated transport to maturing ovaries in which proteins and lipids are accumulated and hepatopancreas may be the source for these constituents circulated through the haemolymph (Kulkarni and Nagabhushanam, 1979; Teshima *et al*., 1988; Okumura and Aida, 2001). Similarly, Sudhakar *et al.* (2009) noticed increased protein, lipid and carbohydrate levels in ovary of unilateral eyestalk ablated crabs, *Portunus sanguinolentus.* Varalakshmi and Reddy (2010) observed increased carbohydrate and protein levels in ovary of fresh water prawn, *M. lanchesteri* due to eyestalk ablation. Wu *et al*. (2013) studied the effect of eyestalk ablation in crab, *Eriocheir sinensis* on physiological and biochemical metabolism and found to induce gonadal maturation. Pervaiz and Sikdar (2014) noticed incresead gonadal development of *Macrobrachium dayanum* due to bilateral eyestalk ablation. Samyappan *et al.* (2015) also found marked decrease in lipid content in the hepatopancreas and a significant increase in ovarian tissue due to unilateral eyestalk ablation in fresh water female crab, *Oziotelphusa senex senex* suggesting active ovarian maturation. However, in some studies lack of response to vertebrate-like steroid hormones in decapod crustacean's ovarian maturation were reported by Teresa *et al*., 2003; Okumura and Sakiyama, 2004; Kirubagaran *et al*., 2005; Gunamalai *et al*., 2006.

In conclusion the results of the present investigation and available literature indicates that *β*- estradiol hormone and eyestalk ablation found to be stimulating the ovarian maturation in fresh water female crab, *B. cunicularis* by increasing the protein levels in the ovary and decreasing in hepatopancreas by transporting the proteins from hepatopancreas to ovary. As compared to *β*-estradiol hormone and eyestalk ablation gives more reliable effects.

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