Fishery Technology
1994, Vol. 31(1) pp: 4-7

Effect of MS-222 on Basal Metabolism of *Penaeus indicus* Seeds

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In laboratory experiments, 150 ppm of MS-222 showed the best effect in delaying the 50 and 100% mortalities respectively to 823 and 1035 min in *Penaeus indicus* seeds of 15-40 mm sizes as against 293 and 420 min in untreated controls. As a result of the treatment, oxygen consumption was reduced from 0.00859 to 0.00385 ml/mg/h, ammonia excretion from 0.00017 to 0.000022 ppm/mg/h and ammonia quotient from 0.01979 to 0.00571. While carbohydrate and lipid in the tissue of anaesthetised prawns decreased to 0.78 and 3.91% (as is) from 0.82 and 4.08 of non-anaesthetised ones respectively, the protein content increased to 19.28 from 17.21%. The changes in the animals indicate that they were at rest under the influence of the chemical and their death was due to oxygen deficiency developed in the water on account of consumption.

Shrimp farming in the country is fast picking up. Shrimp seeds, whether from the wild or from hatcheries, for various reasons, are not available at many farm sites. In most cases, they are being procured from far off places and transported live suffering heavy mortalities. In fish, various anaesthetics are known to reduce stress and mortality during handling and transport. Such work on crustaceans, on the other hand, are very scarce. Tricaine (MS - 222) the methane sulfonate of 3-amino benzoic acid ethyl ester is a chemical used since 1920s for anaesthetising fish. Since no study was carried out on the effect of MS-222 on prawns, an attempt as under was made on the seeds of *Penaeus indicus* H. Milne Edwards.

Materials and Methods

P. indicus of 15 to 40 mm sizes, acclimated to 4 days in the laboratory in well aerated water of 15 ‰ salinity changed on every other day with the left-over feed and faecal matter removed daily formed the test animals. After an initial range finding, concentrations of 50, 100, 150, 200 and 250 ppm MS - 222 in the medium along with control were used for the experiment in which 50 seeds starved for 24 h were released into one litre of water in sealed glass containers. Sodium hydroxide of 1 N was used after Wedemeyer (1970) to rectify reduction in pH of the medium caused by dissolving MS - 222 in it. Oxygen, ammonia and pH were recorded at the beginning and end of the expriment and the time for 50 and 100% mortality were noted down following Mc Farland (1960) to judge the chemical's anaesthetic property. At the end of the experiment, the prawns were sacrificed for biochemical analysis. The prawn tissues were analysed for lipid, protein and carbohydrates by Sulphophosphovanillin method (Barnes & Blackstock 1973), Lowry's method (Lowry *et al.*, 1951) and Phenol-sulphuric acid method (Dubois *et al.*, 1956) respectively. The salinity was estimated by Mohr's method and the oxygen by Winkler's method (both after Strickland & Parsons, 1968) and total ammonia content by Solarzano's (1969) method. The hydrogenion concentration was read using a Toshnival pH meter. All experiments were done in triplicate.

Results and Discussion

The temperature of the medium during the experiment ranged from 22 to 26°C. Dis-

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solved oxygen content was around 4.33 ml/l, ammonia 0.15 ppm and pH between 7.8 and 8.1.

In range findings, MS - 222 at 25 ppm did not have any effect on 50 and 100% mortality times, but at 50 ppm and above, the treated seeds showed higher times of mortality than untreated controls, the highest being at 150 ppm. Higher concentrations than 250 ppm resulted in toxicity (Fig. 1).

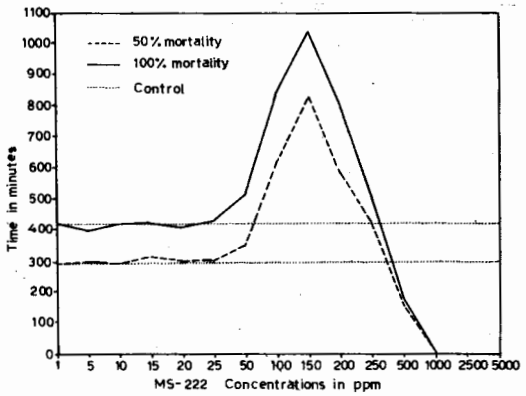

Fig. 1. Time for 50 and 100% mortality of *Penaeus indicus* exposed to different concentrations of MS-222 in range finding

Maximum 50 and 100% mortalities occurred respectively at 823 and 1035 min with 150 ppm concentration as against 293 and 420 min in control without the anaesthetic. The variation of these from the control (Fig. 2) were 180.89 and 146.43% respectively.

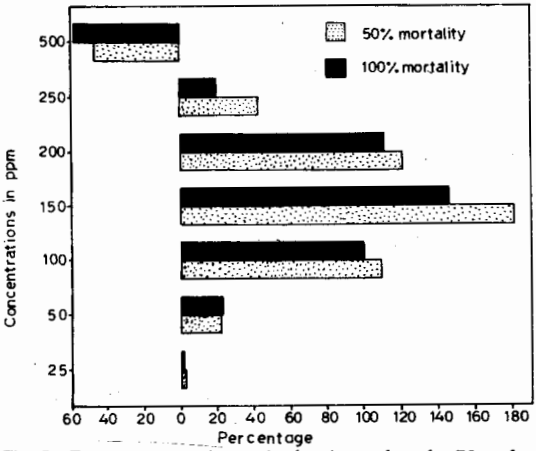

Fig. 2. Percentage variation in the time taken for 50 and 100% moralities in anaesthetised prawns from non anaesthetised ones in control

Rates of oxygen consumption, ammonia excretion and ammonia quotient which were 0.00859 ml/mg/h, 0.00017 ppm/mg/h and 0.01979 respectively in control dropped to 0.00385 ml/mg/h, 0.00002 ppm/mg/h and 0.00571 in 150 ppm concentration (Fig. 3). The percentage variation of these from control at the highest points were 55.18, 87.06 and 71.15 respectively.

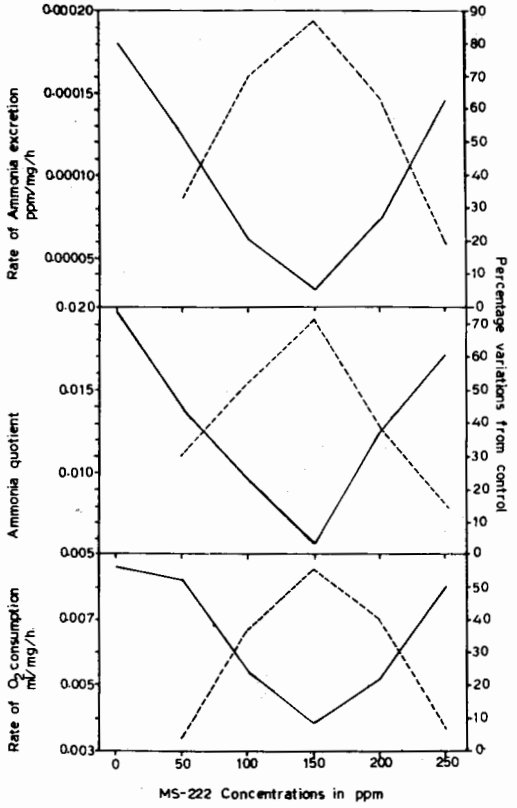

Fig. 3. Rate of ammonia excretion, ammonia quotient and rate of oxygen consumption in MS-222 treated prawns (–) and their percentage variations from those in control without it (---).

Levels of protein, lipid and carbohydrates in g/100 g of body wet weight in the control animals were 17.2, 4.1, and 0.8 respectively (Fig. 4). While the lipid and carbohydrates decreased to 3.9 and 0.78 respectively, in the treated seeds, the proteins increased to 19.28 at 150 ppm. Their decreases were 4.29 and 4.57% from the control respectively (Fig. 5). On the other hand, the protein increased by 12.05%.

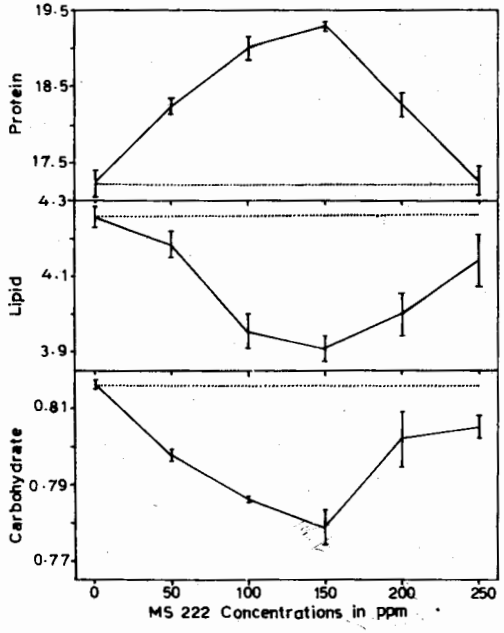

Fig 4. Levels of protein, lipid and carbohydrate contents (in mg/100 mg of body wet weight) with standard deviation

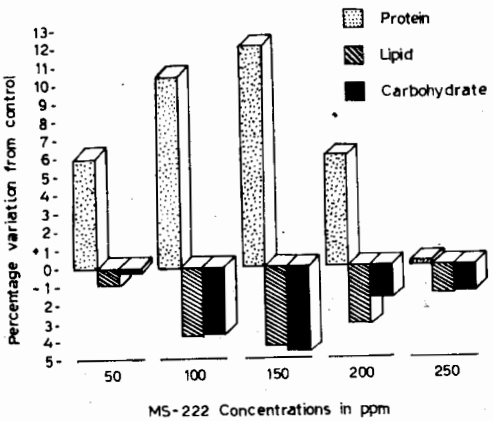

Fig. 5. Percentage variation of protein, lipid and carbohydrates in prawns under the influence of MS-222 from those without it (control).

The reduced rate of oxygen consumption, ammonia excretion and ammonia quotient indicates the animal to be really at rest and hence the fall in lipid content and carbohydrates cannot be attributed to their utilisation for energy. The depletion of lipids and lowering of oxygen consumption indicate a reduced rate of oxidative degradation of lipids for energy purposes through the TCA cycle. The reduced oxygen demand found during the study may indicate a lowered rate of electron transport chain as Krebs cycle intermediates may be diverted towards production of amino acids and thereby proteins. This is indicated by the increased protein content of treated seeds. The carbohydrate depletion reported in the study may also be due to the functioning of the glycolytic cycle.

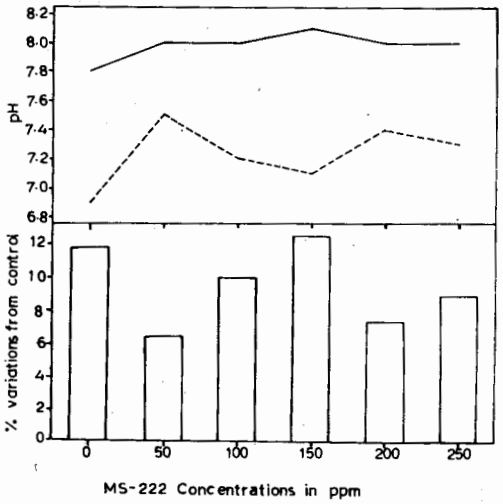

Fig. 6. Changes in pH of the medium with MS-222 (-) and its percentage variation from the control (--) without it.

Ammonia excretion is important as a measure of protein degradation and it is valuable as an index of stress. The rate of ammonia excretion was lower in MS - 222 treated prawns than those in control. According to Krishnakumar (1982), the lethal ammonia levels for seeds of *P. indicus* of 14 - 18 mm sizes was 80 ppm. The level of ammonia reported in the present study was upto a maximum of 75 ppm only. This being below the lethal level, the cause of mortality in the study was not due to ammonia toxicity. Nevertheless, the fall in ammonia concentration with narcotised prawns is indicative of lesser usage of protein. The in-

crease in ammonia quotient during the test as suggested by Kutty (1972) may be a coupling effect of higher ammonia excretion and carbon dioxide production under low oxygen tension. Relative increase in ammonia production during anaerobiosis helps to prevent acidosis, (Kutty, 1967 and 1972). Kripa (1984), comparable to the results obtained here noticed the increase of ammonia quotient with decrease in ambient oxygen. The ammonia quotient decreased with increase in concentrations of MS - 222 as against highest values in control. The MS - 222 thus decreased the metabolism, reduced the rate of ammonia excretion and lowered the ammonia quotient in the treated animals.

The pH in the study was maintained between 7.8 and 8.1. The variation from these to the final value of 6.9 to 7.5 was only 6.2 to 12.3% (Fig. 6). Sarada (1984) suggested that a pH range of 6.0 - 9.0 does not affect the post - larvae of *P. indicus*. So the change of pH also has not caused the observed mortality.

The physiological state of animal is imaged in the metabolism and the rate of oxygen consumption is an index of it. Stress changes the physiology of the animal. But the stress due to sulphonic moiety of MS - 222, as already mentioned, has been eliminated by neutralisation. Since pH was within tolerable limits and ammonia excretion low, the factor that caused death in MS - 222 treated *P. indicus* could be oxygen deficiency.

The tolerance limit of *P. indicus* seeds of 20-30 mm size range to temperature was found to be 38°C by Selvaraj *et al.* (1980). Since the temperature of the medium in this study ranged between 22 and 26°C, the mortality of the prawns was not due to thermal stress.

Selvaraj *et al.* (1980), in their field trials of *P. indicus* seeds transported them 700 km in a duration of 4 days with oxygenation at every 24 h and had less than 5% mortality in 48 h and about 15% at the end of the experiment. By anaesthetising with 150 ppm concentration of MS - 222, it may be possible to transport them safely for longer periods.

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