CHAPTER-VI

THIAMINE REQUIREMENT
I N T R O D U C T I O N

Late in the nineteenth century, Eijkman (1897) (cited, Goldsmith 1964) for the first time identified polynerritis disease in poultry birds termed as 'Beriberi', which Grijns (1901) (cited, Goldsmith, 1964) interpreted as a disease caused by the deficiency or absence from the food of a protective factor, found in the cortical parts of rice. Funk (1912) isolated this antiberiberi factor and named it as 'vitamin B'. Subsequently, this vitamin was redesignated as vitamin $B_1$ or aneurine (Jansen and Donath, 1927) (cited, Halver, 1972) and later it was isolated, characterized and synthesized.

As a derivative of hydrochloride and mononitrate, thiamine is widely used in nutritional studies of aquatic species (Halver, 1957). In tissues, thiamine exists in the form of pyrophosphate or cocarboxylase. Thiamine is very unstable to heat and large quantities have been reported to be lost during feed preparation (Coates, 1976).

In aquatic species, Schmeberger (1941) was the first to cure dietary disease in rainbow trout, using crystalline thiamine and subsequently Vinogradova (1947) produced dietary deficiency in *Palaemon* sps. and *Squilla*. However, specific effect of the vitamin could not be elucidated due to improper information. Later, studies in finfish showed that vitamin $B_1$
to be essential for the normal functioning of nervous system, digestion, growth, fertility and maintenance of good appetite (Halver, 1957; Handler, 1958; Guthrie, 1975). It has a major role in metabolism, especially in the decarboxylation and carboxylation of α-ketoglutaric acid, and also in the transketolase reaction in pentose-phosphate-shunt (Handler, 1958).

William and Spiesz (1938) and Jansen (1954) concluded from the information available till that time that thiamine requirement in all species of animals is some what less than 1 ppm in the food. However, tissue concentrations in different phyla have been reported to vary markedly; for instance freshly boiled prawn has about 0.1 µg/g; raw crab eggs 0.1 µg/g; Pandalus borealis 0.1 µg/g and eggs of Cancer pagurus 0.27 µg/g (Fisher, 1960). These variations in concentrations of thiamine in various species is due to a number of factors such as environmental, the organism's inherent ability to utilise the stored vitamin and the physiological state at a given time (Fisher, 1960; New, 1976a). Significantly, crustaceans also show variations in tissue concentrations, because of the molting cycle (Stern, 1976). Beerstecher (1950) experimentally showed that smaller the species, larger is the amount present and that the regression of these levels on the log body weight fairly follows a negative exponential course, when the vitamin intake is moderate.
Thiamine requirement depends on the composition of the diet, particularly the carbohydrate content. The requirements for practical purposes can be well expressed in terms of calorie intake (Krehlitz, 1969) and minimum requirement works out to be about 0.3 mg/Kcals in the case of higher animals.

Studies in various species have brought to light a number of disturbances in metabolic functions associated with thiamine deficiency in diets, and prolonged deficiency of the vitamin, invariably, results in death of the species. Deficiency signs in salmon include impaired carbohydrate metabolism, nervous disorders, poor appetite, poor growth and increased sensitivity to shock by physical blow on the container or from light flashes (McLaren et al., 1947a; Halver, 1953, 1957, 1969; Coates and Halver, 1958). Thus, for many years controversy had been raging about impairment of the synthesis of fat from carbohydrate during thiamine deficiency. One school of thought believes in the existence of such an impairment (Steyn and Parve, 1967), but the other had the opposite view (Goldsmith, 1964). Stirn et al. (1939) observed that fat exerts a thiamine sparing action.

Most studies on thiamine requirement till date have been done in vertebrates, especially man and domesticated animals and fishes to some extent, but studies in crustaceans are few (Fischer, 1960; New, 1976a). The major constraint for the thiamine requirement studies in aquatic species are primarily centered in the percentage loss during preparation and storing.
of feeds and due to leaching. It has been well established
that thiamine can be easily lost by holding wet diet ingredients
too long for storage or may be lost when the diets are prepared
under slightly alkaline condition (Halver, 1957; Coates, 1976)
or in the presence of sulfide. Wet and frozen diets pose a
different problem because of moisture and subsequent increased
chemical reaction and increased danger for biological hydrolysis
and thus destroying the thiamine molecule. Obviously, wet and
moist diet preparations containing any fresh fish or shellfish
tissue must be used immediately or thiamine loss results
because of thiaminase activity (Halver, 1972). Thiamine is
the most prominent of all the vitamins leaching from the diet.
About 68 to 100% is lost in 24 hrs time (Infanger et al., 1980)
No specific studies have been done to demonstrate experimentally
thiamine requirement in crustaceans. However, Conklin and
Provasoli (1977) reported detrimental effect in Homs, when
thiamine was added in excess.

Thus, taking in view the necessity of the vitamin
for all species including crustaceans, macrodosages are
administered to compensate for the losses incurred during
feed preparation, storing and feeding. However, vitamin needs
in crustaceans show variation with respect to the stage of
life and physiological state. In early stages, more dosage
is required than in adults due to rapid growth and molting
frequency (New, 1976; Heinen, 1984). In certain instances,
excessively high levels of water soluble vitamins in diets
had detrimental effects, as observed in *Artemia* spp. (Provasoli and Shiraishi, 1959). Thus, the present study in juvenile, *P. indicus* was taken up to determine the requirement of thiamine, using graded levels of the vitamins in purified diets.

**MATERIAL AND METHODS**

Thiamine requirement in prawns was studied using graded levels of thiamine hydrochloride in isonitrogenous, purified diets with vitamin free casein as protein source (Table 21). An additional diet was prepared in which both carbohydrate and thiamine were deleted.

The experimental set up, monitoring of environmental conditions, rearing of prawns prior to the experiment and during experimental period were similar to that presented in Chapter I. The mean environmental conditions maintained and the initial length and weight of the animals have been shown in Table 20. All experimental procedures adopted for the present study were similar to those described in earlier chapters. The parameters considered for studying the response of diet and the experimental procedures used for determination of the parameters were similar to those described in Chapter I. Data obtained for various parameters were statistically analysed as described in Chapter I.
### Table 20: Environmental Parameters and Stocking Size of Juvenile Prawns

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>28.6 ± 0.961</td>
</tr>
<tr>
<td>Salinity (%)</td>
<td>21.86 ± 1.86</td>
</tr>
<tr>
<td>pH</td>
<td>8.02 ± 0.56</td>
</tr>
<tr>
<td>Ammonia concentration in the water (NH₄-N mg/l/d)</td>
<td>0.015 ± 0.0035</td>
</tr>
<tr>
<td>Initial length (mm)</td>
<td>18.0 ± 0.84</td>
</tr>
<tr>
<td>Initial weight (mg)</td>
<td>33.6 ± 0.0032</td>
</tr>
</tbody>
</table>

### Table 21: Dietary Composition of Experimental Diets with Graded Levels of Thiamine Hydrochloride

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamine Hydrochloride</td>
<td>0.0* 0.0 0.002 0.004 0.006 0.008 0.01 0.015</td>
</tr>
<tr>
<td>α-Cellulose</td>
<td>0.020 0.020 0.018 0.016 0.014 0.012 0.010 0.005</td>
</tr>
</tbody>
</table>

*Carbohydrates deleted
RESULTS AND OBSERVATIONS

To determine the tentative dietary requirement of thiamine for juvenile *P. indicus*, an experimental study was conducted using purified diets containing graded levels of thiamine (0.0 - 0.1 g/100 g dry diet) and the results as well as significant observations are presented here.

**Survival:**

Survival rates of prawns were significantly (P<0.05) influenced by the levels of thiamine in the diet (Fig. 37). The prawn groups fed on the diet without carbohydrate and thiamine showed significantly (P<0.05) lower survival rate than all other treatment groups fed on diets with various concentrations of thiamine. The prawn groups fed on the diet without carbohydrate and thiamine recorded low percent survival (44.4%), due to high cannibalism. In prawn groups fed on diets with different levels of thiamine, the highest percent survival was recorded with 0.02 g and 0.1 g of thiamine (80%) in the diet. In all other treatment groups percent survival ranged between 50% and 77%. However, no specific trend could be observed in the survival rate with respect to different concentrations of thiamine in the diet.
Fig. 37. Weekly percent survival of prawns fed diets with different levels of thiamine hydrochloride.
FIG. 37

THIAMINE HYDROCHLORIDE (g/100g Dry diet)

FD: Final Day

WCT: Without Carbohydrate and Thiamine

SURVIVAL (%)
Growth:

The mean percent gain in length shown in Fig. 38 was significantly \( (P<0.05) \) influenced by the diets fed to the prawns. However, prawns fed on the diet without carbohydrate and thiamine and those fed on diet with very high concentrations of the vitamin \( (\geq 0.075 \text{ g}) \) recorded significantly \( (P<0.05) \) lower mean percent length gain compared to prawns fed on diets with other concentrations of the vitamin. While the maximum mean percent gain in length was observed (Fig. 38) in prawns fed on the diet with 0.01 g of thiamine (94%), the minimum was recorded in prawns fed on diets with thiamine concentrations of 0.075g (63.6%) and 0.10g(69.4%). It was observed that with the inclusion of thiamine in the diets at a low concentration of 0.01g growth was significantly enhanced; however, higher concentrations of thiamine in the diet proved detrimental to growth.

The mean percent gain in wet weight of prawns (Fig. 38) showed similar pattern as that of mean percent gain in length. Prawns fed on the diet without carbohydrate and thiamine recorded significantly \( (P<0.05) \) lower mean percent gain in wet weight than that of prawns fed on diets with lower concentrations of thiamine, as well as thiamine-free diet. Similarly, at higher concentrations \( (\geq 0.075 \text{ g}) \) of thiamine in the diet, poor mean percent gain in wet weight was recorded. Prawns fed on the diet with 0.01 g of thiamine recorded the
Fig. 38. Percent gain in length and weight, and total biomass (g) of prawns fed diets with different levels of thiamine hydrochloride.
highest mean percent gain in wet weight (521.5%). Although the prawns fed on the diet without both carbohydrate and thiamine recorded the lowest mean percent gain in wet weight (365.9%), this was not significantly different from that recorded by prawns fed on the diet with 0.075g of thiamine (378.7%) and 0.1 g of thiamine (387.4%). In other treatment groups, the mean percent gain in wet weight ranged between 429 and 488.6%. Thus, the prawns fed on the diets without carbohydrate and thiamine and those fed on diets containing thiamine concentrations above 0.075 g in the diet showed significant (P < 0.05) differences in the mean percent gain in wet weight with that of prawns from other treatments.

The experimental diets also had highly significant (P < 0.01) influence on the mean percent dry weight gain of prawns (Fig. 38). The highest percent gain in dry weight was observed in prawns fed on the diet with 0.01 g of thiamine (545.6%) and the lowest in prawns fed on the diet without carbohydrate and thiamine (295%). There was a sharp increase in the percent dry weight gain, when thiamine was included in the diet at a level of 0.01 g. However, further increase in the concentration of thiamine in the diet resulted in decreased percent gain in dry weight.

Specific Food Consumption (SFC):

The Specific Food Consumption (SFC) in prawns was significantly (P < 0.01) affected by the diets containing various levels
Fig. 39. SFC, FCR and PER for diets with different levels of thiamine hydrochloride.
FIG. 39

THIAMINE HYDROCHLORIDE (g/100g DRY DIET)

WCT: WITHOUT CARBOHYDRATE AND THIAMINE
of thiamine (Fig. 39). The prawns fed on diets with 0.01 g and 0.02 g of thiamine showed significantly (P < 0.05) lower SFC compared to those fed with other levels of thiamine in the diet. The maximum SFC was recorded in prawns fed on the diet without carbohydrate and thiamine (5.09%) and the minimum with prawns fed on diets with 0.02 g of thiamine (2.69%), closely followed by prawns fed on the diet with 0.01 g of thiamine (2.95%). In prawns fed on diets with other levels of thiamine, the SFC ranged between 3.71 and 4.77%.

Food Conversion Ratio (FCR):

Similar to SFC, the food conversion ratio was also significantly (P < 0.05) influenced by the diets fed to the prawns. However, no specific trend could be observed in the FCR (Fig. 39) with respect to dietary levels of thiamine. The maximum FCR (2.4) was recorded with 0.075 g thiamine in the diet. However, in all other treatment groups, the FCR non-significantly ranged between 1.0 and 2.1 with the lowest ratio in groups of prawns fed on diet with 0.01 g of thiamine in the diet (1.0).

Protein Efficiency Ratio (PER):

The prawn groups fed on the diet without carbohydrate and thiamine recorded significantly (P < 0.05) lower PER values (1.29) than those fed on diets with different concentrations of the vitamin (Fig. 39). The inclusion of the vitamin at a level of 0.01 g resulted in a sharp increase in PER (2.75).
compared to the vitamin deficient diet (1.63). However, a steady decrease in the PER was observed with further increase in the concentration of thiamine in the diets. Thiamine concentrations between 0.01 and 0.05 g in the diet did not show any significant differences in the PER, which ranged between 1.79 and 1.89. The prawn groups fed on the diet without carbohydrate and thiamine, showed lower values of PER compared to that of prawns fed on the diet deficient in thiamine alone (1.63), indicating that carbohydrate in the diet has some influence in the utilization of dietary protein.

Biochemical Composition:

The moisture, ash, protein, lipid and carbohydrate contents of prawns recorded after the experiment are shown in Fig. 40. Analysis of variance of the data showed that the dietary thiamine level significantly (P<0.05) influence the moisture, ash, protein and lipid contents.

The moisture content was significantly (P<0.05) higher in prawns fed on diets with thiamine concentration of 0.075 and 0.20 g and significantly lower in prawns fed on the diet with 0.01 g of thiamine (74.7%). In all other treatment groups, the moisture content insignificantly varied between 76.3% and 78.9%.

The prawns fed on the diet without carbohydrate and thiamine and those fed on the diets with thiamine concentrations
Fig. 40. Biochemical composition of prawns fed diets with different levels of thiamine hydrochloride.
of 0.01 g or more, significantly ($P < 0.05$) higher ash contents than those fed on the diet without thiamine and those fed with 0.01 g thiamine. The highest ash content recorded in prawns fed on the diet without carbohydrate and thiamine was 19.6%, but this was not significantly different from the ash content recorded with thiamine concentrations ranging from 0.02 g to 0.1 g. The prawns fed on the diet deficient in thiamine and those fed on the diet with 0.01 g thiamine had relatively low ash contents (14.2%).

The protein content in prawns showed an increase on inclusion of the vitamin at a concentration of 0.01 g in the diet. Further increase in concentration of thiamine in the diet did not enhance protein deposition in prawns significantly. The maximum protein content was recorded in prawns fed on the diet with 0.01 g of thiamine (66.4%) and the minimum with 0.05 g (58.7%) and 0.075 g of thiamine (59.3%). The prawns fed on the diet without both carbohydrate and thiamine had relatively lower protein content (61.1%) than prawns fed without thiamine alone in the diet.

The total lipid content in prawns did not show any specific relationship with the increasing concentrations of the vitamin in the diet. The prawns fed on diets with lower concentrations of thiamine (0.01 g and 0.02 g) had significantly ($P < 0.05$) lower lipid levels compared to prawns fed on diets containing higher concentrations (0.03 g and above) of thiamine, except
the prawns fed on the diet with 0.1 g (12.4%) of thiamine, where the lipid level was relatively low. The prawns fed on the diet without carbohydrate and thiamine had relatively lower (15.6%) lipid content than that of prawns fed on the diet deficient in thiamine (17.2%), indicating that carbohydrate in the diet has some influence on the lipid content of prawns.

The total carbohydrate content in prawns increased with the thiamine concentration in the diet up to 0.05 g and thereafter, showed a decrease with further rise in thiamine concentration. However, prawns fed on the diet without carbohydrate and thiamine had significantly higher values (1.17%) than those fed on diet deficient in thiamine (1.02%). Analysis of variance of the data showed that the dietary concentration of thiamine has highly significant (P<0.01) influence on the carbohydrate levels in prawns.

The RNA content of prawns (Fig. 41) was significantly (P<0.05) influenced by the concentration of thiamine in the diet. The prawns fed on diet without carbohydrate and thiamine, and those fed on the diet deficient in thiamine had significantly (P<0.05) lower RNA levels compared to that of prawns fed on diets containing various concentrations of thiamine. However, significant differences were observed between the RNA content on prawns fed on diets containing different concentrations of
thiamine. The maximum RNA content was recorded with 0.01 g of thiamine (2.3 μg/mg) and the minimum in prawns fed on the diet without both carbohydrate and thiamine (1.4 μg/mg).

In contrast to RNA content (Fig. 41), the dry weight/total RNA ratio was significantly (P<0.05) higher in prawns fed on the diet without both carbohydrate and thiamine and also those fed on the diet deficient in thiamine, than that in prawns from other treatments. The highest ratio was observed in prawns fed on the diet without carbohydrate and thiamine (0.70), closely followed by prawns fed on the diet deficient in thiamine (0.69). The recorded ratios from other treatments ranged from 0.43 to 0.50. However, no specific trend was observed in the ratio, with increasing levels of thiamine in the diet.

The DNA content of prawns (Fig. 41), significantly (P<0.05) varied with different dietary levels of thiamine. The prawns fed on the diet without carbohydrate and thiamine and also those on the thiamine deficient diet had significantly (P<0.05) lower levels of DNA than those fed on diets with different concentrations of thiamine. Amongst, the prawns fed on diets with different concentrations of thiamine, the DNA content was highest in those fed on the diet with 0.01 g of thiamine (2.59 μg/mg). In all the other treatment groups, the DNA content ranged between 2.31 and 2.44 μg/mg and thre
Fig. 41. Biochemical composition of prawns fed diets with different levels of thiamine hydrochloride.
FIG. 41

PHOSPHORUS (%)

MAGNESIUM (%)

CALCIUM (%)

NUCLEIC ACIDS DRY WT./NUCLEIC ACIDS (µg) RNA/DNA

NUCLEIC ACIDS DRY WT./NUCLEIC ACIDS (µg) DNA/RNA

THIAMINE HYDROCHLORIDE (g/100g DRY DIET)

WCT: WITHOUT CARBOHYDRATE AND THIAMINE
were no significant differences between them. Prawns fed on the diet without thiamine and carbohydrate had lower DNA contents (1.47 μg/mg) compared to prawns fed on the diet deficient in thiamine (1.61 μg/mg).

Significant differences were also observed in the dry weight/DNA ratio between prawns (Fig. 41) fed on diets without carbohydrate and thiamine, deficient in thiamine, and that of prawns fed with different concentrations of thiamine in the diet. While, the diet without carbohydrate and thiamine gave the highest ratio (0.68), the lowest was obtained with 0.01 g of thiamine (0.39). The ratios recorded for other treatments ranged from 0.4 to 0.43 and showed insignificant differences between them.

The RNA/DNA ratio showed slight variations between treatments (Fig. 41). The highest RNA/DNA ratio was recorded with 0.05 g of thiamine (0.95) and the lowest with 0.04 g of thiamine (0.82) in the diets. In all other treatment groups, the RNA/DNA ratio ranged between 0.83 and 0.89. No specific trend could be observed in the RNA/DNA ratios with respect to different concentrations of thiamine in the diets.

The calcium content in prawns (Fig. 41) was not significantly affected by the dietary concentrations of thiamine and it ranged from 2.77% to 3.02%. The magnesium content in prawns (Fig. 41) recorded from different treatments varied insignificantly from 0.43 to 0.52%, with the highest at 0.01 g thiamine and the
lowest in prawns fed on diet without carbohydrate and thiamine (0.43%). The phosphorus content in prawns (Fig. 41) recorded from various treatments did not show any significant differences between treatments. However, with increasing levels of thiamine in the diet of prawns, the phosphorus content increased up to 0.03 g(1.06%) where it was highest, and thereafter the phosphorus content showed a decreasing trend with further increase in the vitamin level in the diet. The lowest phosphorus content was recorded in prawns fed on the diet without both carbohydrate and thiamine (0.89%). In all other treatments, the phosphorus content ranged insignificantly between 0.93 and 1.03%.

Ammonia Concentration in Water

Mean ammonia concentration in the experimental aquaria showed variation, in relation to the levels of thiamine in the diets (Table 22). The lowest ammonia concentration (0.011 mg/l/d) was recorded in the treatment without carbohydrate and thiamine in the diet. However, with increase in concentration of thiamine in the diets the ammonia concentration in the water increased. The highest mean ammonia concentration was observed in the treatment (0.024 mg/l/d) with thiamine deficient diet.
<table>
<thead>
<tr>
<th>Concentration of thiamine hydrochloride g/100 g dry diet</th>
<th>Mean ammonia concentration in seawater mg/l/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0*</td>
<td>0.011</td>
</tr>
<tr>
<td>0.0</td>
<td>0.024</td>
</tr>
<tr>
<td>0.01</td>
<td>0.013</td>
</tr>
<tr>
<td>0.02</td>
<td>0.014</td>
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<td>0.03</td>
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<td>0.04</td>
<td>0.013</td>
</tr>
<tr>
<td>0.05</td>
<td>0.014</td>
</tr>
<tr>
<td>0.075</td>
<td>0.015</td>
</tr>
<tr>
<td>0.10</td>
<td>0.017</td>
</tr>
</tbody>
</table>

*Carbohydrate deleted.
OBSERVATIONS

Molting:

There were differences in the number of exuviae collected from various treatments (Table 23). Prawns in treatment with 0.01 and 0.02 g of thiamine were found to molt the maximum number of times. However, this number was apparent, since molting in prawns mostly occurred during night and by the time of collection, the exuviae were eaten up by the cohabitants. The lowest number of exuviae were collected from treatment without carbohydrate and thiamine (13 nos.). In all other treatment groups the number of exuviae ranged between 17 to 32 nos., with the low numbers in treatment with the highest concentration of thiamine.

Post-molt deaths (Table 23) were relatively few and did not vary markedly between treatments during the first two weeks of the experimental study. However, from the third week onwards, variation in number of post-molt deaths was evident between treatments. The maximum post-molt deaths occurred in treatments without carbohydrate and thiamine in the diet and those fed on the thiamine deficient diet. In treatments with high concentrations of thiamine in the diets, relatively higher number of post-molt deaths occurred from end of fourth week only. In all other treatments, the post-molt deaths were relatively less.
<table>
<thead>
<tr>
<th>Concentration of thiamine hydrochloride in the diet g/100 g dry diet</th>
<th>Mean nos. of molts of post-molt recovered</th>
<th>Mean nos. of post-molt deaths</th>
<th>Texture of the body</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00*</td>
<td>13</td>
<td>11</td>
<td>SO</td>
</tr>
<tr>
<td>0.00</td>
<td>22</td>
<td>9</td>
<td>H</td>
</tr>
<tr>
<td>0.002</td>
<td>35</td>
<td>14</td>
<td>H</td>
</tr>
<tr>
<td>0.004</td>
<td>36</td>
<td>11</td>
<td>H</td>
</tr>
<tr>
<td>0.006</td>
<td>21</td>
<td>13</td>
<td>H</td>
</tr>
<tr>
<td>0.008</td>
<td>32</td>
<td>13</td>
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</tr>
<tr>
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<td>31</td>
<td>19</td>
<td>H</td>
</tr>
<tr>
<td>0.015</td>
<td>17</td>
<td>8</td>
<td>H</td>
</tr>
<tr>
<td>0.020</td>
<td>18</td>
<td>7</td>
<td>H</td>
</tr>
</tbody>
</table>

H = hard, SO = Soft

*Carbohydrate deleted.
Food Intake:

Food intake in prawns did not vary markedly between treatments during the initial two weeks. However, variation in the amount of left-over feed was observed from the third week onwards, especially in treatments without carbohydrate and thiamine in the diet and thiamine free diet. By the end of fourth week, the experimental prawns in these treatments showed aversion to the feed, when it was introduced in the water, compared to their counterparts fed with other levels of thiamine in the diet, which showed quick responses towards feed, and the left-over food was also comparatively less. During, the penultimate week, prawns fed on 0.075 g of thiamine in diet also started showing aversion towards feed.

Behaviour Towards Light:

Prawns, in various treatments showed distinct responses to table lamp light (1625 x 10^2 lux). In the case of thiamine deficient treatment, the prawns showed quicker responses than their counterparts in other treatment groups. However, prawns fed on the diet without both carbohydrate and thiamine responded passively to the sudden flash of light. In the above treatment groups, the activity showed variation from the third week onwards. In all other treatments, there was no unusual behaviour towards light.
External Morphology:

No marked visible changes could be delineated in prawns fed on the experimental diets at the end of 45 days. Few brown-spots were distributed along the proximal part of abdomen and the gills in prawns fed with both carbohydrate and thiamine deleted diet, and also in the abdomen of prawns fed with thiamine deficient diet. However, these spots were not observed in prawns from other treatments. The hepatopancreas in prawns fed on diets deficient in thiamine and those supplemented with thiamine in diets up to 0.02 g showed distinct 'Y' shaped brown structure underlined with a whitish mass. However prawns fed on diets with higher concentrations of thiamine showed diffused hepatopancreas.

DISCUSSION

Thiamine as thiamine pyrophosphate is involved in the oxidation of α-keto acids. It has important functions in nervous tissue, digestion, growth, fertility and maintenance of good appetite (Mitchell, 1964; Guthrie, 1975). William and Spies (1938), based on the information available till that time, reported that all species of animals require thiamine in their diets. However, recent studies have shown that thiamine requirement of aquatic species is much higher than that of domesticated land animals (Hasting and Cowey, 1977), mainly due
to leaching of the vitamin from diets (New, 1976a; Infanger et al., 1980) into the surrounding water.

The present study shows that juvenile *P. indicus* also require thiamine as an essential nutrient in the diet. Earlier studies, with crustaceans, have also shown that thiamine is essential in the diet of Kuruma prawn, *P. japonicus* (Deshimaru and Kuroki, 1979) the cladoceran, *Moina macrocopa* (Conklin and Provasoli, 1977), the lobster, *Homarus americanus* (Conklin, 1980) and the giant tiger prawn, *Macrobrachium rosenbergii* (Heinen, 1984). However, there are significant differences between the observations of the earlier workers and that of the present study. In most of the earlier studies (Deshimaru and Kuroki, 1979, Heinen, 1984) survival of prawns was found to be unaffected, when fed with diets deficient in thiamine. In contrast, the present study clearly shows that deletion of the vitamin from the diet results in decreased survival rate. Low survival rate, indicates that thiamine deficiency may be induced by breakdown of carbohydrate and protein metabolism (Handler, 1954, Mitchell, 1964; Aoe et al., 1969, Halver, 1980) leading to poor availability of energy for general metabolism.

The survival rate was, however, markedly affected from the third week, onwards, in the case of thiamine deficient diet fed prawns and they became abnormally active. Similar symptoms on feeding with the thiamine deficient diet was also reported in mammals (Mitchell, 1964) and in finfish (Halver, 1957).
Cowey and Sargent, 1972). In contrast, prawns fed with a diet deficient in both carbohydrate and thiamine were found to become passive, show decreased feed intake with the prolongation of the experimental days and high rate of mortality from the fifth week onwards. These results suggest that the animal probably subsists by utilizing body stores of thiamine, during the first two weeks.

Comparatively, high survival rates were recorded in all the prawn groups fed with thiamine in the diets, excepting in groups fed with 0.05 g of thiamine, where significantly lower survival was recorded. The low survival rate in the treatment with 0.05 g group, as a result of number of sudden post-molt deaths that occurred during the sixth week for which the reasons are not clear. In this treatment group (0.05 g), the survival was almost the same as in any other thiamine supplemented treatment groups, till the fifth week. The results clearly indicate that irrespective of the concentrations of thiamine used, survival of prawns is not significantly affected. Deshimaru and Kuroki (1979) also did not report any significant effect of graded levels of thiamine on survival rate of P. japonicus.

However, the growth of prawns was significantly influenced by the concentration of the vitamin in the diet. The highest growth was observed in prawns fed on the diet with 0.01 g thiamine, where the survival was also relatively high. On the other hand, prawns fed on the thiamine deficient diet and
those fed with thiamine more than 0.01 g did not show significant differences in growth between them. Similar results were also reported by Deshimaru and Kuroki (1979) in *P. japonicus* where inconsistent growth was reported with increasing levels of thiamine in the diet. In the case of juvenile *M. rosenbergii* growth and survival of prawns were found to be higher when fed with thiamine deficient diet than with control diet containing 0.05% of thiamine. However, in the present study, prawns receiving the thiamine deficient diet recorded relatively lower growth than prawns fed diet with 0.01 g thiamine. The reduced growth may be due to low activities of carboxylase and RNA transketolase in experimental animals which are dependant on thiamine as coenzyme thus, ultimately affecting the carbohydrate metabolism and poor dietary energy availability as observed by Infanger et al. (1980).

Deshimaru and Kuroki (1979) suggested 12 mg/100 g dry diet of thiamine hydrochloride as preferable level for *P. japonicus*. The present study also shows that in juvenile *P. indicus*, the thiamine requirement is about 10 mg/100 g dry diet of thiamine hydrochloride. However, these levels of thiamine are not comparable to that reported for fishes (McLaren et al., 1947a; Halver, 1972), which required between 1-1.2 mg/100 g of dry diet. These variations in thiamine requirement in crustaceans and fishes could, however, be argued on the basis of the conclusions of Hastings and Cowey (1977).
New (1976a) and Hainen (1984) that domesticated land animals have less requirement for vitamins than aquatic species and that in crustaceans, loss of vitamins from diets is greater than fish diets due to leaching effect. Infanger et al. (1980) observed that thiamine loss is maximum (68-100% in 2 hrs. time) amongst all the B vitamins from the diet. Thus, these observations demonstrate the need for incorporation of higher concentrations of vitamin in the diet of prawns.

Prawns fed on the diet with both carbohydrate and thiamine deficient diet showed very poor growth compared to other treatment groups. This suggests that in the growth of prawns, carbohydrate content in the diet has significant influence.

The growth (almost equal to the prawns fed with thiamine more than 0.01 g in the diet) recorded in prawns fed with diet deficient in thiamine suggests that tissue reserves, and probable gut bacterial synthesis of the vitamin, enabled the prawns to sustain and record good growth. Since the requirement was observed to be very low (0.01 g) compared to other B vitamins, bacterial contribution (Fisher, 1960) might have significantly influenced growth and according to Forster and Gabbott (1971) microbial population increases in the gut, if carbohydrate was added in the diet. However, the synthesis of thiamine by bacteria may not fully satisfy the thiamine requirement of the prawns and so dietary supplementation of thiamine is essential.
as observed in the enhanced growth rate in prawns fed on diets with 0.01 g of thiamine. Excess of thiamine in the diet results in retardation of growth in prawns, which was also observed in other crustaceans like Moina (Comklin and Provasoli, 1977). This may be due to negative feedback mechanism by the excess amounts of thiamine on the various enzymes. Food intake and its utilization have been widely accepted as important characteristics for nutritional studies (Utne, 1979). In many species of mammals and fishes, thiamine deficiency, in few weeks time, results in sudden loss of appetite and weight (Covey and Sargent, 1972). In the present study also, prawns were observed to show a gradual aversion towards the thiamine and carbohydrate deficient diet. There was significant decline in food intake and activity of the prawns from the third week onwards, as the dietary deficiency prolonged.

However, the significant variation in P/R values observed between prawns fed with only thiamine deficient diet and with 0.01 g of thiamine in the diet, suggest that even though food intake was same, yet the dietary protein utilization by the prawns may be partly influenced by the dietary concentration of thiamine. Increasing the dietary levels of thiamine beyond 0.01 g, significantly influenced the SFC, PCR and P/R values and the prawns tend to show poor food intake and protein utilization. This accounts for the poor growth recorded in these treatment groups.
Thiamine concentration in the diet also had significant effect on the body composition, especially on moisture, ash, protein and lipid content of prawns. The moisture and ash contents in prawns fed with diet containing 0.01 g of thiamine were significantly lower than the other treatment group fed prawns. On the contrary, highest protein and significantly high lipid contents were recorded at the same concentration. These biochemical characteristics suggest that the organic matter is efficiently deposited in the tissue at a dietary concentration of 0.01 g of thiamine. Prawns fed with other dietary levels of thiamine however, did not show any significant variations in the moisture content between them.

The present study shows a direct relationship existing between moisture and ash content of prawns. This is evident from the higher moisture and ash contents in prawns fed on both carbohydrate and thiamine deficient diet and with 0.075 g or more of thiamine in the diet, suggesting that under dietary stress, as a result of deficiency or excess of thiamine, organic matter is displaced by water and inorganic substance. However, deficiency in thiamine alone resulted in significantly lower ash content. Possibly, due to the partial utilization of tissue carbohydrates, lipids and proteins, higher moisture and ash contents. The percent ash content in prawns increased significantly when dietary levels
of thiamine was above 0.01 g, indicating that hypervitaminosis. (>0.02 g) may induce imbalances in the utilization of organic nutrients (Mitchell, 1964) resulting in displacement of organic matter with inorganic matter.

There were no significant differences between treatments in the three inorganic constituents namely, calcium, magnesium and phosphorus. This significant variation suggests that thiamine level has no influence on the parameters, but the significantly higher ash levels indicate that other inorganic nutrients may be influenced by thiamine.

Dietary concentration of 0.01 g thiamine seems to be near optimal level for these juvenile prawns for maximum accumulation of protein. The concentration of nucleic acids (RNA-DNA) was also highest at this level, indicating maximum level of protein synthesis at this concentration, resulting in higher growth and PER. The role of thiamine on RNA and protein synthesis has been well established now (Guthrie, 1975; Infanger et al., 1980) and so may be that 0.01 g of thiamine in the diet of prawns could bring out maximum efficiency in protein synthesis resulting in efficient growth.

The relatively lower protein levels recorded in prawns fed with more than 0.01 g thiamine may be due to catabolism of proteins to meet the energy expenditure in overcoming the dietary stress, as a result of hypervitaminosis. It is also
probable that excess of thiamine may impair protein synthesis, thereby resulting in decreased accumulation of proteins. If so, part of assimilated protein may be catabolized, there by enhancing the ammonia excretion. The ammonia concentration in seawater, further indicates increased catabolism of proteins at high concentration of thiamine in the diet.

Prawns fed on the carbohydrate and thiamine deficient diet had relatively lower lipid content indicating that in the absence of carbohydrates, possibly dietary lipids are increasingly utilized for energy requirements. Mitchell (1964) observes that for the metabolism of fat component of diets, excepting glycerol moiety, thiamine demand was less as compared to carbohydrates and it is therefore possible, in the absence of carbohydrates prawns can use lipid as a major energy source. Also, dietary fat has been known to exert thiamine sparing action (Stirn et al., 1939; Reinhold et al., 1944; Holt and Snyderman, 1955). Thus prawns fed on the carbohydrate and thiamine deficient diet and those fed on the diet deficient is only thiamine were perhaps able to sustain for relatively longer periods by utilizing lipids as a major energy source.

The reduced food intake, aversion towards feed, response towards flash of light and striking against the wall of the
aquaria observed in prawns fed on the thiamine deficient diet are similar to the observations reported in mammals (Mitchell, 1964) and fishes (Halver, 1957, 1972) under thiamine deficiency. However, prawns fed with thiamine showed normal activity and there were no significant changes in the food intake.

All these observations suggest that thiamine is required by juvenile prawns and the preferable concentration in the diet could be about 0.01 g/100 g dry diet in the form of thiamine hydrochloride, since the highest growth, survival and protein deposition were recorded at this level of thiamine.

CONCLUSIONS

From the present study based on the growth and other associated parameters studied, it is evident that juveniles of *P. indicus* have a requirement for thiamine in the diet. In earlier studies (Deshimaru and Kuroki, 1979; Heinen, 1984), thiamine has been reported to have insignificant effect on survival when supplemented or deleted from the diet. However, the present study shows that thiamine deficiency affects survival. Also, high dosage of thiamine significantly affect growth, food utilization and body composition of prawns.

Prawns fed on the diets with 0.01 g of thiamine were observed to show the highest survival, growth, best FCR, P/E and higher amount of organic nutrients, amongst all the tested
levels which is much near to the thiamine requirement of
P. japonicus, about 0.012 g/100 g dry diet (Deshimaru and
Kuroki, 1979). However, these values can be altered by number
of abiotic and biotic factors (McLaren et al., 1947a). Also
in fishes it was reported that carbohydrate content in the
diet can influence, the requirement of thiamine, as carbohydrate
metabolism has been reported to have relationship with thiamine
concentration in the diet (Aoe et al., 1967c, 1969).

Amongst the thiamine deficiency, dietary symptoms
observed in prawns, instability and increased sensitivity to
shock by physical blow to the aquaria or from light flashes
are the important ones, which have also been earlier reported
in finfishes (Cowey and Sargent, 1972) but so far not reported
in crustaceans.