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ORIGINAL ARTICLE **OPEN ACCESS**

Impact of ganglionic extract and synthetic hormone injections for metabolizing the lipid content of bivalve *Indonaia caeruleus* (Prashad, 1918) in winter season

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ABSTRACT
Neuroendocrine control play important role in metabolic activities of freshwater bivalves; here the study reports that effects of synthetic hormones (progesterone and estradiol) and ganglionic extract injections in lipid utilization of bivalve Indonaia caeruleus (Prashad, 1918). In winter, sexually mature bivalves of Indonaia caeruleus had been kept in 5 experimental groups for 10 days and they are as follows- 1) Injection progesterone 2) Injection ganglionic extract 3) Injection sham operation 4) Injection estradiol 5) control (normal). Lipid quantitative examination from all experimental groups containing control was measured on 3rd, 6th, & 9th days. This study expresses that, the quantity of lipid was significantly increased in hepatopancreas, gonad and foot in progesterone injected group on 3rd day. Whereas, on 6th day content lipid increased significantly from hepatopancreas, gonad and foot from all the injected experimental groups compared to control. The lipid estimations on 9th day shown significant decrease in mantle and hepatopancreas from all groups compare to control group.
Key words- Lipid estimation, Progesterone, Bivalve, Cerebral ganglion, Extract, Estradiol, Freshwater.

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INTRODUCTION

In some marine as well as freshwater molluscs, bivalves are dominating filter-feeders.They form majority of the biomass and influence the ecosystems structural functionalism [1]. Bivalve molluscs may be essential feeders and additionally they directly affect benthic processes as they used to bury themselves under the sediments. The ecological importance's of bivalves are because of their distribution over large area and filtration capacity[2] and besides this it's economical utilization as a food and fresh-water pearls production [3]. According to the taxonomy given by Vokes [4], the freshwater molluscan fauna is primly belongs to 3superfamiliesUnionacae, Dreissenacae & Corbiculaecae. The freshwater bivalves belongs to Unionaceae are recorded by the candidates members of families - Unionidae and Margaritiferidae. The Unionidae family is larger family and *Indonaia* belongs to this family. The freshwater bivalves have worldwide distribution in flowing and stagnant water body. Filter feeding bivalves are environmentally or ecologically significant because they recycling nutrients, control sestons, and provide a trophic linkage between predators and primary producers [5]. It is well known that the diet of filter feeder bivalves contains majority of phytoplankton (ex. flagellates and diatoms) altogether with some other resources of food like detritus debris and bacteria [6-8]. Even though, food preferences are variable at various stages of life undergone by the bivalves, because of developmental(ontogenetic) changes the food preferences also get changes. These freshwater molluscs have prosperous stock for many essential nutrients such as vitamins, steroids, minerals and proteins. Bivalve molluscs play significant roles to maintain balance of food chain as they preferred by water birds, mammals, reptiles, and fishes as well in the river.

MATERIAL AND METHODS

Sexually mature, active and healthy bivalves, *Indonaia caeruleus* collected from back water of Godavari River during winter. The experiment had been set up and carried till 10 days. Immediately after collection, animals brought to the laboratory and washed as well as brushed to separate the sticky mud, fouling fungal as well as algal biomass. Later, bivalve molluscs having equal size(50-55 mm)have been separated in 3-4clean containers which have good aeration water and keep until 24 hrs. for lab

acclimatization. No food supplied during acclimatization and subsequent experiment. Later post-acclimatization, the separation of animals done with 5 aquaria and adequate quantity of water i.e. 11-12 Lt. /aquaria and aeration also provided. Every group includes 20-25 bivalves and water changed two times everyday having gap of roughly 12 hrs. Injections were prepared i.e. synthetic injection estradiol and progesterone0.1 mg/ml and cerebral ganglionic extract injection made in 1:1 ice cold distill water and ethanol (i.e.20 ganglia in 2ml), it was crushed and centrifuged then supernatant collected to inject bivalves; sham operated injection made by mixing 1:1 solvents (i.e. ethanol and distill water) utilized for preparation of experimental injections. The normal control group of animals kept intact for comparison purpose against experimental group bivalves. After keeping animals in 5 experimental groups, on 1st day the bivalves injected with synthetic hormones estradiol, progesterone and sham operation with 0.1µl/animal; whereas ganglionic extract injected by 0.2 µl/animal.The 5 experimental groups of bivalves are as follows- 1)progesterone injected 2) ganglionic extract injected 3) injection sham operated 4) estradiol injected and 5) normal control. The lipid estimation had been done on 3rd,6th, and 9th day respectively. Always individual 2-3 bivalves dissected by removing posterior and anterior adductor muscles; soft bodied animal removed outside shell and blotting done by blotting paper for proper weighing. Latervarious tissues like – gonad, hepatopancreas, foot and mantle segregated from animal and similar tissues crushed together for intermixing and facilitating weighing. Every tissue type has been taken 100 mg for estimation of lipid. The lipid estimation has been done by sulpho-phospho-vanilline method of Barnes and Blackstock (1973) and cholesterol used as standard. Statistical analysis of all values has been calculated; percentage and significance differences calculated for experimental groups compare to the respective control (normal).

RESULTS

The experimentation outcomes have been demonstrated in (table-1 and Fig. 1- 4(•••=p<0.001; ••=<0.01; •=<0.05)). The physico-chemical factors of water during experiment are as follows– pH (8.1-8.4); hardness (bicarbonate) (112.0- 130.0 ppm); dissolved oxygen (D.O.) content (6.1 – 7.4 mg/l/h) and Temperature (19.0^0C- 24.0^0C). Variation in the biological molecule lipid from various tissues in control, injection of ganglionic extract, hormone estradiol injected and hormone progesterone injected groups on 3rd, 6th and 9th day during winter season has shown in figure 1-4.

In winter, the lipid content from progesterone injected group, on 3rd day, decrease significantly (5.2888 ± 0.1050, 17.13 %, P < 0.01) in mantle and significantly increased (11.3393 ± 0.1503, 31.21 %, P < 0.001) in hepatopancreas, (14.9404 ± 0.1404, 18.09 %, P < 0.01) in gonadal tissue and (7.6215 ± 0.1525, 22.21 %, P < 0.01) in foot. During 6th day, lipid amount increased importantly (8.3505 ± 0.1681, 25.67 %, P < 0.01) in hepatopancreatic tissue, (10.756 ± 0.2252, 13.89 %, P < 0.01) in gonad and (8.4234 ± 0.1786, 61.49 %, P < 0.001) in foot influentially. On 9th day, the lipid content decreased significantly (5.6824 ± 0.0823, 8.24 %, P < 0.05) in mantle and significant increase found (12.8702 ± 0.1581, 11.35 %, P < 0.05) in hepatopancreas compares with controls. In injection ganglionic extract group, lipid content on 3rd day, decreased influentially (5.0263 ± 0.1012, 21.24 %, P < 0.01) in mantle, (7.3299 ± 0.1312, 15.18 %, P < 0.05) in hepatopancreas and (10.9019 ± 0.1940, 13.83 %, P < 0.05) in gonads respectively. During 6th day, the lipid quantity raised crucially (9.5168 ± 0.1210, 43.23 %, P < 0.001) in hepatopancreas, (14.9842 ± 0.3885, 58.66 %, P < 0.001) in gonad and (7.4757 ± 0.1052, 43.33 %, P < 0.001) in foot respectively. The lipid content on 9th day, decreased significantly (4.7785 ± 0.1352, 22.84 %, P < 0.05) in mantle and (8.6712 ± 0.1013, 24.98 %, P < 0.01) from hepatopancreas respectively as compare to control. Lipid quantity from estradiol injection group, during 3rd day, diminuted influentially (4.9243 ± 0.1246, 22.84 %, P < 0.001) in mantle and (11.4851 ± 0.1932, 9.22 %, P < 0.05) in gonadal tissue. Whereas 6th day shown decrease importantly (4.1953± 0.1403, 21.75 %, P < 0.01) in mantle and significant increase found (8.2047 ± 0.1744, 23.48 %, P < 0.01) in hepatopancreas, (11.4851 ± 0.1286, 21.59 %, P < 0.001) in gonad and (6.8925 ± 0.1274, 32.14 %, P < 0.01) in foot respectively. Where 9th day contrasting that, lipid content decreased influentially (5.1721 ± 0.1444, 16.48 %, P < 0.05) in mantle, (8.2047 ± 0.1885, 29.01 %, P < 0.001) in hepatopancreas, and (7.1112 ± 0.1885, 53.45 %, P < 0.001) in gonad respectively as compared with controls.

Table-1: The physico-chemical factors of water

Sr.No.	Season	Months	Temp. (0C)	pH	Hardness (ppm)	D.O. content (mg/lit.)
1	Winter	Dec.	19.0-24.0	8.1-8.27	112.0-118.0	6.1-6.8
		Jan.	18.0-22.0	8.2-8.4	115.0-130.0	6.2-7.4

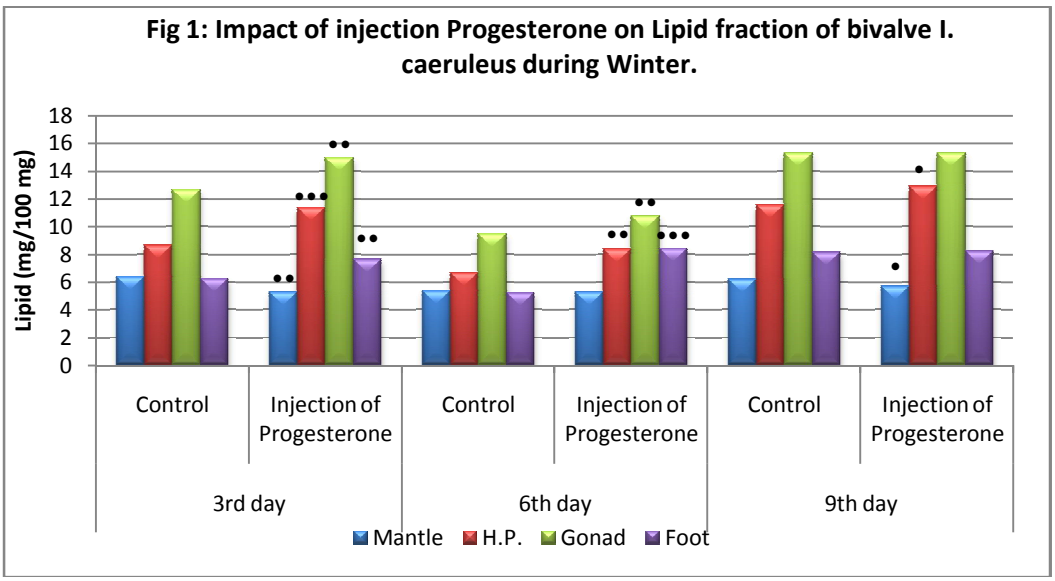


Fig 1: Impact of injection Progesterone on Lipid fraction of bivalve I. caeruleus during Winter.
Lipid (mg/100 mg)
18
16
14
12
10
8
6
4
2
0
Control
Injection of Progesterone
Control
Injection of Progesterone
Control
Injection of Progesterone
3rd day
6th day
9th day
Mantle
H.P.
Gonad
Foot

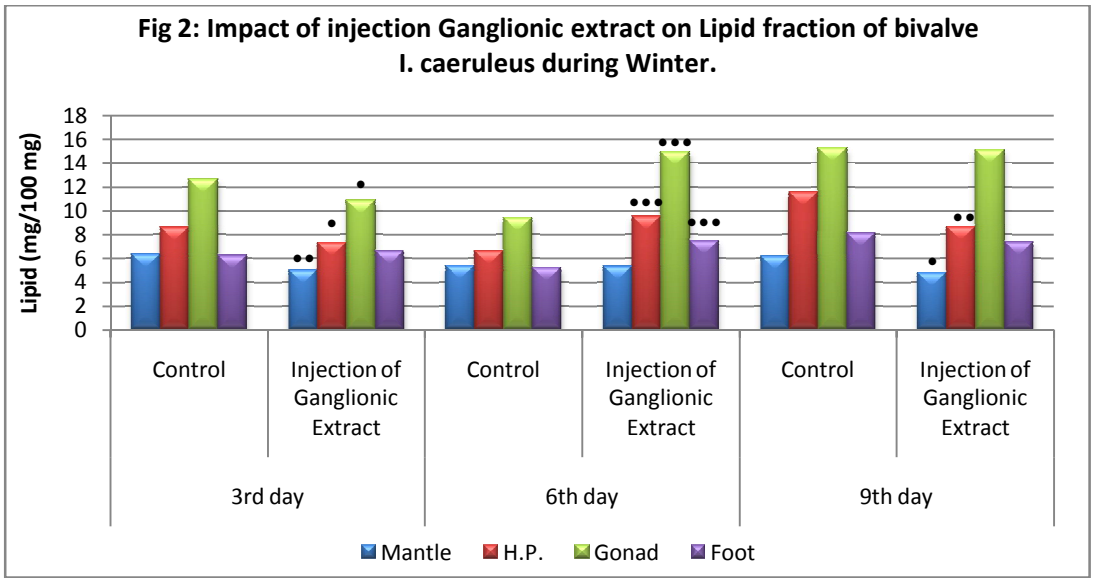


Fig 2: Impact of injection Ganglionic extract on Lipid fraction of bivalve I. caeruleus during Winter.
Lipid (mg/100 mg)
18
16
14
12
10
8
6
4
2
0
Control
Injection of Ganglionic Extract
Control
Injection of Ganglionic Extract
Control
Injection of Ganglionic Extract
3rd day
6th day
9th day
Mantle
H.P.
Gonad
Foot

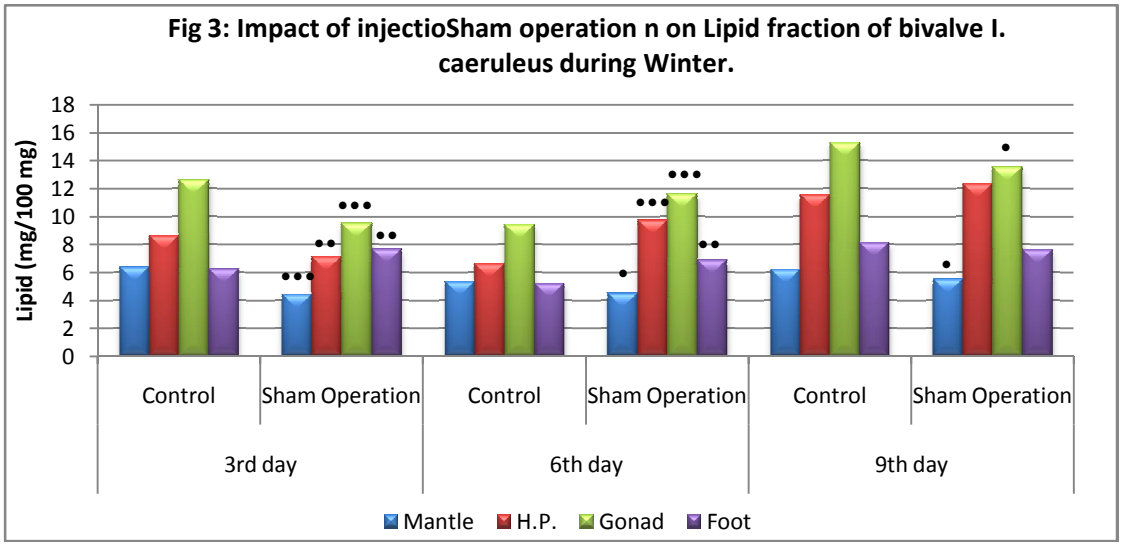
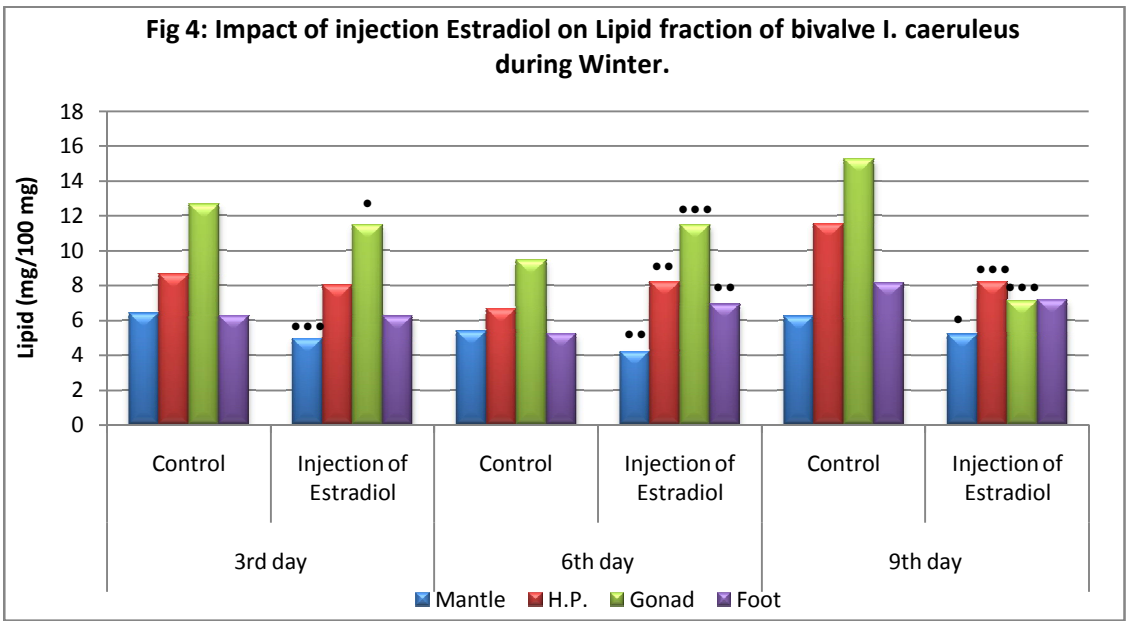


Fig 3: Impact of injectioSham operation n on Lipid fraction of bivalve I. caeruleus during Winter.
Lipid (mg/100 mg)
18
16
14
12
10
8
6
4
2
0
Control
Sham Operation
Control
Sham Operation
Control
Sham Operation
3rd day
6th day
9th day
Mantle
H.P.
Gonad
Foot



DISCUSSION

Lipids used as authoritative source for performing various metabolic roles in bivalve molluscs and act as energy storage because of high caloric cost as well as their essential utilizationin chronic stressful circumstances. Generally a lipid generates more metabolic energy and heat as compared to carbohydrates. Phospholipids, well known as structural lipids and play immense significant part in the cell membrane formation stated by Martinez-Pita [[7-9]. Lipids are vital organic part of the any tissues and play key role in energy metabolism. Later on protein and carbohydrate, lipids have the major energy producing capacity in animals. Among variety of tissues, accumulated lipids represents different forms like lipopolysaccharides, lipoproteins, cholesterol, etc. According to Vijayavel and Balasubramanian [5-7], if there is nosufficient energy in the diet or food, stored lipids present into the body can broken down to match energy requirements. Gametogenic activity in mussels is an energy-requiring activity; it needed transferring of vital nutrients from digested foodstuffs or body storage of the different organs to the reproductive organs. At the time of plentiful food availability, its storage preserved in various body parts before gametogenesis. Biomolecules like carbohydrates, lipid and proteins stored and afterward these biomolecules used for producing gametes at the time of high metabolic demand [7-10]. It has been observed that, storage of lipid in *S. constricta* female gonads shown increased earlier to bulk spawns then conspicuously decreased later on. Variations of lipid reserves during different Seasons in the female gonads have inverse relation to glycogen reserves, shows some glycogen possibly transformed to triglycerides on time of gamete formation [10-11].In this study, it has been observed that glycogen as well as protein contents in hepatopancreas and gonad decreased in monsoon as well as winter season because of synthetic injections used in experimentation suggest that these reserves has been utilized during maturation of gametes. This depletion of protein and glycogen is reflected by increase in the lipid content in gonad tissue by inter-conversion in winter season.

Mori et al. [11] states that metabolic control of proteins, glycogen and lipids inside liver can be controlled by estradiol. In addition Beninger *et al.* [12]shown pathway for nutrients via digestive track to reproductive organ and this type of transfer can change metabolism of the digestive system. A study, [13]stated that, essential difference in the biochemical components in mussels as per variations in season. In winter, both the male and female follicles of *Indonaia caeruleus* gonad show ripen gametes with partially shed gametes in some of the follicles. Whereas, the lipid globules has been considerably decreased in quantity.

CONCLUSION

It can be conclude that integrated effect of different seasonal environmental factors and experimental injections may affect significantly on the lipid content metabolism via neuroendocrine control; as ganglion regulate the metabolism of different tissues during reproductive cycle of the bivalves.

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