Variation in the protein metabolism due to injections of cerebral ganglionic extract and equivalent commercial hormones (progesterone and estradiol) in freshwater bivalves *Indonaia caeruleus* (Prashad, 1918) during winter season.

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Abstract

During seasonal changes in the environmental parameters and considering the metabolic shifts in freshwater bivalve molluscs, we report here the effect of ganglionic extract and commercial hormones injections induced changes in protein content from mantle, hepatopancreas, gonad and foot of freshwater bivalve mollusc, *Indonaia caeruleus* (Prashad, 1918) from Godavari river. During winter season, the adult bivalve mollusc, *Indonaia caeruleus* (50-55 mm shell length) were subjected to the five respective experimental groups are as follows- 1) injection of commercial hormone progesterone 2) injection of ganglionic extract 3) injection of sham operation 4) injection of estradiol and 5) control (normal) for 10 days. The protein estimation in bivalves from all four groups (including control) was measured on 3rd, 6th, and 9th day.

The study revealed that, the protein content was significantly increased from mantle and gonad as well as hepatopancreas and foot in progesterone and ganglionic extract injected group respectively. The protein content showed significant increase from hepatopancreas in progesterone injected group and similarly hepatopancreas and gonadal tissue showed significant increase in ganglionic extract injected group on 6th day. During 9th day, the protein content decreased significantly from mantle tissue in all experimental groups as compared to control.

Keywords: - Cerebral ganglionic extract, Progesterone, Estradiol, Protein estimation, Freshwater bivalve.

Introduction

The freshwater mussels (Order Unionoida) are distributed worldwide in lotic and lentic habitats. As filter feeders, freshwater mussels are ecologically important; they control seston, recycle nutrients, and provide a trophic link between primary producers and predators (Nalepa, Gardner & Malczyk, 1991). It has been known that the diet of suspension feeding bivalves consists mainly of phytoplankton (e.g. diatoms, flagellates) together with other sources of food such as bacteria and detritus debris (e.g. Parrish et al., 1998; Budge et al., 2000). However, the diet varies at different stages of the life history of the bivalve, owing in part to ontogenetic changes in feeding.

The mussels are ecologically important because of their widespread distribution and biological filtration activity (K. Lewandowski, A. Stanczykowska, 1975 and K. Kasprzak, 1986) and also economically, used as food and in the production of freshwater pearls (N. V. Subba Rao and A. Dey, 1989). Bivalve molluscs

are potential sources of valuable proteins, carbohydrates and minerals and are abundantly available in India. The biochemical composition of mollusc is influenced by its size, growth and reproductive status. Bivalves play an important role in the ecosystem equilibrium and constitute an important economic end point. The bivalves have not been the subject of intense studies despite the presence of rich diversity of edible and commercial species in India.

Organic constituents like protein act as key substances for different metabolic activities. The protein main organic nutrient used to build up different body tissues. The organic would be exported to make compensatory adjustments to both the components of energy gain and energy loss fate of changes in the environmental conditions (Vedpathak, 1989).

These bivalves are filter feeder, these bivalves in temperate climate undergo seasonal cycles in their physiological condition in association with their reproductive cycle and the nutritional requirement of these animals may also vary seasonally with the changing reproductive condition (Kreeger, 1993). Studies on changes in biochemical constituents in relation to the reproductive cycle in bivalve molluscs have been carried out extensively and have reviewed by many workers (Bayne, 1976; Sastry, 1979). Though considerable data is accumulating on several aspects of endogenous and exogenous regulation in reproduction and energy metabolism in bivalve molluscs, the data appears to be restricted exclusively and specially for marine dioecious species. Very little work on involvement of neurosecretion in reproduction and energy metabolism reported in case of freshwater species (Kulkarni, 1987).

Materials and Methods

Site selection have been done on the back water of Godavari river for collecting active, healthy and sexually mature bivalves, *Indonaia caeruleus* throughout the year in different seasons. The experimentation has been set up and carried out for 10 days during summer season. As soon as after collection of the animals from banks of Godavari river, animals brought to the laboratory and washed with tap water to remove access muddy coarse particles and brushed to remove the sticky mud, fouling fungal and algal biomass. After cleaning the animals of 50-55 mm in shell length were selected and separated in 2-3 small containers having well aerated water and kept them for 24 hours for laboratory acclimatization. No food was given to the animals during laboratory acclimatization and subsequent experimentation.

After laboratory acclimatization, the animals were separated in five (5) different aquaria with sufficient water quantity (11-12 liter) and aeration for providing oxygenated water to every animal. Each group was having 20-25 animals and water has been changed twice in a day with regular interval of 12 hours approximately and at the same time spawning, behavior and mortality if any observed on every day of experimentation. Injections were prepared before every experimentation i.e. commercial hormone injection progesterone and estradiol 0.1 mg/ml respectively; injection of cerebral ganglionic extract was prepared in 1:1 ice cold distilled water and ethanol (*i.e.*20 ganglia in 2ml ice cold distilled water and

ethanol), it was centrifuged and supernatant collected for injecting purpose; sham operated injection was prepared by using 1:1 solvent (i.e. ice cold distilled water and ethanol) used for dilution of other experiment injections. The control (normal) group has been kept as it is for comparing with the other injected (experimental) groups. After separation of animals in five groups, the aquaria were labeled and the animals injected with commercial hormones progesterone, estradiol, sham operated control with 0.1µl quantity; except ganglionic extract injection group, it was injected by 0.2 µl quantity (0.2 µl extract/animal *i.e.* equivalent to 2 ganglia/animal).

The five respective experimental groups are as follows- 1) injection of commercial hormone progesterone 2) injection of ganglionic extract 3) injection of sham operation 4) injection of estradiol and 5) control (normal). After injecting each group on 1st day of experiment, the protein estimation has been done on 3rd, 6th, and 9th day respectively and every time individual 2-3 animals dissected carefully to remove anterior and posterior adductor muscles; animal taken out from shell valve and blotted on filter paper and weighed on weighing balance. Then different tissues – mantle, hepatopancreas, gonad and foot were separated from animal body and crushed well the same tissues for intermixing and facilitate weighing. 100 mg of each tissue have been taken for estimating protein. Protein has been estimated by Lowry's et al., (1951) method by using Bovine serum albumin (BSA) as a standard. All values were subjected to statistical analysis; significance as well as percentage differences were also calculated for experimental group with compare to the intact control.

Results

The results of the experiments were shown in (Fig. 1- 4 and table 1). The physico-chemical characteristics of the water used in experiments during winter season were – Temperature (18.0^{0}C- 24.0^{0}C); pH (8.13-8.42); hardness in terms of bicarbonate (112-130 ppm) and dissolved oxygen content (6.10 – 7.45 mg/l/h).

During winter season, on 3rd day, the protein content increased significantly (11.1506 ± 0.2025, 31.41 %, $P < 0.001$) from mantle, (12.4733 ± 0.2197, 16.103 %, $P < 0.05$) from gonad and (16.733 ± 0.1521, 13.14 %, $P < 0.05$) from foot respectively. On 6th day, the content significantly decreased (7.258 ± 0.3083, 13.102 %, $P < 0.05$) from mantle and increased significantly (11.6343 ± 0.2237, 32.053 %, $P < 0.001$) from hepatopancreas. The protein content on 9th day, found decrease significantly (8.9883 ± 0.1815, 17.51 %, $P < 0.01$) from mantle and significantly increased (13.5676 ± 0.1559, 32.23 %, $P < 0.001$) from hepatopancreas compared to control. In ganglionic extract injected animals, the protein content on 3rd day, increased significantly (11.6593 ± 0.1014, 15.94 %, $P < 0.01$) from hepatopancreas as well as (17.7653 ± 0.1615, 20.12 %, $P < 0.001$) from foot and significant decrease found (8.3013 ± 0.2755, 22.73 %, $P < 0.01$) from gonad. Whereas on 6th day, the content increased significantly (11.2013 ± 0.1787, 21.14 %, $P < 0.001$) from hepatopancreas as well as (15.4756 ± 0.0954, 56.26 %, $P < 0.001$) from gonad. On 9th day, the protein decreased significantly (8.276 ± 0.1208, 24.05 %, $P < 0.001$) from mantle as well as (17.689 ± 0.0855, 9.55 %, $P < 0.05$) from foot, and significantly increased (11.7863 ± 0.352, 14.87 %, $P < 0.01$) from hepatopancreas as

compared to controls. The protein content in hormone estradiol injected animals, on 3rd day decreased significantly (9.09 ± 0.0408, 9.61 %, $P < 0.05$) from hepatopancreas as well as (7.36 ± 0.1064, 31.49 %, $P < 0.001$) from gonad respectively. On 6th day, the content found significantly decreased (12.2956 ± 0.1167, 13.74 %, $P < 0.05$) from foot. On 9th day, the protein content decreased significantly (7.8433 ± 0.1338, 23.56 %, $P < 0.01$) from hepatopancreas, (7.2073 ± 0.2532, 45.86 %, $P < 0.001$) from gonad and (13.9493 ± 0.2815, 28.63 %, $P < 0.001$) from foot respectively as compared to control. The glycogen content during winter season, in hormone progesterone injected animals, on 3rd day, decreased significantly (2.0929 ± 0.0756, 35.77 %, $P < 0.01$) in hepatopancreas and (0.8923 ± 0.0256, 23.61 %, $P < 0.01$) in foot; whereas significant increase found (3.3196 ± 0.29.38, 39.88 %, $P < 0.05$) in gonad. On 6th day, the content increased significantly (4.3533 ± 0.1271, 102.95 %, $P < 0.001$) from mantle as well as (2.0316 ± 0.0503, 48.73%, $P < 0.01$) in hepatopancreas respectively.

Table.1

Sr. No.	**Seasons**	**Months**	**Temperature (0C)**	**pH**	**Hardness (ppm)**	**Dissolved Oxygen content (mg/lit.)**
1	**Winter**	December	19-24	8.13-8.27	112-118	6.10-6.80
		January	18-22	8.25-8.42	115-130	6.22-7.45

Fig.1

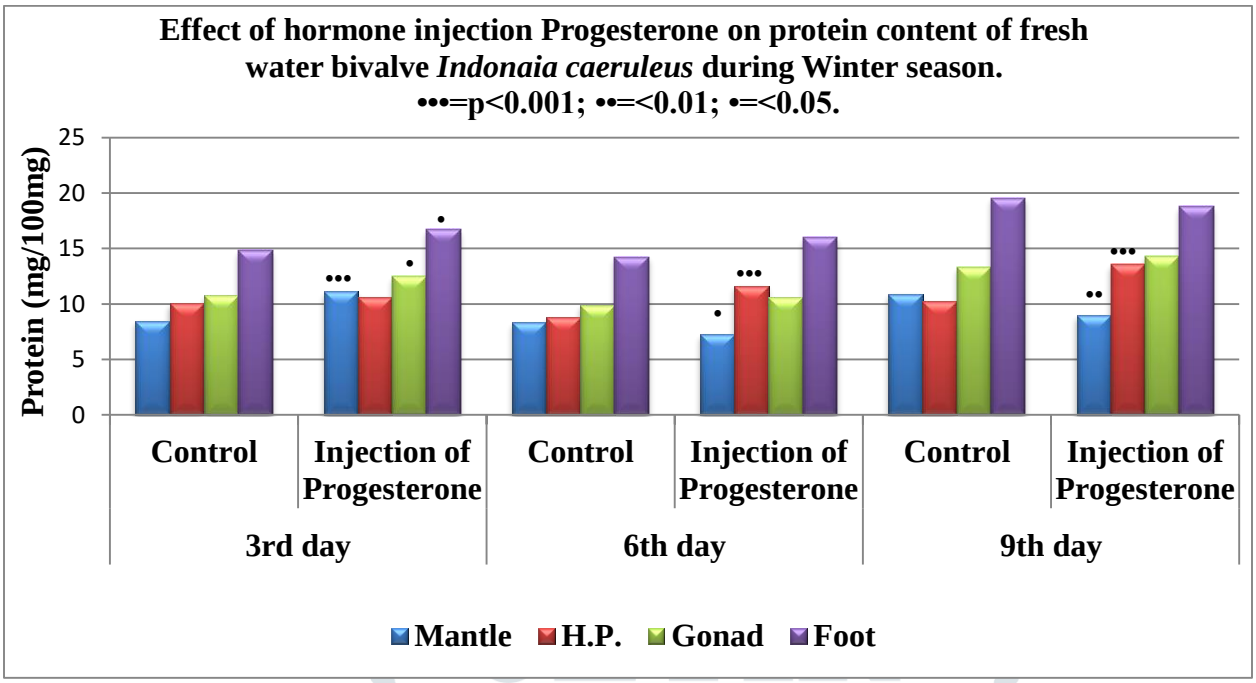

Effect of hormone injection Progesterone on protein content of fresh water bivalve *Indonaia caeruleus* during Winter season.
•••=p<0.001; ••=<0.01; •=<0.05.
Protein (mg/100mg)
25
20
15
10
5
0
Control
Injection of Progesterone
Control
Injection of Progesterone
Control
Injection of Progesterone
3rd day
6th day
9th day
Mantle
H.P.
Gonad
Foot

Fig.2

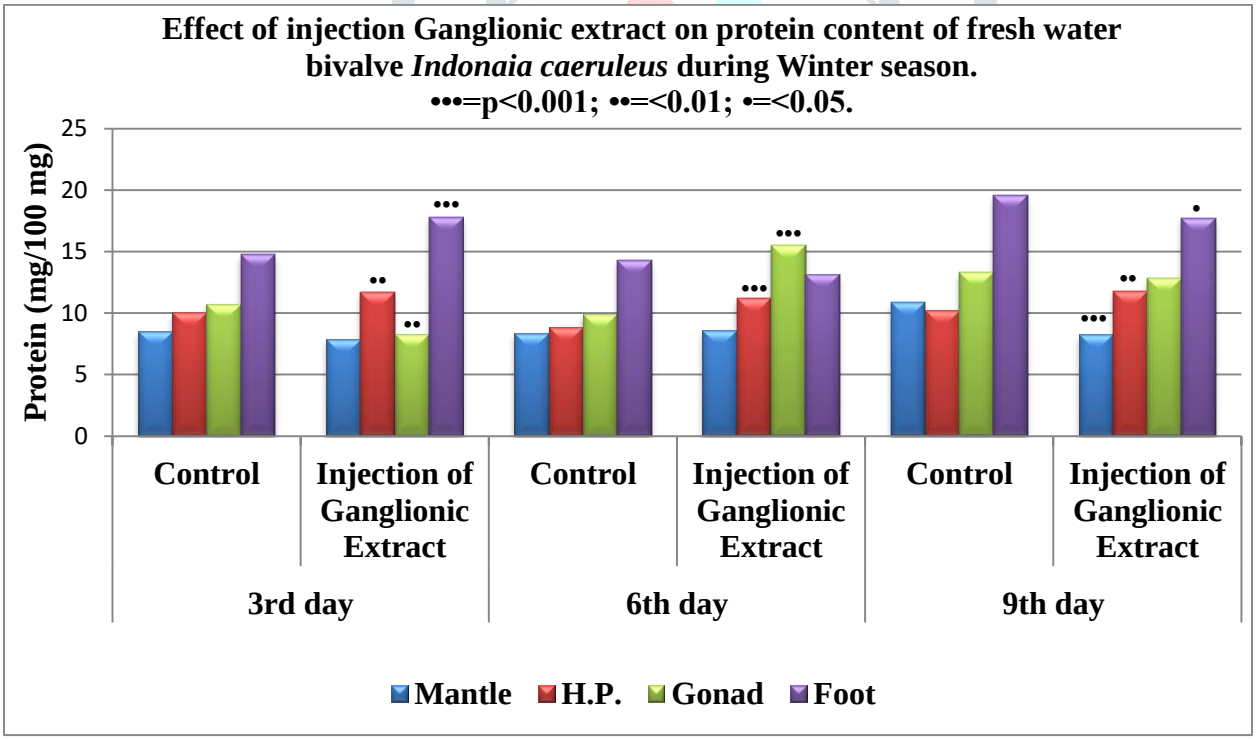

Effect of injection Ganglionic extract on protein content of fresh water bivalve *Indonaia caeruleus* during Winter season.
•••=p<0.001; ••=<0.01; •=<0.05.
Protein (mg/100 mg)
25
20
15
10
5
0
Control
Injection of Ganglionic Extract
Control
Injection of Ganglionic Extract
Control
Injection of Ganglionic Extract
3rd day
6th day
9th day
Mantle
H.P.
Gonad
Foot

Fig.3

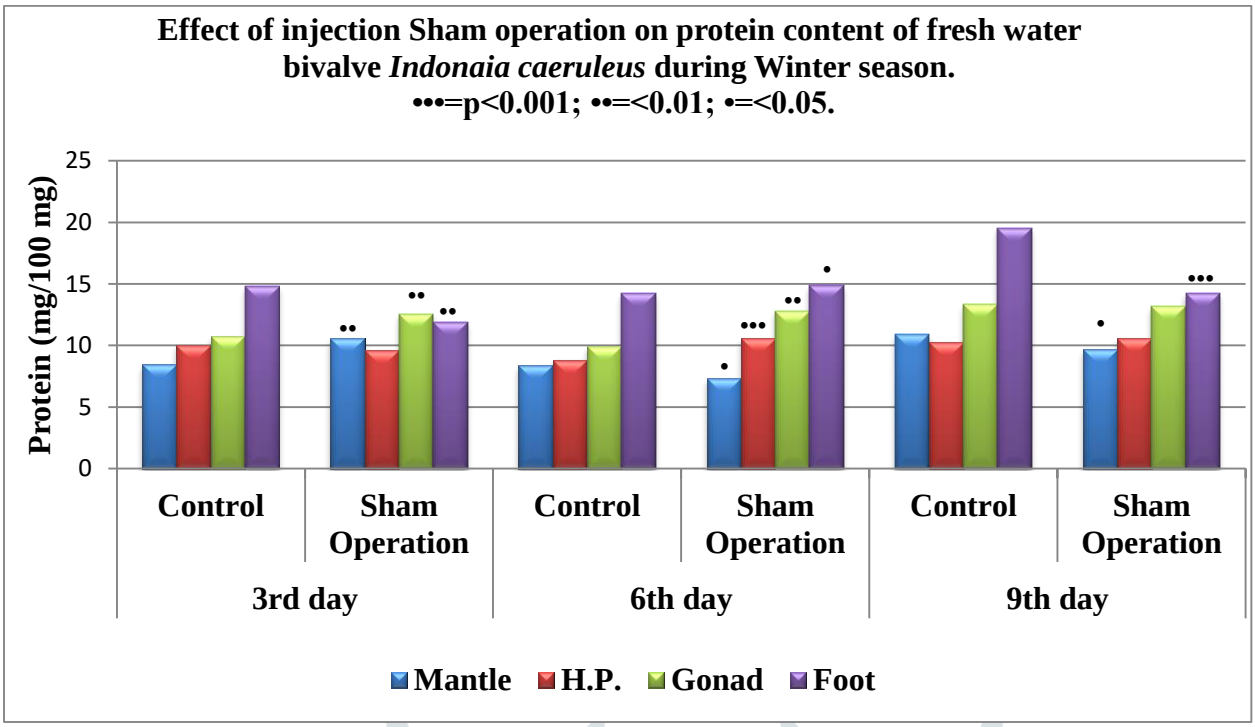
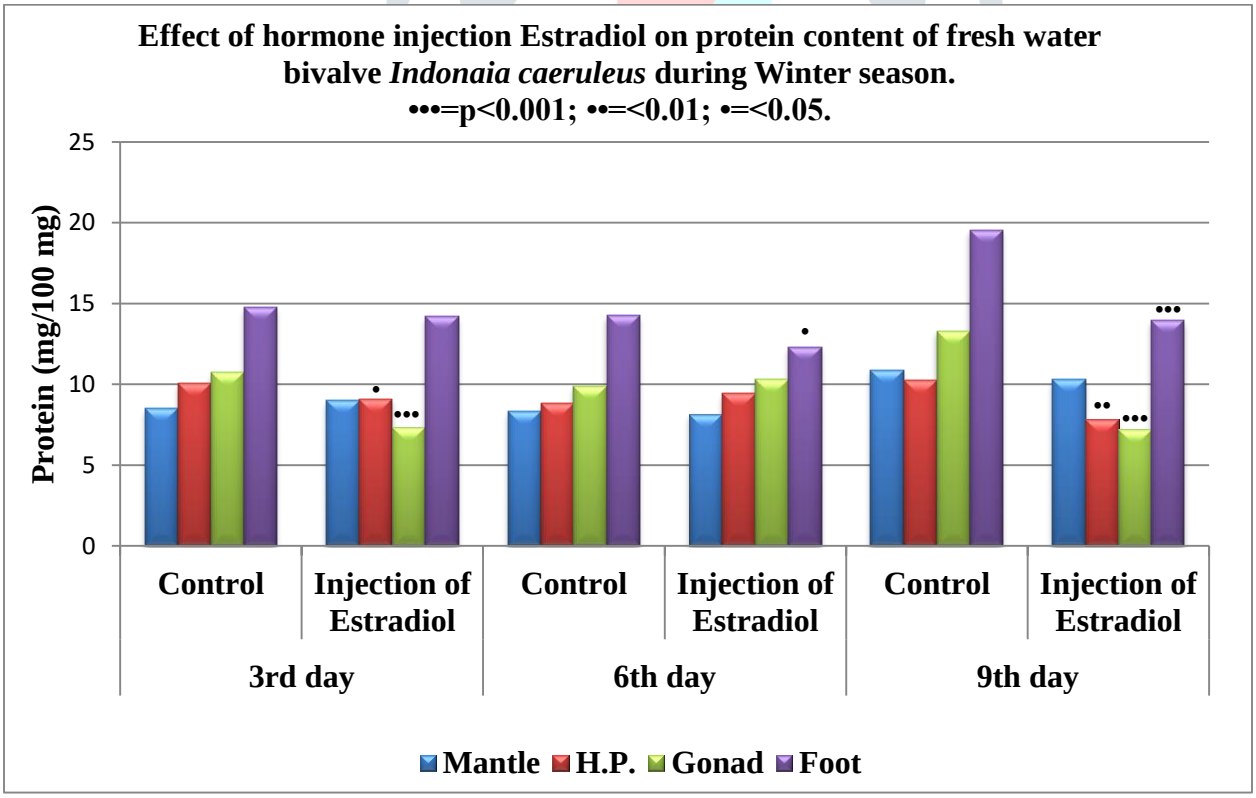


Fig.4



Discussion

The nutritional composition is an important factor governing the growth of living beings. According to Vijayavel and Balasubramanian (2006), growth represents a net outcome of a series of processes such as

digestion, assimilation, metabolism, and excretion. In this context, Brett and Groves (1979) produced a hypothetical model for average partitioning of dietary energy for organisms, which starts with an intake of 100 calories*of which 20 calories are lost as feces and 7 calories as nitrogenous waste. The cost of digestion and assimilation of the food is 14 calories for specific dynamic function, leaving a net energy of 59 calories to be used by the organism. This net energy is apportioned between maintenance activities, metabolism, and growth. Sufficient nutrients are thus essential for proper metabolism and growth. Biochemical components such as protein, carbohydrates and lipids are very essential for body growth and maintenance. Protein in tissues occurs in the form of amino acids and other metabolites, which serves as building blocks. The energy gain or depletion from the body is due to the changes in the amount of protein content of tissues (Gonzalez et al. 2001).

Protein content within each species changed more dramatically over the year than did the other biochemical constituents. Although glycogen is regarded as the major form of energy reserve in bivalves (de Zwaan & Zandee, 1972), protein reserve may be used simultaneously with carbohydrate, or even as the primary energy source (Ansell, 1972; Riley, 1976). S. B. Dongre and N. B. Dongre (2012) have observed effect of cerebralectomy on biochemical components from gonad of bivalve *L. corrianus*, and tentatively suggested that the cerebral ganglion in perhaps elaborate some factors which trigger the metabolic demand and control reproduction.

For Indian bivalves, Nagabhushanam and Mane (1975) reported correlation in organic constituents and the annual reproductive cycle of *Katelysia opima.* The entire body analyses for protein, lipid and glycogen showed that during February and September the lipid content increased and glycogen content decreased - such a condition is seen when the clams reach maturing stage. In another bivalve, *Mytilus viridis* Mane and Nagabhushanam (1975) reported that gonads of mature mussels store high levels of protein during August and January but the content decreases in the spent gonads of the mussels during September and March.

Fluctuations in the levels of sex steroids were correlated with the reproductive cycle in *S. constricta*, suggesting that they may be involved in regulating the reproduction of this species. H. Yan et al., (2010) found during their study oestradiol-17b and testosterone levels increased slightly before gametogenesis, indicating that they may be associated with sex determination and participated in the initiation of the gametogenetic cycle of *S. constricta*. Synthesis of lipid, glycogen and total proteins in molluscs may also be under the regulation of steroids (Mori, Muramatsu, & Nakamura, 1972a, b). Therefore, administration of sex steroids in molluscs may accelerate the metabolic rate in the gonad, stimulating gonadal differentiation (Croll & Wang, 2007).

Conclusion

Among the biochemical constituents, protein can be very crucial as it can utilize simultaneously with glycogen as a primary source as well as it can be converted easily to other form of biomolecules for energy production as well as to form building blocks of various tissues. It can be concluded that due to injections of ganglionic extract and commercial hormones metabolism raised up to fulfill different physiological needs including initiation of gametogenesis. Endocrine control of carbohydrate metabolism cannot be considered separately from the metabolism of protein and fats, both of which are potential source of carbohydrates by gluconeogenesis and both of which can be formed from carbohydrate residues.

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