

Effect of natrum muriaticum on biochemical constituents of ovary of selected ornamental fishes

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Abstract

The quantitative estimation of the proteins, lipids and carbohydrates on the ovary of ornamental oviparous fishes like goldfish, rosy barb and viviparous fishes like molly, guppy has been carried out by using homeopathy preparation of natrum muriaticum of 30 centesimal potency. The biochemical analyses revealed the changes in the components; however total lipid content of the ovary showed a gradual increase from 0 day to 4th day of the experiment.

Keywords: Oviparous fishes, Viviparous fishes, Biochemical analyses, Natrum muriaticum

1. Introduction

Biochemical composition of gonads forms an important pre-requisite in understanding the physiology of pre spawning and post-spawning fish [khan,2005]. Various endogenous and exogenous factors play a dynamic role in the biochemical composition of fishes. Fishes show seasonal variations in the cellular constituents of liver, muscle, and gonad due to variations in the external (environments) and internal (sex steroid) factors [www.shodhganga.inflibnet.ac.in]. There are several biochemical parameters such as the utilization of yolk protein, lipid and carbohydrates constitute a major part required for successful embryonic development [Jones,1998]. Previous studies were done using the hormones for biochemical analysis of tissues. However, there is paucity of information regarding the homeopathy induction is lacking in ornamental fishes. Thus, the present study aims to find out the effect of homeopathy preparation natrum muriaticum on biochemical compounds namely proteins, carbohydrates and lipids are analyzed in viviparous and oviparous fish during the breeding time (i.e.,) 0 day and 4th day.

2. Review of Literature

Generally changes in chemical composition of body have been known to reflect storage or depletion of energy reserves. The values of body

composition in fishes vary within and between species, with fish size, sexual condition, feeding, time of the year and activity [Hoar and Randall,1988].

Various workers have studied the protein synthesis in the target organ like liver and ovary [Medda et al.,1980]. Protein is the most important constituent of fish eggs. It serves two primary functions; provides amino acids for tissue growth and supplies energy (via) catabolic process [Wallace, and Selman, 1981]. Fish protein contains all essential amino acids which are easy to digest. It is a important constituent of living cells, it changes depending upon the stage of maturity of the gonads, state of nutrition and age, time of year and environmental condition [Rojas, 2011]. Lipids serve as the major structural element in cell membranes as well as means of storing energy. Some of the specialized lipids serve as pigments (retinal cofactors, vitamin K), detergents (bile salts), and hormones (steroid hormones) [Petersen and Emmerson, 1977]. Lipids are actively involved in reproductive phases of teleost fishes. Lipids are the primary energy storage material in fish. Fish store the lipids in various organs; particularly in muscles and liver [Hoar and Randall, 1988]. Fish eggs contain relatively little carbohydrates when compares with the amounts of protein and lipid. A large proportion of total egg carbohydrates are associated with egg membranes. They are present in both a free state and complexed with yolk proteins. It is tend to be the most utilized yolk nutrient [Weatherly and Gill,1987]. Carbohydrates also plays a major role in the reproductive phase of both viviparous and oviparous teleost, but only limited information available on carbohydrates synthesis and utilization [Petersen and Emmerson, 1977].

3. Materials and Methods

Natrum muriaticum is commonly known as table salt or sodium chloride. Salt is the second most common substance in nature. It is an important

component in regulating the balance of body fluids and tissues. It will increase the production of red blood cells and albumin, a protein [www.naturalhealthstore.com]. Induced breeding studies were done by using natrum muriaticum 1000 centesimal potency in blackmolly and goldfish [Vishakan,2002]. He suggested that it was found more effective in induced breeding potency with little side effect than other synthetic medicines and it is economical and easily available. To understand the influence of natrum muriaticum 30 centesimal potency is used in few fish like goldfish, rosy barb, whitemolly and guppy were chosen for induced breeding purpose.

Sexually mature female gold fish, rosy barb, molly and guppy were acclimated to 10 days in separate plastic tub prior to the experiment. On the day of experiment similar size fishes weighing 1.7 – 2.2g of rosy barb, Gold fish 8 – 9 g, Molly 2.4 – 3 g, and Guppy 1.5 – 1.7g were chosen for the study. The fishes were divided into two groups i.e egg layer and live bearer. In each group two types of fishes are used i.e., gold fish, rosy barb and guppy and molly respectively. In each type totally 12 fishes were taken. Among this six were used as experimental, six fishes as control. The homeopathy preparation natrum muriaticum of 30c potency was brought from local homeopathy medical shop. From natrum muriaticum 30c potency 0.025% dilution was prepared. 0.1ml of natrum muriaticum 30c was diluted to 0.025% by adding 400ml of water (i.e., 0.1ml is added in 400ml of water). The homeopathic preparation medium was prepared in six plastic troughs considered as experimental ones and six troughs containing only ground water as control. The fishes were introduced into medium one in each.

The experiment was carried out for three days i.e., the medium was changed morning at 8 am and evening at 4 pm. Evening only ground water was used. For the control animals water was changed. At the same time feeding was done. On 0 day and 4th day of the experiment fishes were autopsied, the ovary was removed and analysed for the total protein, lipid and carbohydrate content by using the standard methods. Total protein was estimated by [Lowery et al., 1951] method. Total lipid was estimated by methanol chloroform method described by [Folsch et al., 1957]. Carbohydrates were estimated by anthrone method suggested by [Dubois et al., 1956]. Each value represented the mean value of six samples for each species.

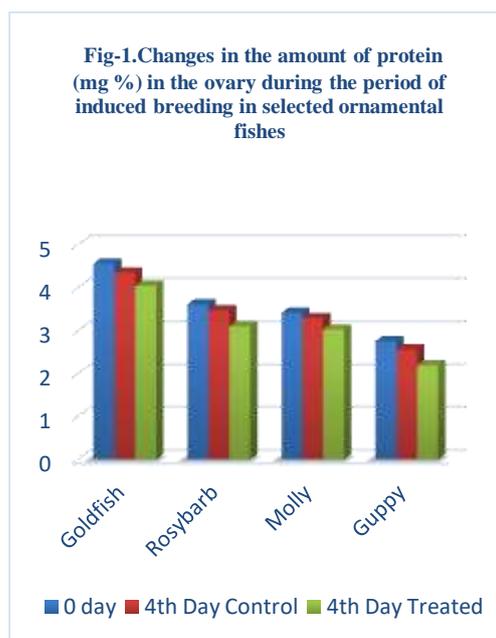
4. Results and Discussion

The present investigation showed that levels of protein (Table1 and Fig. 1) are found to vary in control and treated goldfish was 4.56±0.09mg% on 0 day. The level has decreased to 4.35±0.12mg% in control and 4.04±0.26mg % in treated on 4th day of the experiment. Both control and experimental gold fish didn't show a significant difference (P>0.05) in

the protein concentration during the time of the experiment. The total protein content in the ovary of rosy barb was 3.61±0.13 mg % at 0 day. There is a decrease in the level of protein in control and treated i.e., 3.47±0.04mg % and 3.10±0.15 mg% respectively (Table1 and Fig. 1) on 4th day. There is no significant difference (P>0.05) was noticed between control and treated fish. The level of protein in Molly was 3.41±0.05 mg % at 0 day. It decreases to 3.29±0.15 and 3.02±0.13 mg % in control and treated fishes. In guppy total protein in the ovary was 2.75±0.08at 0 day. The protein level was reduced to 2.56±0.11mg % and 2.19±0.04 mg% in control and treated fishes. There is no significant difference (P>0.05) was observed between control and treated fishes of molly and guppy.

Type of fish	Days of Sampling	Control (Mean±S.D)	Treated (Mean±S.D)
Goldfish	0 Day	4.56±0.09	4.56±0.09
	4 th Day	4.35±0.12	4.04±0.26
Rosy barb	0 Day	3.61±0.13	3.61±0.13
	4 th Day	3.47±0.04	3.10±0.15
Molly	0 Day	3.41±0.05	3.41±0.05
	4 th Day	3.29±0.15	3.02±0.13
Guppy	0 Day	2.75±0.08	2.75±0.08
	4 th Day	2.56±0.11	2.19±0.04

Table-1. Changes in the amount of protein (mg %) in the ovary during the period of induced breeding in selected ornamental fishes



The lipid concentration at 0 day was 2.60 ± 0.05 mg% in gold fish. The content value at 4th day was 2.73 ± 0.07 mg% in control fishes steady rise in treated fish was 3.04 ± 0.03 mg% (Table 2 and Fig.2). The starting level was 2.60 ± 0.03 mg% at 0 day in rosy barb and the level has increased to 2.70 ± 0.07 mg% in control and 2.75 ± 0.38 mg% in treated fishes at 4th day. With regard to lipid concentration there was no significant difference ($P > 0.05$) was observed between control and treated fishes on 4th day of Gold fish and rosy barb. The total lipid content of ovary in molly fish at 0 day was 2.60 ± 0.06 mg% and minimum increase at 4th day of control was 2.79 ± 0.07 mg%, maximum level was found in treated fish i.e., 3.02 ± 0.06 mg% (Table 2 and Fig 2). In guppy concentration of lipid at 0 day was 2.11 ± 0.07 mg%. At 4th day control was 2.32 ± 0.03 mg% and 2.34 ± 0.03 mg% in treated fishes. Both control and treated fishes show no significant difference ($P > 0.05$) in lipid content on 4th day of the experiment in Molly and Guppy.

Type of fish	Days of Sampling	Control (Mean±S.D)	Treated (Mean±S.D)
Goldfish	0 Day	2.60 ± 0.05	2.60 ± 0.05
	4 th Day	2.73 ± 0.07	3.04 ± 0.03
Rosy barb	0 Day	2.60 ± 0.03	2.60 ± 0.03
	4 th Day	2.70 ± 0.07	2.75 ± 0.38
Molly	0 Day	2.60 ± 0.06	2.60 ± 0.06
	4 th Day	2.79 ± 0.07	3.02 ± 0.06
Guppy	0 Day	2.11 ± 0.07	2.11 ± 0.07
	4 th Day	2.32 ± 0.03	2.34 ± 0.03

Table-2. Changes in the amount of lipid (mg %) in the ovary during the period of induced breeding in selected ornamental fishes

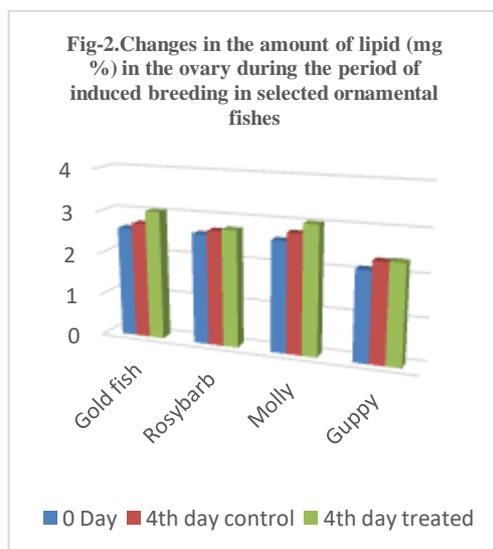


Fig-2. Changes in the amount of lipid (mg %) in the ovary during the period of induced breeding in selected ornamental fishes

The total carbohydrate content of ovary in gold fish, the value was 2.86 ± 0.06 mg% at 0 day and they declined at 4th day of control 2.74 ± 0.03 mg% and 2.62 ± 0.01 mg% of treated fishes (Table 3 and Fig 3).

In rosy barb concentration of carbohydrate was 2.77 ± 0.03 mg% at 0 day. The level was more or less similar in control and treated i.e., 2.71 ± 0.02 mg% and 2.60 ± 0.04 mg%. There was no significant difference ($P > 0.05$) among control and treated fishes on 4th day of experiment in gold fish and rosy barb. In molly, carbohydrate content was 2.46 ± 0.05 mg% at 0 day. The level was similar in control and treated fishes at 4th day 2.32 ± 0.03 mg%, 2.30 ± 0.04 mg% (Table 3 and Fig 3). Similar results were obtained in guppy. At 0 day, the level of carbohydrates was 2.25 ± 0.02 mg% and 4th day control fish showed 2.19 ± 0.06 mg% level, 2.06 ± 0.05 mg% in treated fishes. There was no significant difference ($P > 0.05$) was observed between control and treated fishes of molly not in guppy.

Type of fish	Days of Sampling	Control (Mean±S.D)	Treated (Mean±S.D)
Goldfish	0 Day	2.86 ± 0.06	2.86 ± 0.06
	4 th Day	2.74 ± 0.03	2.62 ± 0.01
Rosy barb	0 Day	2.77 ± 0.03	2.77 ± 0.03
	4 th Day	2.71 ± 0.02	2.60 ± 0.04
Molly	0 Day	2.46 ± 0.05	2.46 ± 0.05
	4 th Day	2.32 ± 0.03	2.30 ± 0.04
Guppy	0 Day	2.25 ± 0.02	2.25 ± 0.02
	4 th Day	2.19 ± 0.06	2.06 ± 0.05

Table 3- Changes in the amount of carbohydrate (mg %) in the ovary during the period of induced breeding in selected ornamental fishes

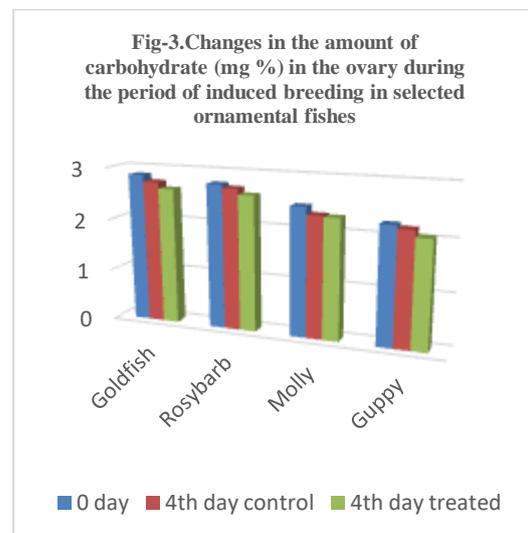


Fig-3. Changes in the amount of carbohydrate (mg %) in the ovary during the period of induced breeding in selected ornamental fishes

The changes in physical properties of the organ all primarily attributed to the changes in chemical composition [Weatherly and Gill, 1987]. The present study showed biochemical compounds like protein, lipid and carbohydrate levels during the breeding time in gold fish, rosy barb, molly and guppy which were induced by homeopathy preparation natrum muriaticum 30c potency.

In the present studies, both in oviparous and viviparous fishes, the homeopathy preparation caused a decrease in protein and carbohydrate level and an increase level in lipid constituents at the end of the experiment. According to [Thomas et al., 1988] there is a continual loss of protein from the yolk mass as it is transferred to the developing tissues. As growth proceeds and the metabolic rate increases a large protein of yolk protein is shunted into energy production and bulk protein quantities decline. This is especially evident after hatch, in accordance with the higher levels of activity and energy demand. This trend has been reported for Salmonids. Among other studies of oxygen consumption and nitrogen metabolism [Kaushik, 1982] also supported an increased use of protein for energy production after hatching. In the present studies also the value at the starting of the experiment was high and decline at the end of the experiment.

Lipid is the next most abundant dry constituent of most of the fish eggs. Lipid levels were high in cymatogaster during the later stages of gestation. In *Zoarces arces* viviparous, the concentration of lipid is high during mid-late gestation [Devlaming et al., 1983]. In the present result also, lipid concentration of both oviparous and viviparous fish slight increase from 0 day to 4th day of the experiment. Lipid contents of ovary were found to be increasing with the time after treatment of homeopathy preparation. The increase in lipid content in treatment fish is likely to be due to the intermediary metabolism and the resulted in lipid quantity will aid to provide energy for maturation of ovarian tissues. Similar findings were reported in fingerlings of *Catla catla* treated with sub lethal doses of modern detergents have shown significant alternate in their biochemical constituents especially increase in lipid contents [Basheer MJ, 2000]. Lipids supply energy which is necessary for growth and for maintenance of the embryo [Kamler et al., 1982].

The increase in the lipid content of four fishes confirm previous observations in *Carassius auratus* and *Poecilia sphenops* [Vishakan, 2002] and in *Epinephelus diacanthus* [Chandrasekhara Rao and Krishnan, 2011]. In *Schizothorax niger*, the quantitative estimation of the total proteins, lipids and carbohydrates throughout the reproductive cycle revealed the fluctuating trends during the different stages of maturity (24.16 ± 0.87) proteins in stage II and minimum value (4.86 ± 0.40) in stage IV, carbohydrates were maximum (0.93 ± 0.60) in stage II and minimum (0.43 ± 0.08) in stage IV. However,

lipid percentage of the ovary showed a gradual increase from stage I (5.33 ± 0.58) to stage IV (16.33 ± 1.04) [Ghulam et al., 1988]. In our studies also, the lipid value straightly increased from the starting of experiment till then end of the experiment they did not decrease like protein and carbohydrate.

Carbohydrates are in minor percentage of the total composition of the ovary [Hoar and Randall, 1988]. The low values of carbohydrates recorded in the present study supports the view that carbohydrate plays an insignificant role as energy reserve in aquatic animals. Carbohydrate plays a minor role in the energy reserves of *Ambassis gymnocephalus* and the depletion is due to spawning and it is also negligible when compared to lipid and protein [Roja, 2011].

In the present experiment, the value of carbohydrate present in the ovary decreased from the starting (0 day to 4th day) to till the end. The decline in carbohydrate value indicates the impact of natrum muriaticum, mobilization of glucose to provide energy for the reproductive stage.

5. Conclusion

Marked changes in the biochemical constituents of all the four fishes were observed during the breeding seasons. It should be finally mentioned that the observed metabolic changes during breeding reflects a shift from regulation of oogenesis to the regulation of breeding. So natrum muriaticum can be used for induced breeding of these fishes which is very cheap, easily available. By using this preparation production of ornamental fishes can be increased and in turn offer good opportunities for entrepreneurs.

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