Journal of Environmental Biology
©Triveni Enterprises, Lucknow (India)
Free paper downloaded from: www.jeb.co.in

October 2007, 28(4) 771-778 (2007)
For personal use only
Commercial distribution of this copy is illegal

Effects of estradiol-17β and 17α, 20β-dihydroxy-4-pregnen-3-one on different phospholipids metabolism and histological changes in ovary during reproductive growth in the catfish, *Heteropneustes fossilis* (Bloch)

P. B. Singh*, Vandana Singh, Shailly Srivastava and S. Pandey
***pratap_b_singh@rediffmail.com**

Department of Zoology, Tilak Dhari (PG) College, Jaunpur-222 002, India

(Received: October 07, 2005 ; Revised received: May 20, 2006 ; Accepted: June 17, 2006)

Abstract: *Effect of cumulative doses of estradiol -17β (E_2- 7, 14 and 28 mg/kg body weight) and 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β P- 7, 14 and 28 mg/kg body weight) on total phospholipids (TP) and various phospholipids- phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidylethanolamine (PE) on liver, plasma and ovary were investigated during the reproductively active preparatory and prespawning phases of the annual reproductive cycle in the freshwater female catfish, H. fossilis. The effect of E_2 on TP was generally stimulatory and has pronounced effect than 17α,20β P during both the phases. The levels of PC was promoted high during prespawning phase by E_2 comparatively very less than by 17α,20β P in studied tissues during both the phases. The levels of PS after E_2 treatments was maximum in all tissues during prespawning phase whereas 17α,20β P was effective only in liver during this phase. The PI was elevated in liver during preparatory phase but its elevation was in all studied tissues during prespawning phase after E_2 treatments. The levels of PI was most effective in ovary during preparatory phase in response to 17α,20β P. The levels of PE was declined in liver but elevated in ovary after E_2 treatments during both the phases. Treatments of E_2 during preparatory phase showed greater number of vitellogenic oocytes as compared to 17α,20β P treatments. The present finding has demonstrated that estradiol-17β has more pronounced effects than the 17α,20β P in regulation of different phospholipids and ovarian recrudescence during reproductively active phases and among the phospholipids the PC is the main phospholipids of vitellogenin / ovarian lipids in H. fossilis.*

Key words: *Sex steroids, Progestagens, Phospholipids metabolism, H. fossilis*
PDF file of full length paper is available with author

Introduction

Earlier reports of De Vlaming *et al*. (1977) have indicated that the estradiol-17β therapy causes increase in ovarian size during prespawning phase. Seasonal changes in lipid and cholesterol (Singh and Singh, 1979; Lal and Singh, 1987b; Sehgal and Goswami, 2005; Rajendra Phartyal *et al.*, 2005) and seasonal correlative changes between lipid and sex steroids have been well documented (De Vlaming *et al*., 1984; Singh and Singh, 1990; Shankar and Kulkarni, 2006). Further report of Lal and Singh (1987a), has demonstrated that estradiol was found to be most potent in inducing and testosterone in suppressing the plasma levels in female catfish, *Clarias batrachus*. Wiegand and Peter (1980), have reported that estradiol-17β raised the levels of plasma lipid phosphorus.

Most previous studies were restricted to either normal profile of lipids or histological changes in gonads, brain tissues or membrane fluidity / permeability. No information is available for any teleost on the effect of estradiol-17β (a female sex steroid hormone-E_2) and 17α,20βP (a maturational inducing steroid hormones) on different phospholipids and histological changes in ovary during reproductive active phases (preparatory and prespawning) when the parameters of interest have maximum steroidogenic and lipogenic activity in the freshwater stinging catfish, *Heteropneustes fossilis* (Bloch). Therefore we explored the comparative effects of estradiol-17β and 17α,20βP on different phospholipids – phosphatidylcholine (PC), phosphatidyserine (PS), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and histological changes in ovary during ovarian recrudescence when the maximum mobilization of lipid takes place from liver to ovary via plasma under the influence of hormones.

Materials and Methods

Experimental fish: The original research reported herein was conducted under ethical guidelines for the treatment of animals in behavioral research and teaching (Anonymous, 1998) established for animal usage by Tilak Dhari College, Jaunpur (UP). Female catfish, *H. fossilis* (65-70 g and length 21-22 cm) were collected from a pond of the same brood stock during preparatory and prespawning phases and maintained in earthen pots supplied with a constant flow of dechlorinated tap water and enjoyed natural photoperiod and temperature during different phases. They were fed *ad libitum* with minced goat liver comprising 20% protein, 5% lipid, 15% carbohydrate, the remaining 60% being water, minerals and vitamins *etc.*

Chemicals: Analytical grade chemicals were obtained from BDH, (India). Thin layer chromatography (TLC) pre-coated plastic sheets (E. Merck Silica gel G_{60} F254. 20 cm x 20 cm x 0.20 mm) were obtained from BDH. Phospholipids standards were obtained from Sigma Chemical Co., UK. Estradiol-17β and 17α,20β-dihydroxy-4-pregnen-3-one were received as a gift from Professor Adelino V. M. Canario, Portugal.

Table - 1: Treatments of hormones during preparatory and prespawning phases

Batches	Hormone treatments
1	Control without any treatment
2	Control fish vehicle injected
3	Fish injected with estradiol-17β 1 µg/g body weight
4	Fish injected with estradiol-17β 2 µg/g body weight
5	Fish injected with estradiol-17β 4 µg/g body weight
6	Fish injected with 17α,20βP 1 µg/g body weight
7	Fish injected with 17α,20βP 2 µg/g body weight
8	Fish injected with 17α,20βP 4 µg/g body weight

After 10 days acclimatization following experiments were performed. The freshwater female catfishes were divided into 8 batches each comprising 5 fish in an earthen pot having 20 litre water in each at room temperature (Table 1).

All the injections in sesame oil medium were given intraperitoneally to the fish on alternate days for fifteen days during both the phases. Changes in various lipids were measured against total amount received (7, 14 and 28 mg/kg body weight) of each hormone. At the end of the experiment, fishes were bled by caudal incision and blood samples were collected in heparinized glass culture tubes. Plasma was separated by centrifugation at 5000 rpm at 4°C for 15 min and stored at -20°C until assayed for various phospholipids- PC, PS, PI and PE. Livers and ovaries were individually removed, washed in 0.6% saline, blotted and kept at -20°C for further analysis of phospholipids. A part of ovary from fish of each batch was fixed in Bouin's fluid for the histological examination for the purpose of maturation status. The sections were cut at 6-7 µm and stained with haematoxylin and eosin stain.

Tissue (25 mg) and plasma (100 µl) were extracted for total lipids (Folch *et al*., 1957) and then total phospholipids (TP) were separated from total lipids (Freeman and West, 1966). The TP was further separated into various lipids (Vitello and Zanetta, 1978) and quantified spectrophotometrically (Marzo *et al*., 1971).

Statistical analysis: Data were expressed in mg/g of tissues or mg/ml plasma. Values were expressed as mean ± SEM (n = 5). For statistical analysis of the data, analysis of variance and Student's 't' test at the probability level of 0.05 was employed (Bruning and Kintz, 1977).

Results and Discussion

Changes in phospholipids: Effect of estradiol-17β (E_2) and 17α,20β-dihydroxy-4-pregnen-3-one (17α,20βP) on the concentration of different phospholipids in liver, plasma and ovary during reproductively active preparatory phase and prespawning phases of the annual reproductive cycle in the freshwater female catfish, *H. fossilis* (Fig. 1-5).

Total phospholipids (TP): During preparatory phase, E_2 at 14 mg/kg body weight elevated TP levels in liver but decreased at its lower dose in plasma, while promoted in ovary at all tested dose of E_2. The TP was elevated in liver, plasma and ovary by E_2 treatments at all doses during prespawning phase. The levels of TP was decreased in liver and plasma but elevated in ovary in response to 17α,20βP treatments during preparatory phase. During prespawning phase, hepatic and ovarian levels were recorded high but declined in plasma in response to 17α,20βP treatments.

Phosphatidylcholine (PC): During preparatory phase, the PC was elevated in liver and ovary in response to E_2 treatment at 14 and 28 mg/kg bw but declined at 7 mg/kg body weight. The prespawning phase showed the trend of elevation of PC in all studied tissues after E_2 treatments. The level of PC was declined in liver and plasma but elevated in ovary in response to 17α, 20βP treatments during preparatory and prespawning phases (except dose level 14 mg/kg body weight during prespawning phase which showed promoted levels of PC).

Phophatidylserine (PS): The level of PS was increased at 14 and 28 mg/kg bw of E_2 in liver and ovary during preparatory phase. During prespawning phase, the PS level was increased in liver and ovary during both the phases while elevated in liver, plasma and ovary in response to E_2 treatments. The ovarian PS was recorded high during preparatory phase while the hepatic and ovarian concentration of PS was also promoted during prespawning phase after 17α, 20βP treatments.

Phophatidylinositol (PI): During preparatory phase**, t**he level of PI was promoted in liver at all doses of E_2 while their elevations were recorded in liver and ovary during prespawning phase. The plasma levels of PI showed low levels in response to E_2 during this phase. The ovarian levels of PI was enhanced at all tested doses of 17α, 20βP during preparatory phase but its elevation was noticed in liver and plasma during prespawning phase in response to 17α, 20βP treatments.

Phosphatidyl ethanolamine (PE): During preparatory phase, plasma and ovarian levels of PE was elevated but declined in liver whereas during prespawning phase was elevated in liver and ovary in response to E_2 treatments. During preparatory phase, the elevated levels of ovarian PE were recorded high but during prespawning phase, promoted levels of PE in liver and ovary were noticed in response to 17α, 20βP treatments.

Histological examination of ovary:

The treatments with estradiol-17β during preparatory phase indicated greater number of vitellogenic oocytes when compared to 17α, 20βP treatments.

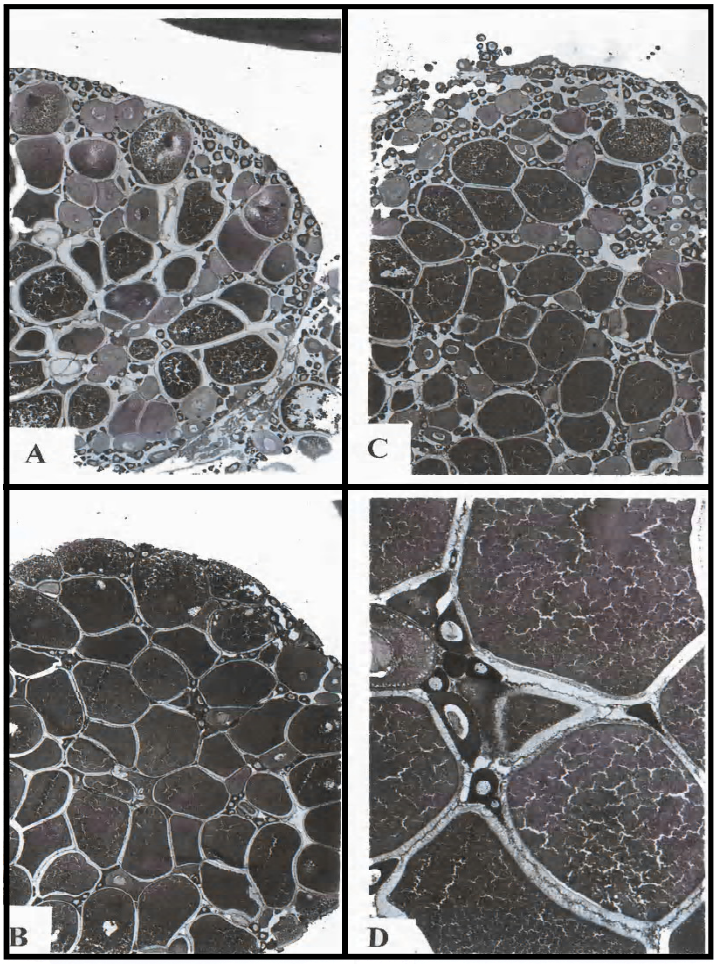

Fig. 1: Showing maximum number of vitellogenic oocytes in the ovaries by estradiol-17β comparative to 17α, 20βP treatments during preparatory phase in *H. fossilis*. A = Control x 60, B = Estradiol-17β x 60, C = 17α, 20βP x 60, D = Vitellogenic follicles after estradiol-17β treatments magnified of B x 240

It has been well established that polar lipids (PC, PS, PI and PE) are a major component of biomembranes. In keeping with other poikilothermic organism, freshwater fish alter the composition of their biomembrane lipids in response to changes in environmental temperature (Henderson and Tocher, 1987; Tocher, 2003). Reports are available that estradiol-17β levels increased lipids simultaneously with the appearance of yolky oocytes in the ovary of *Leptocottus armatus* (De Vlaming *et al*., 1984). Lal and Singh (1987a) have reported that estradiol-17β was most potent in inducing and testosterone in suppressing the plasma lipids in gonadectomized catfish, *C. batrachus*. These authors have indicated that progesterone could not affect plasma phospholipids in *C. batrachus*. Wiegand and Peter (1980) have reported the decreased levels of phospholipids and neutral lipid-triglycerides in sexually regressed *C. auratus*. Another report of Singh and Singh (1986), has demonstrated that the injection of *Mystus gonadotropin* elevated the levels of phospholipid in *H. fossilis*. In retrospect of the present findings it can be suggested that estradiol-17β enhanced the levels of different phospholipids by stimulating liver, which is ultimately deposited in growing oocytes during reproductively active phases. Our findings also indicate that the effect of estradiol-17β was more pronounced than the 17α,20βP during hepatic lipogenesis in this species during gonadal recrudescence.

Reports of Fremont and Riazi (1988), have indicated that the fish vitellogenin comprises 18% of total lipid of which 2/3 is phospholipid and the remaining fractions is largely triglycerides, sterols and sterol esters. The reports of Leslie and Buckley (1976) have shown that in the goldfish liver, PC was major constituent of total phospholipid, the rest being PE, PI and PS in decreasing order. In another report Singh and Singh (1991), have also indicated that oLH - RH and mGTH enhance the levels of total phospholipids in *H. fossilis*. In the present findings E_2 treatments indicated that liver is stimulated for the synthesis of TP, PC, PS, PI and PE during oogenesis required for vitellogenin as well as for membrane lipids. Reports of Sundararaj *et al*. (1982) have indicated that purified carp GtH acted as vitellogenic in hypophysectomized *H. fossilis* during preparatory phase, which stimulate E_2 by the ovary and enhanced the hepatic lipogenic activity lends to further support by our studies.Here it can be concluded that in *H. fossilis*, E_2 and 17α,20βP plays a vital role in regulating lipid dynamics.

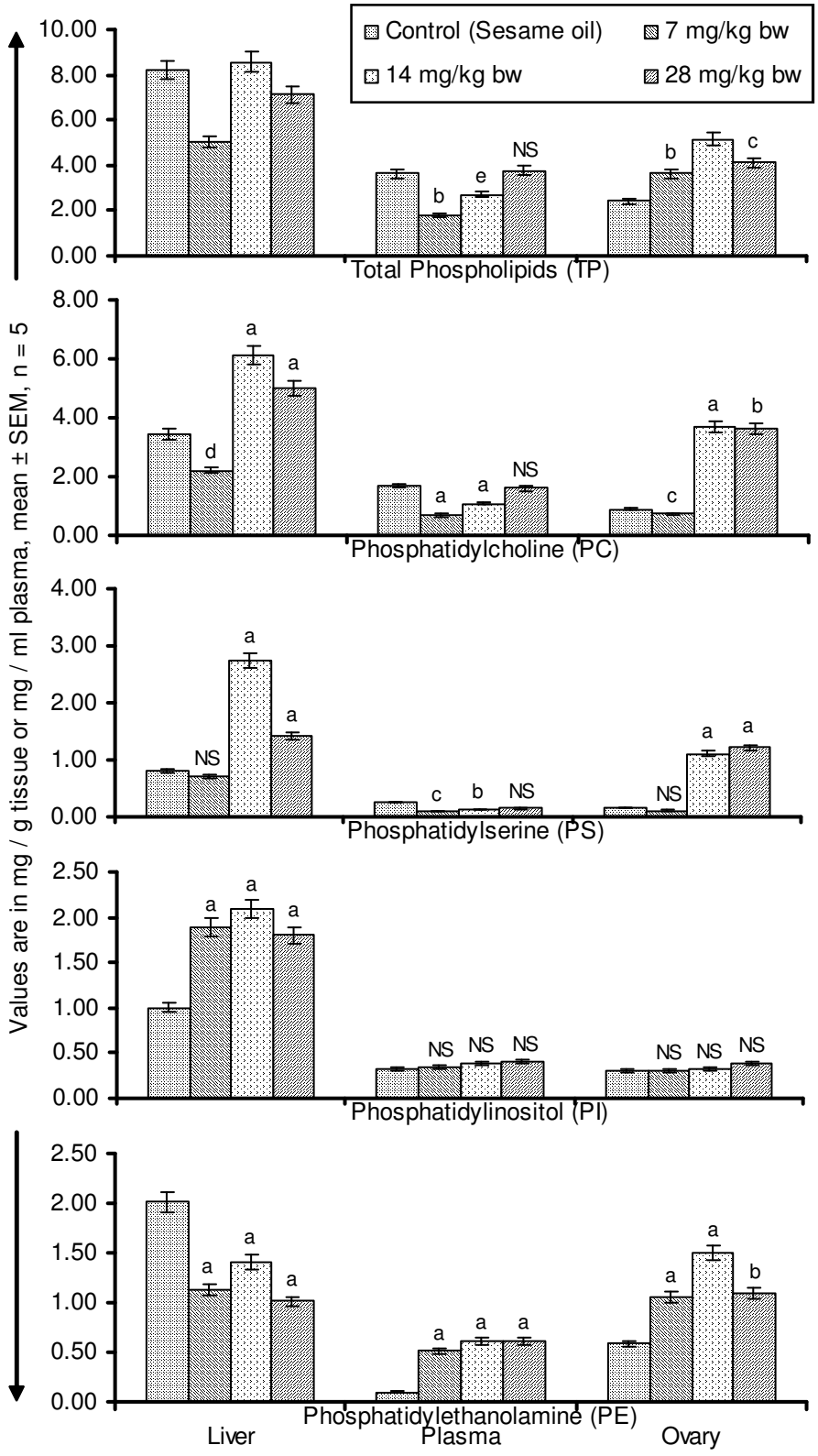

Fig. 2: Effect of cumulative dose levels (7, 14 and 28 mg/kg body weight) of estradiol 17β (E_2) on the concentration of different phospholipids in liver, plasma and ovary during preparatory phase of the annual reproductive cycle in the freshwater female catfish, *Heteropneustes fossilis* (Bloch). Control vs treated were compared by Student's 't' test. $^{a}p>0.001$, $^{b}p>0.005$, $^{c}p>0.01$, NS = Not significant ($p<0.05$)

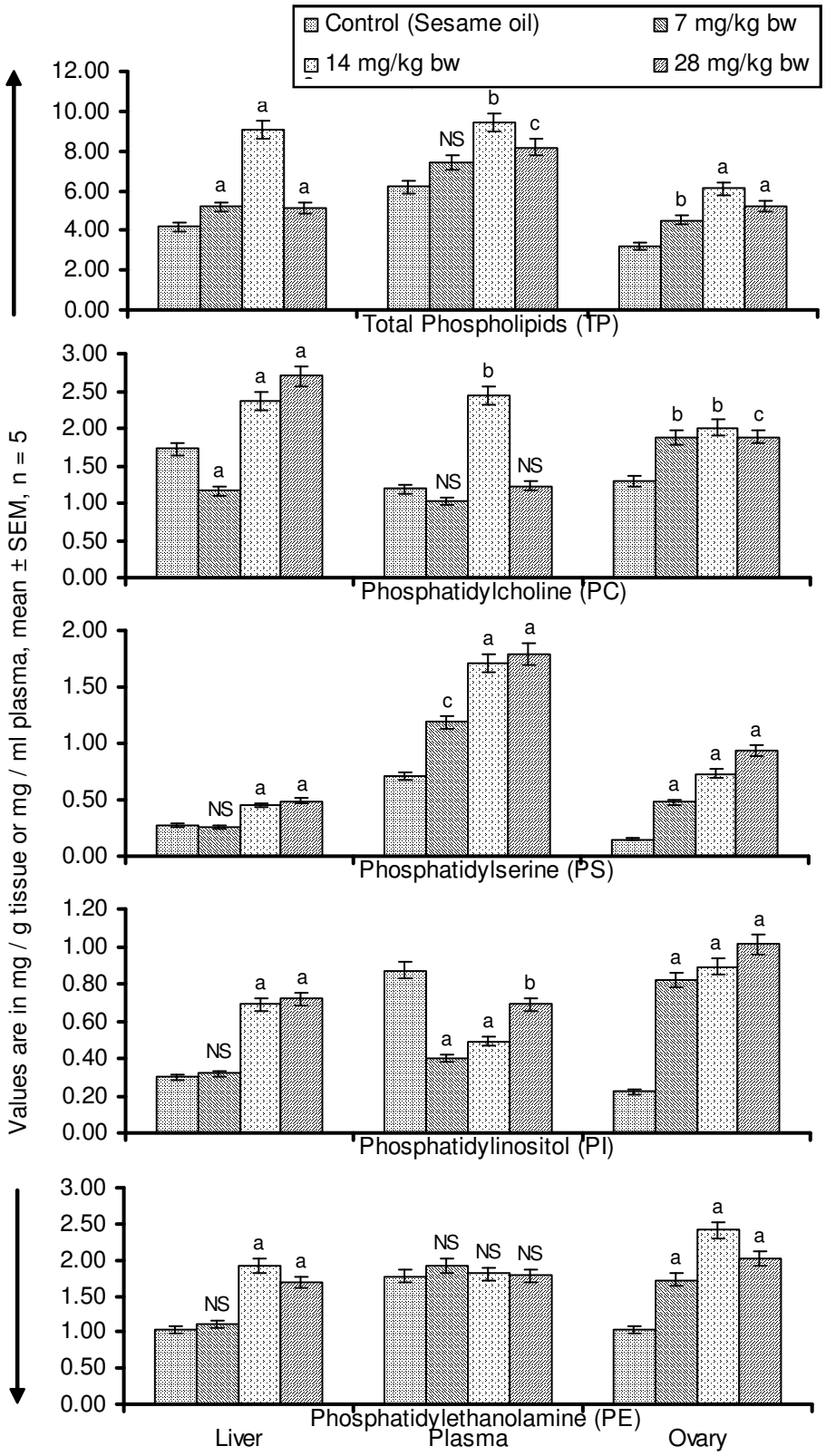

Fig. 3: Effect of cumulative dose levels (7, 14 and 28 mg/kg body weight) of estradiol 17β (E_2) on the concentration of different phospholipids in liver, plasma and ovary during prespawning phase of the annual reproductive cycle in the freshwater female catfish, *Heteropneustes fossilis* (Bloch). Control vs treated were compared by Student's 't' test. [a]p>0.001, [b]p>0.005 , [c]p>0.01, NS = Not significant (p<0.05)

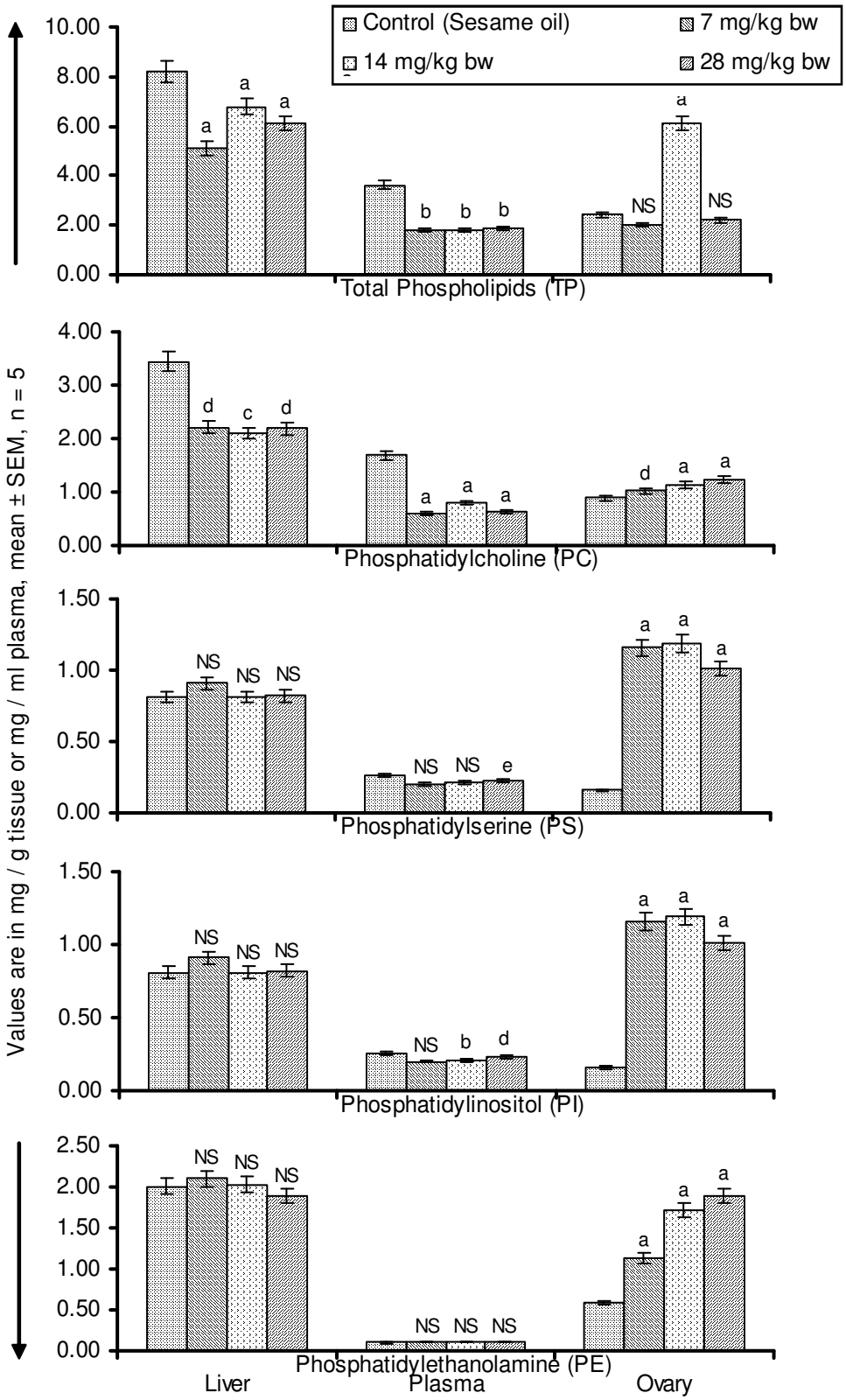

Fig. 4: Effect of cumulative dose levels (7, 14 and 28 mg/kg body weight) of 17α,20βP on the concentration of different phospholipids in liver, plasma and ovary during preparatory phase of the annual reproductive cycle in the freshwater female catfish, *Heteropneustes fossilis* (Bloch). Control vs treated were compared by Student's 't' test. [a]p>0.001, [b]p>0.005, [c]p>0.01, [d]p>0.02, [e]p>0.05, NS = Not significant (p<0.05)

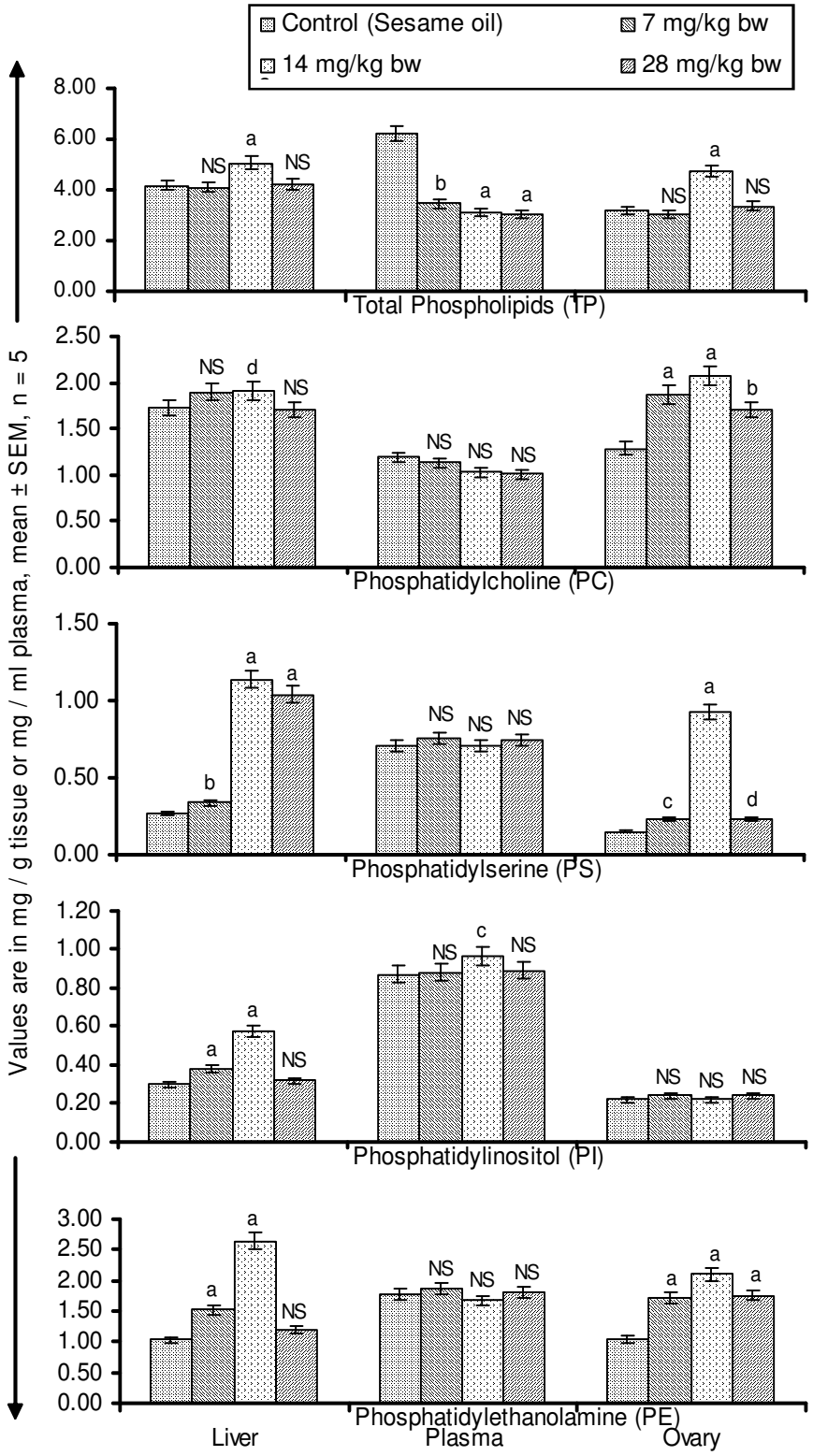

Fig. 5: Effect of cumulative dose levels (7, 14 and 28 mg/kg body weight) of 17α,20βP on the concentration of different phospholipids in liver, plasma and ovary during prespawning phase of the annual reproductive cycle in the freshwater female catfish, *Heteropneustes fossilis* (Bloch). Control vs treated were compared by Student's 't' test. $^{a}p>0.001$, $^{b}p>0.005$, $^{c}p>0.01$, $p>0.05$ NS = Not significant ($p<0.05$)

The report of Wiegand and Peter (1980), has shown that progesterone increased the plasma triglycerides but has no effect on phospholipids in *C. auratus*. The report of Kim and Kalkhoff (1975) and Velette *et al.* (1978), have indicated that progesterone activates the lipoprotein lipase responsible for the removal of triglycerides from plasma. Takashima *et al.* (1972) in *Salvelinus fontinalis* and De Vlaming *et al.* (1977) during prespawning phase of *C. auratus* have reported increased liver lipids in response to estrogen treatments. Here it is suggested that steroids may regulate the activity of hepatic lipase, an enzyme bound to the endothelial cells of liver sinusoids. Hepatic lipase has a central role in the removal / mobilization of phospholipids from liver to ovary *via* plasma. The effect of E_2 was more as compared to 17α,20βP which is evident from the histological examination of ovary having greater number of vitellogenic oocytes in this species. These suggestions receive support from the fact that administration of mGtH increased the vitellogenic oocytes and sex steroids- testosterone, estradiol-17β and 17α-hydroxyprogesterone (Singh *et al.,* 1993) in *H. fossilis*. It has been well established that the secretion of E_2 by the ovary (under maturational gonadotropin control) which stimulates the liver for the secretion of vitellogenin / different phospholipids, ultimately deposited in growing oocytes (under the influence of vitellogenic gonadotropin) during preparatory and prespawning phase, thus affecting vitellogenesis and spawning in this species. However, stimulation of lipogenesis in the rainbow trout ovary another synthesizing site besides liver, has been also reported by several workers in response to carbohydrate rich gonadotropin *in vitro* (Wiegand and Idler, 1982, 1984). In our laboratory the effect of E_2 and 17α,20βP on various phospholipids in ovariectomized immature *H. fossilis* is in progress for a clear picture of phospholipids biosynthesis.

In conclusion, retrospect of above finding it is suggested that the estradiol-17β has more pronounced effects than the 17α,20βP in regulation of different phospholipids and ovarian recrudescence during reproductively active phases and among the phospholipids PC is the main phospholipid of vitellogenin / ovarian lipids in *H. fossilis*.

Acknowledgments

A grant-in-aid from UGC MRP (F.5.1.3 (75)/2004(MRP/ NRCB) dated 31 March, 2004) New Delhi-222 001 to PBS is greatly appreciated. We are grateful to Dr S. S. Srivastava (Former DAAD Fellow) for the correction of English of this manuscript.

References

Anonymous.: Guidelines for the treatment of animals in behavioral research and teaching. *Anim. Behav*., **55**, 251-257 (1998).

Bruning, J.L. and B. L. Kintz: Computational Handbook of Statistics, 2nd Edn. Scott, Foresman Glenview, IL (1977).

De Vlaming, V.L., M.J. Vodicnik, G. Bauer, T. Murphy and D. Evan: Estradiol-17 effect on lipid and carbohydrate metabolism and on induction of yolk precursor in goldfish, *Carassius auratus*. *Life Sci.*, **12**, 1945-1951 (1977).

De Vlaming, V.L., R. Fitz Gerald, G. Delahunty, J. Ceeh, K. Silman and M. Barkley: Dynamics of oocyte development and related changes in serum estradiol-17β, yolk precursor and lipid levels in the teleostean fish, *Leptocottus armatus*. *Comp. Biochem. Physiol.*, **77**, 599-610 (1984).

Folch, J., M. Lees and G.H. Sloane-Stanley: A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.,* **226**, 497-509 (1957).

Fremont, L. and A. Riazi: Biochemical analysis of vitellogenin from rainbow trout (*Salmo gairdneri*). Fatty acid composition of phospholipids. *Reprod. Nut. Develop.,* **28**, 939-952 (1988).

Freeman, C.P. and D. West: Complete separation of lipid classes on a single thin layer plate. *J. Lipid Res.*, **7**, 324-327 (1966).

Henderson, R.J. and D.R. Tocher: The lipid composition and biochemistry of freshwater fish. *J. Lipid Res*., **26**, 281-347 (1987).

Kim, H.J. and R.K. Kalkhoff: Sex steroid influence on triglycerides metabolism. *J. Clin. Invest.*, **56,** 888-896 (1975).

Lal, B. and T.P. Singh: Effect of gonadectomy and some sex steroids on various classes of lipids and their metabolism in the catfish *Clarias batrachus*. *Zool. Jb. Physiol*., **91**, 479-488 (1987a).

Lal, B. and T.P. Singh: Changes in tissue lipid levels in the freshwater catfish, *Clarias batrachus* associated with reproductive cycle. *Fish Physiol. Biochem*., **3**, 191-201(1987b).

Leslie, J.M. and J.T. Buckley: Phospholipid composition of goldfish (*Carassius auratus* L.) liver and brain and temperature dependence of phophatidylcholine synthesis. *Comp. Bioch. Physiol*., **53**, 335-337 (1976).

Marzo, A., P. Ghirarch and D. Serdini: Simplified measurements of monoglycerides, diglycerides, triglycerides and fatty acids in biological samples. *Clin. Chem*., **17**, 145-147 (1971).

Rajendra Phartyal, L., B. Singh, S.V. Goswami and N. Sehgal: *In vitro* production of vitellogenin by estradiol-173β in isolated hepatocytes of catfish, *Clarias gariepinus*. *Fish Physiol. Biochem*., **31**, 241-245 (2005).

Sehgal, N. and S.V. Goswami: Vitellogenin exists as charge isomers in the Indian freshwater murrel, *Channa punctatus* (Bloch). *Gen. Comp. Endocrinol.,* **141**, 12-21 (2005).

Shankar, D.S. and R.S. Kulkarni: Effect of cortisol on female freshwater fish *Notopterus notopterus*. *J. Environ. Biol.,* **27**, 727-731 (2006).

Singh, P.B. and T.P. Singh: Effect of partially purified catfish gonadotropin on lipid metabolism in the freshwater catfish, *Heteropneustes fossilis*. Proc. 4th FAOB Congress, The Federation of Asian and Oceanian Biochemists held at Singapore on Nov. 30-Dec. 5, 1986. pp. 340-341 (1986).

Singh, A.K. and T.P. Singh: Seasonal fluctuation in lipid and cholesterol content of ovary, liver and blood serum in relation to annual sexual cycle in *Heteropneustes fossilis* (Bloch). *Endocrinologie*, **73**, 47-54 (1979).

Singh, P.B. and T.P. Singh: Seasonal correlative changes between sex steroids and lipids level in the freshwater female catfish, *Heteropneustes fossilis* (Bloch). *J. Fish Biol*., **37**, 793-802 (1990).

Singh, P.B. and T.P. Singh: Impact of oLH- RH and mGtH on lipid metabolism and steroidogenesis in the freshwater catfish, *H. fossilis*. *Zool. Jb. Physiol*., **95**, 221-232 (1991).

Singh, P.B., D.E. Kime and T.P. Singh: Modulatory actions of *Mystus* gonadotropin on γ-BHC induced histological changes, cholesterol and sex steroid level in *Heteropneustes fossilis*. *Ecotoxicol. Environ. Saf*., **25**, 141-153 (1993).

Sundararaj, B.I., P. Nath and G.E. Burzawa: Synthesis of vitellogenin and its uptake by the ovary in the catfish, *H. fossilis* (Bloch) in response to carp gonadotropin and its subunits. *Gen. Comp. Endocrinol.*, **46**, 93-98 (1982).

Takashima, F., T. Hibiya, P.V. Nyan and K. Aida: Endocrinological studies on lipid metabolism in rainbow trout. II. Effect of sex steroid, thyroid powder and adrenocorticotropin on plasma lipid content. *Bull. Jpn. Soc. Sci. Fish*, **38**, 43 (1972).

Tocher, D.R.: Metabolism and functions of lipids and fatty acids in teleost fish. *Rev. Fish. Sci*., **11**, 107-184 (2003).

Velette, A., A. Verine, L. Varese and J. Boxer: Effects of ethynyl estradiol and progesterone on triglyceride metabolism in the female rat. *Endocrinol.,* **103**, 1647-1653 (1978).

Vitiello, F. and J.P. Zanetta: Thin layer chromatography of phospholipids. *J. Chromatogr*., **166**, 637-640 (1978).

Wiegand, M.D. and R.E. Peter: Effects of sex steroids on plasma lipids in the goldfish, *Carassius auratus*. *Can. J. Zool*., **58**, 967-972 (1980).

Wiegand, M.D. and D.R. Idler: Synthesis of lipids by the rainbow trout (*Salmo gairdneri*) ovary *in vitro*. *Can. J. Zool*., **60**, 2683-2693 (1982).

Wiegand, M.D. and D.R. Idler: Stimulation of lipogenesis in rainbow trout ovary by carbohydrate rich gonadotropin *in vitro*. *Gen. Comp. Endocrinol*., **53**, 418-426 (1984).