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EFFECT OF ESSENTIAL AMINO ACID L-LYSINE ON BIOCHEMICAL PARAMETERS OF FRESHWATER BIVALVE *LAMELLIDENS MARGINALIS* (LAMARCK, 1819)

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ABSTRACT:

With an average increase in global production of bivalves by 5% per year (Pawario, 2010), one of the limiting factors of its industry is the limited information on their nutritional requirement. It has been reported that marine bivalves have the ability to absorb certain dissolved nutrients either through their general body surface or through specialized organs like ctenidia. This has opened an avenue of understanding the nutritional requirements as well as the effect of individual nutrients on bivalves. With this view point, this study aims in understanding the effect of essential amino acid lysine on the biochemical changes in different tissues of freshwater bivalve *Lamellidens marginalis* (Lamarck, 1819).

The freshwater bivalves were divided into four groups as A, B, C and D. 'A' group of bivalves was maintained as control. Group 'B', 'C', and 'D' of bivalves were exposed to concentration 25mg L-Lysine/l of water (25.045 ppm), 50mg of L-Lysine /l of water (50.089 ppm) and 100mg of L-Lysine/l of water (100.179 ppm) respectively for 28 days. The animals were then sacrificed and dissected open to remove gills, foot and hepatopancreas. The tissues were homogenized and the homogenate was used for estimation of biochemical parameters like carbohydrate, lipid and protein. The data obtained was analyzed by one way ANOVA; $p < 0.001$. it was found that there was significant difference between the Carbohydrate

($F_{(gill)}$ = 23.600; $F_{(foot)}$ = 5.776 ($p \leq 0.003$); $F_{(hepatopancreas)}$ =45.422), Lipid ($F_{(gill)}$= 3.971 ($p \leq 0.0197$); $F_{(foot)}$ =16.697; $F_{(hepatopancreas)}$ = 6.005 ($p \leq 0.004$) and protein ($F_{(gill)}$= 27.357; $F_{(foot)}$ = 29.704; $F_{(hepatopancreas)}$ =20.002) content in different tissues subjected to different concentration of amino acid L-Lysine. From the present study it is clear that bivalve when exposed to different concentration of L-Lysine showed significant biochemical changes.

KEY WORD: *L-Lysine, Biochemical parameters, Lamellidens marginalis, Bivalve.*

INTRODUCTION:

The bivalves are in great demand in diverse fields such as shell industry, pearl industry, lime and handicrafts. Their meat, rich in protein, is consumed by humans as well used as feed in varied aquaculture. Global aquaculture production of bivalves has consistently increased from 72.8% in 1993 to 87.3% in 2005 with respect to overall bivalve production. Simultaneously, the wild harvest production showed a downward trend from 21.5% to 12.7% for the said duration (Pawiro, 2010). The bivalve industry is thus relying more on aquaculture production for optimum harvest. One of the important aspects of aquaculture is nutrition. Bivalves are filter feeders. Research on nutritional requirements of bivalves is limited. One of the reasons is difficulties in defining 'balanced diet' within an artificial food particle of suitable size for such filter feeders. This paucity of data on their nutritional requirement is one of the limiting factors for bivalve industry in producing artificial food for their mass production (Knauer and Southgate, 1999).

The nutritional requirement as well as its role has been explored to certain extent in marine bivalves. It has been reported that different life stages of marine bivalves are capable of absorbing dissolved nutrients, such as amino acids (Manahan, 1983), glucose (Nell *et al.*, 1983), fatty acids (Bunde *et al.*, 1978), and vitamins (Nell *et al.* , 1983). This absorption of dissolved nutrients occurs across the body wall or via specialized organs such as ctenidia (Pajor and Wright, 1989) of molluscs. Absorption of dissolved nutrients by bivalves has opened a new avenue of understanding their nutritional requirements as well as the effect of individual nutrients on them. With this view point, this study was aimed in understanding the effect of essential amino acid lysine (absorbed in the body via general body surface) on the biochemical changes in freshwater bivalve *Lamellidens marginalis* (Lamarck, 1819).

An essential amino acid, Lysine (basic amino acid, $C_6H_{14}N_20_2$, Molecular Weight: - 146.19) is important for proper growth of humans and animals. It lowers cholesterol level and helps in absorption of calcium. Nutritional requirement of Lysine varies from animal to animal. Lysine

deficiency studies in animals reported reduced growth (Kliger, 2010), lowered immunity (Khalil *et al*., 2010), stress-induced anxiety (Smriga, 2002), increased mortality (Kliger and Krehl, 2010), and fin-rot (Ketola, 1983). Researches are being carried out to know its dietary requirement to bring out optimal growth in animals. It has been found that dietary supplementation of Lysine has influence on growth performance, biochemical composition and protein metabolism (Walton *et al*., 1984).

MATERIAL AND METHODS:

Chemicals:

L-Lysine, crystalline pure (98%) free base, was brought from HiMedia Laboratories Pvt. Ltd. Other chemicals of analytical grade were brought locally. L-Lysine concentrations were prepared by dissolving required amount of it in distilled water.

Animal (collection and maintenance):

Lamellidens marginalis were collected from Barvi Dam, Badlapur. Immediately after bringing them to laboratory, the shells of these bivalves were brushed and washed with 48 hrs. aged water to remove algal biomass, mud and other waste material. The bivalves were then acclimated under laboratory condition for 15 days. During this period they were kept in freshwater aquarium (Temperature 28 ± 2^0C; pH 7.15 ± 0.4; DO 7.8 ± 0.93 mg/lit; Salinity 0.86 ± 0.07 parts per thousand) under normal photoperiod of 13L and 11D. The active acclimated bivalves of size 8.6 ± 0.7 cm and weight 49.74 ± 3.66 gm were selected for the experiment.

Experiment:

The acclimated bivalves were grouped in four different groups as A, B, C and D with each group having eight of them kept in plastic troughs. ‘A’ group of bivalves was maintained as control. ‘B’ group of bivalves was exposed to concentration of 25mg L-Lysine/l of water (25.045 ppm), ‘C’ group of bivalves was exposed to concentration of 50mg of L-Lysine /l of water (50.089 ppm) and ‘D’ group of bivalves was exposed to concentration of 100mg of L-Lysine /l of water (100.179 ppm) for 28 days. During this period the bivalves were fed with paste of finely powdered artificial diet (Shingadia and Shaktivel, 2006) twice daily. The ratio of bivalve to water was l bivalve per litre. The water with appropriate concentration of the L-Lysine was changed every day.

Tissue analysis:

After the exposure period, the bivalves were dissected open to remove the gills, hepatopancreas and foot. The tissues were homogenised to analyse the biochemical contents in each tissue.

Protein analysis (extraction and estimation): - The protein was extracted using 1 N NaOH and later neutralised with 1N HCl. The preparation was centrifuged at 2000 rpm for 10 mins and an aliquot of

1 ml from the supernatant diluted with 9 ml of Distilled water was used for protein estimation. The protein estimation was carried out by Lowry's method (Lowry *et al*., 1951).

Carbohydrate analysis (extraction and estimation): - Carbohydrate extraction was done by using 2.5N HCl and later neutralised with solid sodium carbonate. The preparation was centrifuged and an aliquot of 0.5 ml from the supernatant diluted to 10 ml with distilled water was used for carbohydrate estimation. The carbohydrate estimation was carried by Anthrone method (Seifter *et al*., 1950).

Lipid analysis (extraction and estimation): - Lipid analysis was carried out by Folch method (Folch *et al*., 1957). The tissue was homogenised with chloroform methanol (2:1) and later agitated for 15-20 mins at room temperature. The homogenate was filtered and the filtrate was washed with 0.9% NaCl solution. The filtrate was vortexed and centrifuged to separate the two phases. The lower phase was siphoned and evaporated.

Statistical analysis: Data were expressed as mean ± standard deviation and analysed by 2-way ANOVA ($P < 0.001$), & Student's t-test ($P < 0.05$).

RESULTS AND DISCUSSION:

Protein: -The protein content observed in various tissues of *Lamellidens marginalis* during the experiment is given in table no. 1. The maximum protein content was observed in gills followed by foot and hepatopancreas in control. All the three tissues showed the same trend for protein content changes in different treatment concentrations. There is steady decrease in protein content from control to 50.089 ppm but an increase in protein content for the 100.179 ppm is seen for all the tissues. The protein content in 50.089 ppm concentration showed significant decrease for all the tissues. The protein content for foot in 100.179 concentration (10.03 $\pm$ 0.593) was the highest, and it was significantly more than the protein content of foot for control (8.89 $\pm$ 0.352). This observation is in sync with hyper foot activity for the same concentration.

Experiments involving treatment by lysine via diet showed increase in whole body protein content in *Etroplus suratensis* (Palavesam *et al*. , 2008**),** *Clarius gariepinus* (Fasakin *et al*., 2006), *Rachycentron canadum* (Zhou *et al*. , 2007), *Myxocyprinus asiaticus* (Lin *et al*., 2012), *Ctenopharyngodon idella* (Yang *et al*. , 2010). In all the above experiments tissue specific studies were not carried out.

Any stressful condition alters the biochemical composition. *Lamellidens marginalis* when exposed to different stressful conditions showed steady decrease in protein content with increasing concentration or duration of exposure of stressors. *Lamellidens marginalis* when exposed to acute concentration of tributyltin chloride showed significant and steady decrease in protein content as compared to control for 24hrs, 48hrs, 72hrs, and 96hrs exposure (Jagtap *et al*., 2011). The same was seen when they were

exposed to detergent wheel (Shingadia and Shaktivel, 2006), ammonia (Ramesh *et al*., 2011), colour pigments (Phadnis *et al*., 2013). The % metabolic transformation of U-^{14}C lysine injected in rats fed with excess lysine supplemented diet, showed significant decrease in radioactivity recovery in the protein fraction of both liver and muscle as compared to the rats fed with balanced lysine supplemented diet (Yamashita and Ashida, 1971).

The observation obtained in the current experiment is neither in accordance with experiments involving feeding animals with dietary lysine nor in accordance with experiments involving exposure of *lamellidens marginalis* to various toxicants. To get an insight, more elaborative work involving enzyme assay associated with protein metabolism as well as stress protein assay is needed.

The data obtained from biochemical analysis was analyzed by one way ANOVA; $p < 0.001$. It was found that there was significant difference in protein content of different tissues subjected to control and different concentration of amino acid L-Lysine. The details of it is given in table no. 2

Carbohydrate: - The observation for carbohydrate content in three different tissues of *Lamellidens marginalis* for control and treatment concentrations is given in table no. 1. The carbohydrate content was higher in hepatopancreas as compared to other tissues as it is the storage house for carbohydrates as glycogen. At 25.045 ppm concentration, the hepatopancreas showed significant decline in carbohydrate content, while for the same concentration the other two tissues showed significant increase in carbohydrate content with reference to control. For the other two treatment concentrations, in hepatopancreas there is steady increase in carbohydrate content whereas there is steady decrease in foot and gills for the same. The decrease in carbohydrate content in of 25.045 ppm concentration for hepatopancreas probably may be due to mobilization of carbohydrates from it to other tissues like gills and foot which showed increased carbohydrate content for the same concentration.

Very limited references are available on the effect of lysine on carbohydrate content / metabolism. The carbohydrate content increased in *Etroplus suratensis* when fed with protein rich diet supplemented with L-Lysine (Palavesam *et al*., 2008). The carbohydrate / glycogen content decreased when treated with stressors like copper sulphate (Padewar *et al*., 2011; Satyaparameshwar *et al*., 2006), phosphamidon (Moorthy *et al*., 1983), colour pigments (Phadnis *et al*., 2013), ammonia (Ramesh *et al*., 2011).

Detailed biochemical analysis involving glycogen, pyruvic acid, lactic acid content supplemented with assay of enzymes such as succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (G-6-PDH) activity can give a better picture on the effect of L-Lysine on carbohydrate metabolism in *Lamellidens marginalis.*

The data obtained from biochemical analysis was analyzed by one way ANOVA; $p < 0.001$ as given in table no. 2. It was found that there was significant difference in carbohydrate content subjected to control and different concentration of amino acid L-Lysine for different tissues.

Lipid: - The observation for lipid content in different tissues of *Lamellidens marginalis* is given in table no. 3. The lipid content is more in foot for control and treatment concentrations as compared to other tissues. The lipid content of foot and hepatopancreas for treatment concentration 25.045 ppm showed significant decrease with respect to control whereas for the same concentration, gills showed an increase in lipid content. For the rest treatment concentrations, the foot and hepatopancreas showed increase in lipid content. In case of gills for the rest of the treatment concentrations, the lipid content decreased. For foot as well as hepatopancreas of all the treatment concentrations, the lipid content was found to be less than that of control.

The lipid content of *Etroplus suratensis*, when fed with protein rich diet supplemented with lysine showed increase in lipid content with reference to the animals fed with protein rich diet sans lysine, but the lipid content in both the test groups showed significant decrease in lipid content as compared to animals fed with control diet (Palavesam *et al*., 2008). Juvenile Chinese sucker *Myxocyprinus asiaticus*, showed significant decrease in whole body lipid content when fed with increasing levels of dietary lysine (Lin *et al*., 2012). Juvenile grass carp, *Ctenopharyngodon idella*, showed decrease lipid content in whole body, white muscle and liver when fed with diet supplemented with lysine and methionine. There was significant decrease in whole body lipid content as compared to the grass carps fed with control diet not supplemented with lysine and methionine (Yang *et al*., 2010).

In case of Juvenile cobia *Rachycentron canadum* whole body lipid content was not affected by dietary lysine (Zhou *et al*., 2007). Similar result was also seen in juvenile yellow cat fish, *Pelteobagrus fulvidraco*, (Cao *et al*., 2012). Stressors like ammonia also caused decrease in lipid content in *Lamellidens marginalis* (Ramesh *et al*., 2011). The data obtained from biochemical analysis was subjected to one way ANOVA; $p < 0.001$. It was observed that there was significant difference in lipid content subjected to control and various concentration of amino acid L-Lysine for different tissues. The details of it is given in table no. 2.

CONCLUSION:

The objective of this study was to know the biochemical changes induced by L-Lysine on *Lamellidens marginalis* via absorption through general body surface. From the present study it is clear that bivalve when exposed to different concentration of L-Lysine showed significant biochemical changes. The observations obtained need to be fortified with studies involving various

enzyme assays, stress protein assays, study of influx of amino acids via general body surface and gills, factors affecting such influx, change in L-Lysine content in free as well as in total amino acid in the different tissues. Such studies can help in understanding the biochemical changes brought in by L-Lysine on *Lamellidens marginalis.* Finding the optimum concentration of different amino acids and other nutrients for bringing out optimum growth, or enhancing a particular function (e.g. increased rate of pearl formation or increased absorption of toxicants for detoxification of the water body or its own detoxification) in bivalves through a different route of entry may be studied by this method.

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Table No. 1

Biochemical content in mg % of different tissues of *Lamellidens marginalis* exposed to differe concentrations of L-Lysine for the duration of 28 days.

Tissues	**Control**	**25.045 ppm**	**50.089 ppm**	**100.179 ppm**
Protein				
Gills	**9.13 ± 0.74**	**6.65* ± 0.85**	**5.78* ± 0.66**	**6.049* ± 0.71**
Foot	**8.89 ± 0.35**	**8.41 ± 0.21**	**7.95* ± 0.50**	**10.03* ± 0.59**
Hepatopancreas	**8.07 ± 0.84**	**7.30 ± 0.89**	**5.01* ± 0.57**	**6.41* ± 0.90**
Carbohydrate				
Gills	**0.964 ± 0.13**	**1.294* ± 0.16**	**0.868 ± 0.24**	**0.816* ± 0.11**
Foot	**1.530 ± 0.10**	**1.857* ± 0.28**	**1.588 ± 0.13**	**1.549 ± 0.09**
Hepatopancreas	**5.698 ± 0.30**	**3.503* ± 0.42**	**4.360* ± 0.28**	**5.237* ± 0.27**
Lipid				
Gills	**1.114 ± 0.177**	**1.285 ± 0.134**	**1.057 ± 0.161**	**0.985 ± 0.106**
Foot	**2.47 ± 0.111**	**2.057* ± 0.127**	**2.128* ± 0.125**	**2.414 ± 0.157**
Hepatopancreas	**0.816 ± 0.075**	**0.65* ± 0.054**	**0.733 ± 0.081**	**0.783 ± 0.075**

'*' - indicates significant difference at $P \leq 0.05$ (Student t – test) with reference to Control.

Table No. 2				
Results of one way Anova test; $p < 0.001$ for various tissues of *Lamellidens marginalis.*				
Biochemical parameters	**Tissue**	***F***	***P-value***	***F crit***
Protein	**Gills**	**27.357**	**0.001**	**2.947**
	Foot	**29.704**	**0.001**	**2.947**
	Hepatopancreas	**20.002**	**0.001**	**2.947**
Carbohydrate	**Gills**	**23.600**	**0.001**	**2.947**
	Foot	**5.776**	**0.003**	**2.947**
	Hepatopancreas	**45.422**	**0.001**	**3.098**
Lipid	**Gills**	**3.971**	**0.020**	**3.009**
	Foot	**16.697**	**0.001**	**3.009**
	Hepatopancreas	**6.005**	**0.004**	**3.098**

Chart 1: Protein content in mg % of different tissues of Lamellidens marginalis exposed to different concentrations of L-Lysine for the duration of 28 days.

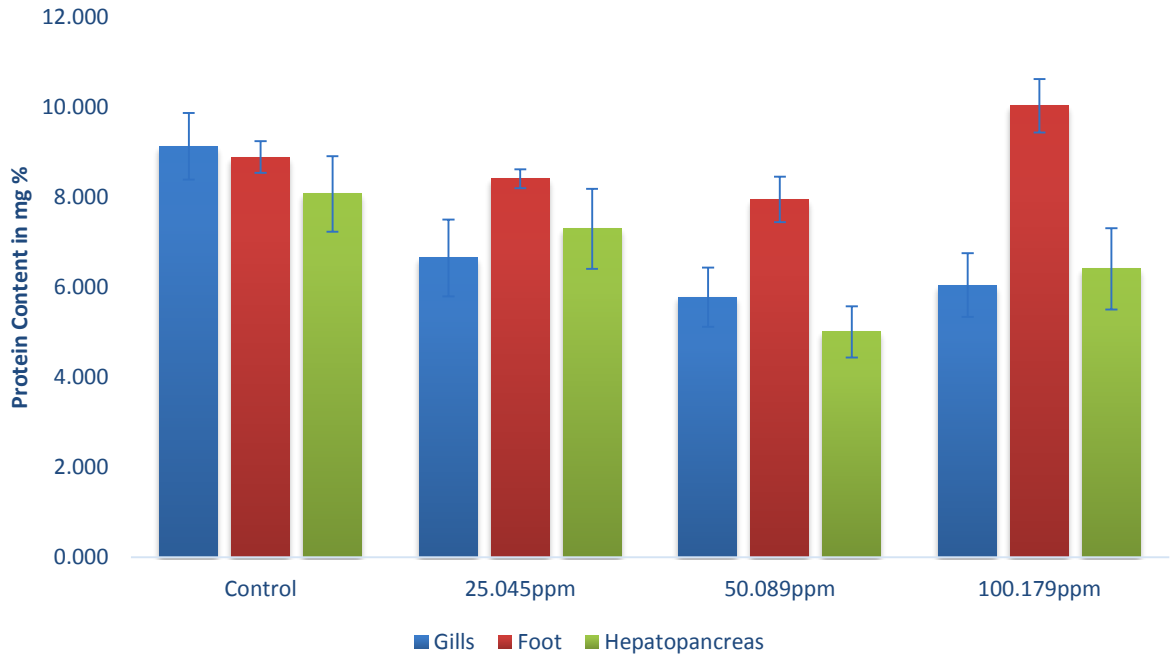

Chart 2: Carbohydrate content in mg % of different tissues of Lamellidens marginalis exposed to different concentrations of L-Lysine for the duration of 28 days.

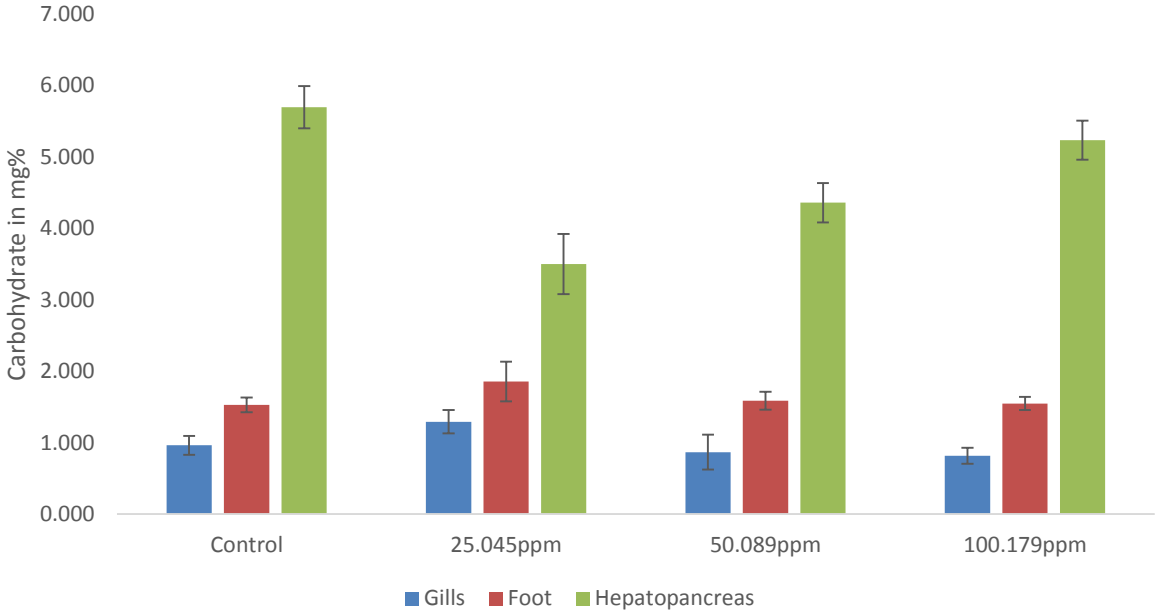

Chart 3: Lipid content in mg % of different tissues of Lamellidens marginalis exposed to different concentrations of L-Lysine for the duration of 28 days.