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Effect of contaminated fish meal diet on estrous cycle, hormones and biochemical changes in ovary and uterus of *Rattus norvegicus*

Usha Anandhi D.* and Jyothi S. Bhatt
Reproductive Physiology Unit
Department of Zoology, Bangalore University, Bangalore, (Karnataka) - India

Abstract

The aim of the present investigation was to evaluate the adverse effects of consumption of contaminated fish meal diet on estrous cycle, hormones and biochemical constituents in ovary and uterus of Albino rats, *Rattus norvegicus*. Rats were randomly distributed into five groups (n=6), Group I served as control, while group II, III and IV received combination of fish meal ratios and group V only fish meal diet for 90 days respectively. Result revealed fish meal diet arrested the normal estrous cycle at diestrous phase and reduction in protein, glycogen, and cholesterol in ovary and increase cholesterol content in uterus of experimental groups. Gradual decrease of estrogen indicated the fall of hormonal levels in all experimental groups ($p< 0.05$) and increase of progesterone led to prolonged diestrous phase. These changes indicates alteration in the physiology of the animal to toxic substance in contaminated fish meal diet which has a negative implication on the reproductive performance of rat.

Key-Words: Fishmeal, ovary, uterus, protein, glycogen, cholesterol, estrogen, progesterone, *Rattus norvegicus*

Introduction

Natural waters are usually contaminated with mixtures of metals and other toxic compounds. Exposures to different metals cause various behavioral and physiological changes in fish (Sola *et al.,* 1995).Fishes are exposed to high level of metal ions in aquatic environment, their tissues tend to take up metal ions through various routes from their surroundings. There are two main routes of metal acquisition from water and diet (Bury *et al.,* 2003). Nutrients play an important role in the performance of various physiological processes associated with the attainment of sexual maturity (Shashikala *et al.,* 2000). Fish is highly nutritive and rich source of animal proteins for human consumption (Sandhu *et al.,* 2005). Contaminated fish with toxic metals are of great concern for public health and for the health of animals that eat fish which disrupt reproduction in mammals (Colborn *et al.,* 1993). Mammalian ovary is the most dynamic organ and also a significant site of toxic damage affecting fertility of female reproductive system (Karen *et al.,* 2005).

*** Corresponding Author**
E.mail: ushaanandhi@rediffmail.com, jothi1511@gmail.com

Uterus is a major hormone responsive reproductive sex organ in mammals and its structure and function depends on the ovarian estradiol secretion (Couse *et al.,* 1999). Biochemical parameters are sensitive index and can constitute important diagnostic tool in toxicological studies (Singh *et al.,* 2001). The risk or benefit of contaminated fish meal consumption is complicated as most assessments are based on analyses of fish. Thus, the purpose of the present study was to evaluate biochemical changes in rat ovary and uterus with contaminated fish meal Intoxication.

Material and Methods

Albino rats, *Rattus norvegicus* weighing about 40-50gms of 4 – 5 weeks procured from Raghavendra enterprises, Bangalore, were housed in plastic cages and maintained at room temperature of 24°C ± 1° C and photoperiod of 12L/12D. Animals were divided into 5 groups of 6 each (n=6). Group I received vehicle only (Rat feed procured from Amruth mice and feed, India) and served as control. Group II, III and IV received different ratios of fish meal at the doses of (1:1, 2:1and 3:1) and Group V fed with fish meal alone. The experiment was carried out for 90 days. Vaginal Smear from the control and experimental animal was observed (9am-10am) throughout the experimental period. The animals were sacrificed after

90 days the ovaries and uterus were dissected out freed from adhering tissues, weighed and processed for biochemical analysis of Protein (Lowry *et al.,* 1951), Glycogen (Dubois *et al.,* 1956) and Cholesterol (Zlatkis *et al.,* 1958). The values are expressed as mg/g wet weight of tissue. Hormone assay of Serum estrogen and progesterone was done using ELFA (Enzyme Linked Fluorescent Assay). Experiments conducted during these studies comply with the ethical approval.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) and subjected to one way ANOVA, followed by Tukey's HSD using SPSS Version 17. Values at $p<0.05$ were considered significant.

Results and Discussion

Fish meal is considered to be one of the best ingredients, due to its compatibility with the protein requirement of fish (Alam *et al.,* 1996). Nutritional deficiencies have been shown to alter reproductive function in rats (Simonich *et al.,* 1995 and Baligar *et al.,* 2001). Cyclic changes of the vaginal smear observed in estrous cycle gives a reasonable index of ovarian activity and hormonal synthesis of estrogen and progesterone. In the present study Group II and III showed gradual reduction in number of estrous cyclicity, whereas, more significant reduction was observed in group IV and V when compared to control (Table 1). This result revealed different doses of contaminated fish meal diet fed to cyclic rats resulted in the cessation of estrous cycle in diestrous phase and decreased the cornfield cells in the vaginal smear. (Asmathabanu *et al.,* 1997; Math *et al.,* 1998) observed increase in the prolonged diestrous phase of the estrous cycle in rats treated with organophosphate pesticide. The present study is in accordance with Rao *et al.,* (2002). A significant decrease in protein and glycogen content was noticed in ovary and uterus of treated groups. Heavy metals and pesticides caused depletion in protein content in rats which might be due to defective protein synthesis or energy diversification to meet the impending energy demand when the animal is under toxic stress (Neff 1985). In the present study significant reduction of protein and glycogen was in group IV and V (F=75.32; df=4, 25; $p<0.05$, F=432.58; df=4, 25; $p<0.05$ and F=841.23; df=4, 25; $p<0.05$, F=2650.05; df=4, 25; $p<0.05$) when compared to control (Fig.1 & 2). Decrease of protein and glycogen content might be due to interaction of heavy metals which alter the physiology of organisms by affecting important aspects of cellular metabolism (Stohs *et al.,* 1995). Cholesterol acts as precursor molecule during steroidogenesis in the ovary. The present study revealed decrease in ovarian cholesterol and increase in uterine cholesterol in group IV and V. (F=230.51; df=4, 25; $p<0.05$, F=274.92; df=4, 25; $p<0.05$) (Fig.3). Decrease of cholesterol in the tissues resulted in degeneration of follicles in the ovary which cause reduction in hormone synthesis and increase in the uterus may be due to necrotic changes. Further, reducing level of circulating hormones which contribute to altered physiology of the reproductive system in turn affects steroid metabolism. Smith *et al.* (2005) opined increasing cholesterol in uterus was found to have a significant deleterious effect on uterine contractile activity. Synthesis of ovarian hormones governs the stages of the estrous cycle and their inter-conversion are under the control of ovarian hormones estrogen and progesterone secreted by matured follicles and corpus luteum, which in turn controlled by the secretion of pituitary gonadotropins and hypothalamic-releasing factors. The present result revealed reduction in estrogen and gradual increase in progesterone in all experimental groups (F= 146.58; df= 4, 25 $p<0.05$) (Fig.4). Further, Group IV and V showed significant increase of progestrone level during diestrous phase (F= 90.60; df= 4, 25 $p<0.05$) (Fig.5). The increase of progesterone level due to high plasma levels prevents events for the initiation of the next estrous stopping the estrous cycle in the diestrous phase. Similar results were also reported in monocotrophs treated mice Soratur *et al.* (1999). Thus, in conclusion the general health condition of the animal should take into account the biochemical modulations, hormone concentration and ovarian cycle irregularities induced by fish meal diet which affects the reproductive performance in rats.

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Table 1: Effect of contaminated fish meal on estrous cycle in albino rats

Groups	No. of Rats	Number of cycle	Duration in days (Mean±SD)				
			Proestrous	Estrous	Metaestrous	Diestrous	Diestrous index
CONTROL	6	5.64±0.45a	5.46±0.29a	7.05±0.49a	4.58±0.27a	11.61±0.75a	38.70
1F:1C	6	4.63±0.25b	3.66±0.13b	5.41±0.19b	3.53±0.23b	14.55±0.49b	48.50
2F:1C	6	3.41±0.22c	2.61±0.19c	4.75±0.08b	3.42±0.15c	17.46±0.35c	58.20
3F:1C	6	2.80±0.16d	1.87±0.11d	3.88±0.11c	2.88±0.06d	20.25±0.42d	67.50
FISHMEAL	6	2.11±0.21e	1.16±0.03e	3.30±0.17d	2.13±0.04e	21.83±0.49e	72.76
F value	-	151.47	532.03	189.65	150.90	378.54	-
df	-	4,25	4,25	4,25	4,25	4,25	-

Mean values fallowed by different letters with in a column are significantly different at $p<0.05$.

$$\text{Diestrous index} = \frac{\text{Number of days with clear diestrous smear}}{\text{Total duration of experiment (Days)}} \times 100$$

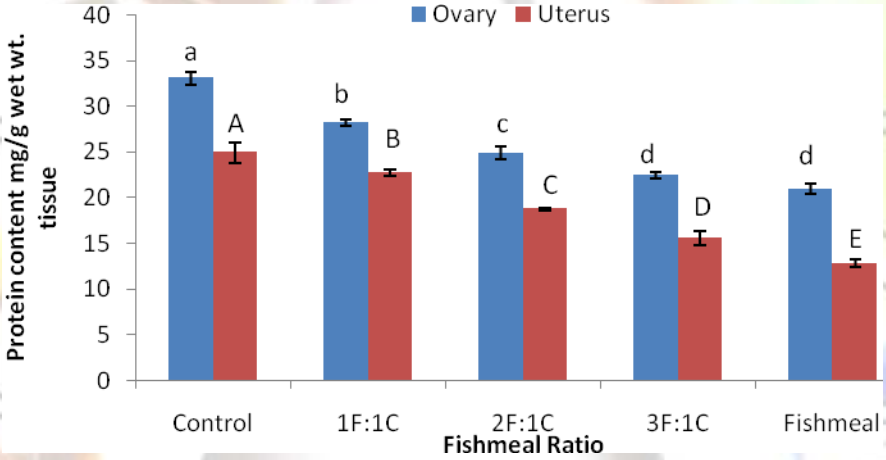

Fig 1: Protein concentration (mg/g) in ovary and uterus of Albino rats treated with contaminated fishmeal diet. Bars fallowed by different small and capital letters are significantly different in protein content in ovary and uterus respectively

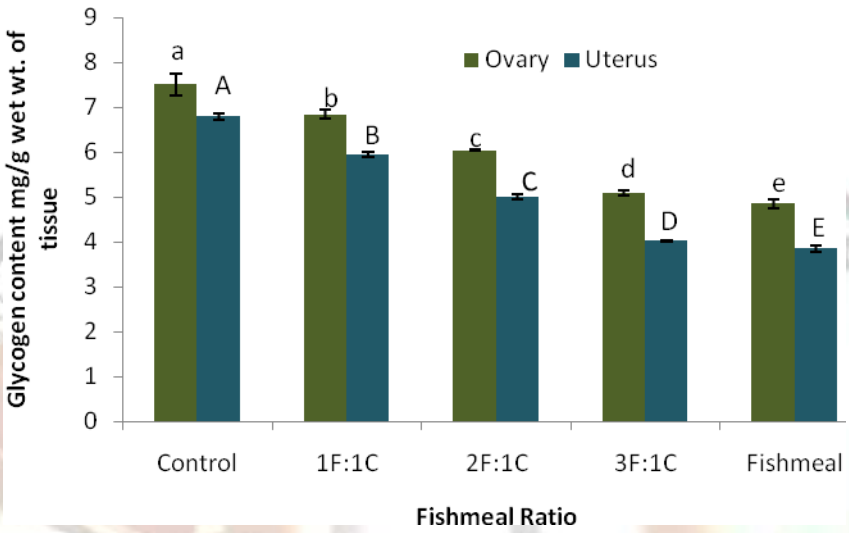

Fig. 2: Glycogen concentration (mg/g) in ovary and uterus of albino rats treated with contaminated fishmeal diet. Bars fallowed by different small and capital letters are significantly different in protein content in ovary and uterus respectively

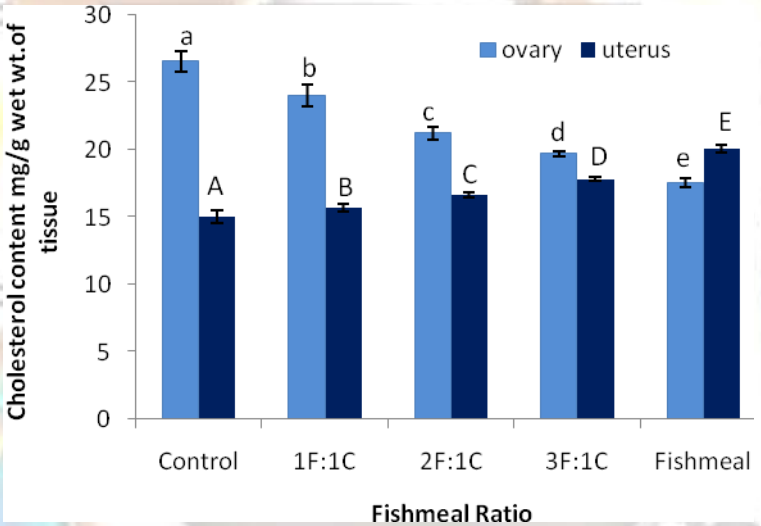

Fig. 3: Cholesterol concentration (mg/g) in ovary and uterus of albino rats treated with contaminated fishmeal diet. Bars fallowed by different small and capital letters are significantly different in protein content in ovary and uterus respectively

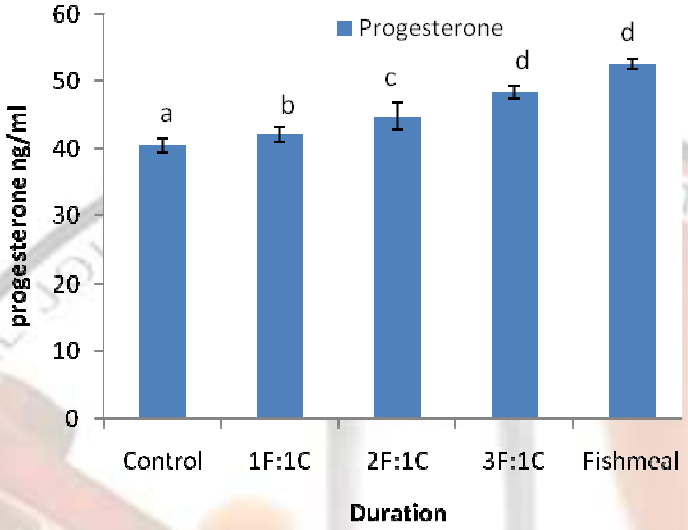

Fig. 4: Serum Progesterone (ng/ml) in albino rats treated with contaminated fishmeal diet. Bars fallowed by different small and capital letters are significantly different in protein content in ovary and uterus respectively

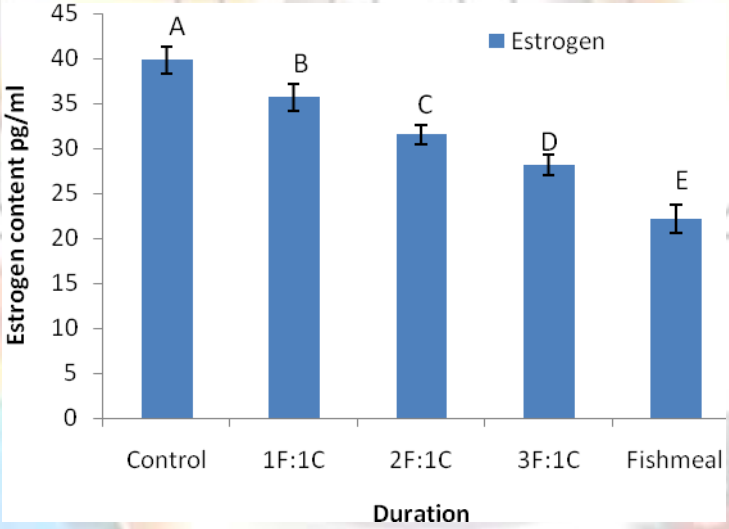

Fig. 5: Serum Estrogen (pg/ml) in rats treated with contaminated fishmeal diet. Bars fallowed by different small and capital letters are significantly different in protein content in ovary and uterus respectively