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CHANGES IN THE AMMONIA EXCREATION DUE TO ABLATION OF CEREBRAL GANGLIA AND INJECTION OF THEIR EXTRACTS IN FRESHWATER BIVALVE MOLLUSCS *LAMELLIDENS CORRIANUS*

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ABSTRACT:

In mollusca, interactions with the environment are handled by the nervous system (including the sense organs) and the muscular system (the muscles that making the foot and those that attach the animal to its shell). Both systems, of course, have been of different forms in different group of animals. In these freshwater bivalve molluscs like *Lamellidens corrianus* ammonia excretion play an important role in the physiology; just like exogenous factors i.e. temperature, P^H, salinity etc. endogenous factors are too important for maintaining the homeostasis. Both the groups of animals i.e. cerebral ganglia ablated and cerebral ganglionic extract injected showed pronounced effect on ammonia excretion. Experiments were carried out in summer, monsoon and winter seasons.

Key words: Ammonia excretion, *Lamellidens corrianus*, seasonal changes.

INTRODUCTION:

As *Lamellidens* molluscs lives in freshwater many authors have quoted that ammonia in general is a major nitrogenous excretory product of bivalves and there occurs a profound difference in loss of nitrogen between different sizes and seasons (Bishop *et al*., 1983). This indicate shifts in physiological capacity with change in temperature, season and reproductive cycle that affect the nitrogen economy and the metabolic rate in somewhat disparate fashions. The O:N ratio is an index of protein utilization in energy metabolism. A few investigators also demonstrated the probable role of ammonia in the settlement of larvae of different bivalves. According to Coon *et al*., (1990) ammonia solution (pH 8.0) (2.5 mM concentration) induced stereotypical settlement behavior of larvae. It suggested that ammonia increased the intracellular pH. Fitt and coon (1992) stated that the actual concentration of NH_3 was associated with the surface for the oysters *Crassostrea virginica* and *Crassostrea gigas*. Increased protein catabolism is indicated by high level of ammonia excretion and decline in oxygen : nitrogen ratio (Bayne, 1976a) and thus changes in the rates of nitrogen excretion are best understood in the contest of physiological energetic and nitrogen balance, when related to overall metabolic rate by means of the oxygen : nitrogen (or O:N) ratio. According to Khalil (1994) in *Tapes decussalus* ammonia excretion rate varied with body weight, temperature, and starvation. Ammonia excretion rates were steady during six days of starvation and higher excretion rate was depended on the temperature. The ammonia excretion rate was higher for starved calms than for fed calms of all sizes and at different temperatures, weight specific ammonia excretion rates were related to dry flesh weight of starved calms but were not related to fed ones in *T. decussates* (Khalil, 1994).

MATERIALS AND METHIDS:

The freshwater bivalve molluscs, *Lamellidens corrianus* (Lea) were collected from Jayakwadi backwaters (Nathsagar) at Paithan, 45 km. away from Aurangabad. After brought to the laboratory, the shells of the bivalves were brushed and washed with fresh reservoir water so as to remove the fouling algal biomass and mud. The animals of 80-85 mm shell length were selected for experiment and they were acclimatized for 24 h. at laboratory condition in fresh aerated reservoir water (with renewal of water at the interval of 12-13 h.) and stocking capacity was given during this period and no food was given to the bivalves during laboratory acclimatization and subsequent experimentation. After 24 h., reservoir water was once again renewed and aeration was given. After a lapse of 1 h. the animals extended their organs (foot, mantle, siphons) to maximum and soon surgical operations and injection of the ganglionic extract were done. For removal of both the cerebral ganglia (bilateral cerebralectomy) following method was used. Active animal was chosen from the aquarium and a wedge (4-5 mm thick) was kept between the valves of the shell. Both the cerebral ganglia were removed by performing minimum injury to the animals within 30 seconds, with the help of fine, pointed sterilized forceps. For injection of ganglionic extracts, cerebral ganglionic extract was prepared in ice cold distilled water (10 ganglia in 1ml cold distilled water was centrifuged and the supernatant (0.2 ml/animal i.e. equivalent to 2ganglia/animal) was injected into the foot (muscular region) of normal control and gangliatomized (both cerebral ganglia ablated) bivalves. In sham operated control animals were injected by 0.2ml cold distilled water. The result for control and sham operated groups were similar and hence a comparison was made between gangliactomized and control group and extract injected to normal control as well as ablated and control group of animals only. Soon after the operation and injection of ganglionic extracts to normal control, extirpated 30 animals of cerebralectomy, 30 animals of extract injected, and 30 animals of extract injected to ablated bivalves were transferred to separate aquaria. Each aquarium contained 15 liter well aerated reservoir water, and experiment was run for 12 days. The water from each aquarium was changed at an approximate interval of 12 – 13 h. throughout experimental period. The behavior and mortality of the bivalves were recorded before each change of water from all the aquaria. The experiments were conducted for 12 days on freshly collected animals in each season i.e. summer (April - May), monsoon (July - August), and winter (December –January). The adult bivalves were placed individually in respiratory jars with 1 liter water. The rate of ammonia excretion was measured on 2nd, 7th and 12th day according to phenol-hypochlorite method of Solorzano (1969). Every time four individual animals of each group were used and mean of triplicate of water samples were estimated for each group. The statistical analysis was done to express final data. The atomic equivalent values of ammonia excretion obtained for the same individual. All the values were subjected to statistical analysis for confirmation using student ‘t’test (Dowdeswell, 1957). Statistical and percentage differences were also calculated in experimental animals.

RESULTS:

Water parameters used in the experiments i.e. temperature, pH, hardness in terms of carbonate and dissolved oxygen contents during different seasons, are given in table-1. The temperature of water was found lowest 18.5-20.5^{o}C in January (winter) and highest 31.5-33.0^{o}C in May (summer), during the study period. The pH of water was ranged between 7.4 -8.8 throughout the study period. The hardness of the water was found 80-105 ppm in April and 125-145 ppm in September.

Table 1
Physico – chemical characteristics of water during summer seasons

Sr. No.	Seasons	Months	Temperature (ºC)	pH.	Hardness (ppm)	Dissolved oxygen content (mg/lit.)
1	Summer	April	30.0 – 32.5 ºC	7.4 – 7.8	80 – 105	5.50 – 5.90
		May	31.5 – 33.0 ºC	7.5 – 7.8	83 – 112	5.25 – 5.77
2	Monsoon	August	25.0 – 28.0 ºC	7.8 – 8.4	120 – 140	6.35 – 7.80
		September	24.0 – 27.0 ºC	7.9 – 8.7	125 – 145	7.35 – 8.35
3	Winter	December	22.0 – 23.0 ºC	8.3 – 8.6	108 – 125	7.55 – 8.10
		January	18.5 – 20.5 ºC	8.3 – 8.8	113 – 134	8.15 – 8.40

The changes in the rate of ammonia excretion in the bivalves *Lamellidens corrianus from* control, ablation of both cerebral ganglia and ganglionic extract injected groups during different seasons were given in the table 2. All the values of the rate of ammonia excretion were expressed as μg-NH_3-N per liter per hour. In control groups, during summer season the rate of ammonia excretion was (0.0052 ± 0.0024) on 2nd and (0.0083 ± 0.0016) on 7th day. The rate of ammonia excretion showed significant increase (0.0214 ± 0.0070, $P < 0.001$) on 12th day compared to 2nd day. The rate of ammonia excretion showed (58.32 %) increased on 7th and (308.19%) on 12th day compared to 2nd day. During monsoon season the rate of ammonia excretion in control group was (0.0192 ± 0.0037), on 2nd, (0.0126 ± 0.0038) on 7th and (0.0214 ± 0.0036) on 12th day. The rate of ammonia excretion showed (34.08%) decrease and (11.32 %) increase on 7th and 12th day respectively. During the winter season, the rate of ammonia excretion in control group was (0.0105

± 0.0014), on 2nd day, (0.0105 ± 0.0014), on 7th and (0.0131 ± 0.0022) on 12th day. The rate of ammonia excretion does not showed any significant change on 7th day while it showed (25.0%) increase on 12th day compared to 2nd day. During the summer season the rate of ammonia excretion in ganglia ablated animals was increased significantly (0.0118 ± 0.0043, $P < 0.01$, 125%) on 2nd day, (0.0166 ± 0.0033, $P < 0.05$, 79.99 %) on 7th day, whereas the rate showed no significant decrease (0.0100 ± 0.0054, 53.05 %) on 12th day, compared to respective control. But the rate of ammonia excretion in ganglionic extract injected groups was increased (0.0148 ± 0.0022, 183.33 %) on 2nd day, and significantly increased (0.0168 ± 0.0029, $P < 0.05$, 94.74 %) on 7th day, and decreased non significantly (0.0122 ± 0.0014, 43.83 %) on 12th day compared to respective control. During monsoon season, the rate of ammonia excretion in ganglia ablated animals was decreased significantly (0.0061 ± 0.0041, $P < 0.05$, 68.18 %) on 2nd day, decreased non significantly (0.0113 ± 0.0046, 10.34 %) on 7th day and decreased significantly (0.0087 ± 0.0062, $P < 0.05$, 59.16 %) on 12th day compared to respective control. The rate of ammonia excretion in ganglionic extract injected group was decreased non significantly (0.0113 ± 0.0059, 40.90 %) on 2nd, increased significantly (0.0223 ± 0.0080, $P < 0.001$, 75.83 %) on 7th day and decreased non significantly (0.0157 ± 0.0031, 26.50%) on 12th day compared to respective control. During winter season, in ganglia ablated animals, the rate of ammonia excretion was decreased non significantly (0.0087 ± 0.0014, 16.66 %) on 2nd day. While it was increased significantly (0.0175 ± 0.0014, $P < 0.01$, 66.66 %) on 7th day and increased non significantly (0.0175 ± 0.0051, 33.33 %) on 12th dat. The rate of ammonia excretion was increased significantly (0.0245 ± 0.0037, $P < 0.01$, 133.33 %) on 2nd, (0.0315 ± 0.0014, $P < 0.001$, 200 %) on 7th day and (0.0262 ± 0.0037, $P < 0.01$, 100%) on 12th day in cerebral ganglionic extract injected groups.

Table 2

Effect of ablation of cerebral ganglia and injection of their extracts on the rate of ammonia excretion of *Lamellidens carrianus* during different seasons. (Bracket Values represents percentage difference)

= P <0.001, .. =P<0.01, ... =P<0.05

	Summer season				Monsoon season				Winter season			
Days	Control	Ablation of cerebral ganglia	Injection of distilled water	Injection of cerebral gangilonic extra	Control	Ablation of cerebral ganglia	Injection of distilled water	Injection of cerebral gangilonic extra	Control	Ablation of cerebral ganglia	Injection of distilled water	Injection of cerebral gangilonic extra
On 2nd	0.0052 ±0.0024	0.0118 ±0.0043 (125) ..	0.0087 ±0.0024	0.0148 ±0.0022 (183.33)	0.0192 ±0.0037	0.0061 ±0.0041 (68.18) ...	0. 0227 ±0.0037	0.113 ±0.0059 (40.90)	0.0105 ±0.0014	0.0087 ±0.0014 (16.66)	0.0140 ±0.0014	0.0245 ±0.0037 (133.33) ..
On 7th	0.0083 ±0.0016 (58.32)	0.0166 ±0.0033 (79.99) ...	0.0118 ±0.0016	0.0168 ±0.0029 (94.74) ...	0.0126 ±0.0038 (34.08)	0.0113 ±0.0046 (10.34) ..	0.0161 ±0.0038	0.0223 ±0.0080 (75.83) .	0.0105 ±0.0014	0.175 ±0.0014 (66.66) ..	0.0140 ±0.0014	0.0135 ±0.0014 (200) .
On 12th	0.0214 ±0.0070 (308.19)	0.0100 ±0.0054 (53.05)	0.0280 ±0.0063	0.0122 ±0.0014 (42.83)	0.0214 ±0.0036 (11.32)	0.0087 ±0.0062 (59.16) ...	0.0249 ±0.0036	0.0157 ±0.0031 (26.50)	0.0131 ±0.0022 (25.00)	0.0175 ±0.0051 (33.33)	0.0166 ±0.0022	0.0262 ±0.0037 (100) ..

DISCUSSION:

In the present study on *Lamellidens corrianus*, in control group rate of ammonia excretion was increased (0.0083 ± 0.0016) on 7th and significantly increased (0.0214 ± 0.0070) on 12th day compared to 2nd day during summer. Similarly the rate of ammonia excretion increased (0.0214 ± 0.0036) on 12th day during monsoon and (0.0131 ± 0.0022 NH_3-N/l/hr) on 12th day during winter. Increase in the rate of ammonia excretion might be due to starvation, because during starvation there is more protein catabolism hence ammonia excretion rate increases. Increased ammonia excretion indicated increased protein catabolism during starvation (Krishnakumar and Damodharan, 1986). They also stated that the mussel is dependent on protein for energy production, which is less than healthy *Mytilus edulis* from Cochin backwater. In addition, they further stated that the ratio might vary according to gametogenic cycle, nutritive reserves and nature of food. The rate of ammonia excretion showed significant increase in cerebral ganglia ablated as well as ganglionic extract injected groups in all the seasons. Rate of ammonia excretion showed more increase in extract injected group during winter. It is well known fact that the high stress conditions during drought or severe winter conditions has been conquered by several lineage of gastropods and sand bivalves, and their ability to enter the resistant or dormant stages (low food) protein catabolism increased. Many workers have compared the excretion rates of bivalves related with their environment. Boucher and Boucher (1990) have demonstrated the ammonia exchange processes of *Crassostrea gigas* and sediment. They compared the oyster biomass and lighting conditions (light and dark) with oxygen and ammonia exchange rates. The oxygen consumption increased with increasing biomass but the respiration rate was decreased, whereas, ammonia released never matched the rate predicted by adding sediment efflux to oyster excretion. The O:N ratio was influenced by oyster biomass. Further, the author suggested that both the organism and sediment were involved in regulation process (Boucher and Boucher, 1990). Food availability and the cells rapidly filled with material. With the close of monsoon, from October NM absorption phases extended till November NM. This was correlated with longer feeding period under short supply of food and further extended till February FM, showing tubules in either holding or absorption phases. The water temperature decreased with the onset of winter and no feeding occurred for a short period of November FM when the tubule showed re-organization phase (Saokar, 1994).

The present work was undertaken with the view to understand the impact of endogenous parameters particularly neuroendocrine on the aspects of rate of ammonia excretion. It reveals that cerebral ganglia ablation and cerebral ganglionic extract injection might posse's some effect on ammonia excretion which varied seasonally also affected by some exogenous factors.

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