NUTRITIONAL STUDIES IN JUVENILE PENAEUS INDICUS WITH REFERENCE TO PROTEIN AND VITAMIN REQUIREMENTS

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CERTIFICATE

This is to certify that the thesis entitled "NUTRITIONAL STUDIES IN JUVENILE PENAEUS INDICUS WITH REFERENCE TO PROTEIN AND VITAMIN REQUIREMENTS" is the bonafide record of the work carried out by Mr.C.GOPAL under my guidance and supervision and that no part thereof has been presented for any other Degree.

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DECLARATION

I hereby declare that this thesis entitled "NUTRITIONAL STUDIES IN JUVENILE *PENAEUS INDICUS* WITH REFERENCE TO PROTEIN AND VITAMIN REQUIREMENTS" has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

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(C. GOPAL)

C O N T E N T S

P R E F A C E

Man in his quest to explore food had turned towards the oceans and during the last five decades, there has been phenomenal increase in the exploitation of the marine fisheries resources. According to FAO statistics (1983), marine fish landings of the World stagnated at about 73 million tonnes per year during the last decade. It is generally believed that harvest of fisheries resources from the oceans is either at or near the maximum sustainable yield (Lawrence, 1981); whereas the demand for marine fish and fishery products is on the increase.

Crustaceans, as a group, contribute significantly to the marine fish landings (about 15% of the total), with the shrimps and prawns constituting bulk of the catch (about 87.3%). The landings of shrimps and prawns had been increasing steadily since 1960. In 1982, a production of 1.69 million tonnes was recorded, which was about 71% higher than the production of 1.2 million tonnes recorded during 1972. During the last five years, the World production stayed at an average between 1.6 and 1.7 million tonnes.

Among the top 20 countries, producing 83% of World catch of shrimps and prawns, India holds the premier place, with a production of about 214, 980 tonnes during 1983 (FAO, 1983). However, prawn catches have remained steady during the

years from 1974 to 1984, with production fluctuating between 164.2 and 250.3 thousand tonnes. This has generated considerable concern among fisheries researchers, planners and development personnel,who have started looking for new avenues to meet the ever-increasing demand for prawns and prawn products. Culture of prawns in coastal saline water impoundments appears to be a promising avenue.

The success of any aquaculture production system depends upon the sum total interaction of all abiotic and biotic factors which directly or indirectly influence production. These interrelated parameters have also marked influence on the success of prawn culture operation. Many of the problems associated with aquaculture production can be coped up through manipulation of nutrients in feed formulations, taking in view the cost of production of feed and animal biomass per unit area.

In most of the cultivated species certain amount of food is obtained from the natural environment, yet, in high density culture systems, supplementary feeding becomes inevitable for better farm production. This can be in the form of natural diets or may be in the form of compounded diets obtained from different natural ingredient sources of both animal and plant origin. Compounded diets also referred to as artificial diets are either complete (containing all the required nutrients in adequate levels) or supplemental (meant to provide additional energy rich ingredients) to animals receiving some natural food. However, inspite of several years of research in this field,

majority of the formulations proved rather unsatisfactory, when compared to live or natural feeds for the production of juveniles for stocking purposes. This is mainly due to imbalance of essential nutrients in these empirically formulated feeds.

Thus, it is clear that knowledge of the nutritional requirements of the cultured species is a prerequisite for the formulation of practical feeds. However, the study of prawn nutrition is complicated by multivariate factors with imposing dimensions. Goodwin and Hanson (1975) consider 11 major variables, namely, stage of growth, species of shrimp, water quality and temperature, feed stability (binding), presentation, percentage and derivation of protein, health of shrimps, effect of feeds which occur naturally in the rearing environment and feeding rates, all of which synergistically affect the overall production rate of prawns.

The Indian white prawn, Penaeus indicus H. Milne Edwards is one of the most suitable cultivated species. Extensive studies have been made in the past on the biology, fishery, life-history and culture techniques of the species. However, only very few studies have been carried out on the nutritional requirements of this species, though a number of studies have been carried out on the efficacy of a variety of compounded feeds. Besides, the vitamin requirements of prawns in general and penaeid prawns in particular have been very poorly understood, inspite of their importance in the metabolism.

Therefore, nutritional studies were carried out in the juveniles of the Indian white prawn, *P. indicus* H. Milne Edwards with the following objectives:

- to study the effect of different levels of proteins in purified diets on the growth, feed efficiency and body composition and to determine the optimal protein requirements of juveniles.
- to evaluate the nutritive value of cheaply available protein rich ingredient sources (plant and animal origin) in diets.
- to study the deficiency symptoms associated with the deletion of water-soluble vitamins (ascorbic acid, choline, thiamine, pyridoxine, niacin, pantothenic acid, riboflavin and inositol) from the diet.
- to study the dietary requirements of important water-sluble vitamins (ascorbic acid, choline, thiamine, pyridoxine, niacin and pantothenic acid) using graded levels of the test vitamin in purified diets.

The thesis has been organised into nine chapters. Chapter I deals with the protein requirement using purified diet with casein as protein source. Chapter II relates to the nutritive value of cheaply available protein sources, which can form alternative protein sources for large-scale feed production for *P. indicus*. Chapter III deals with the effects

of deletion of some of the water-soluble vitamins from the diets. Chapter IV to IX deals with the quantitative vitamin requirements of the prawn for ascorbic acid, choline, thiamine, pyridoxine, niacin and pantothenic acid.

All the experiments were carried out in the laboratory and the following parameters were studied; survival, growth, specific food consumption, food conversion ratio, protein efficiency ratio and body chemical composition. In addition to these, ammonia excretion rates by prawns were recorded in few experiments. Also, histological studies were carried out in prawns from ascorbic acid and choline requirement experiments, to study the effect of these vitamins on the cellular structures. All the data obtained were statistically analysed and represented in the graphs.

The present investigation shows that the juvenile prawns have a protein requirement between 35 and 40% in the diets. Amongst the tested protein sources, both of plant and animal origin, it appears that animal protein sources, in general, and a mixture of animal protein sources in particular have significant effect on growth. However, plant protein sources like soybean meal and groundnut oil-cake too can form alternative protein sources in mixed diet formulations.

The vitamin requirement studies show that amongst water-soluble vitamins-ascorbic acid, choline, thiamine,

pyridoxine, niacin and pantothenic acid are indispensable. In most of the studies, the survival and growth were affected significantly when the prawns were fed without these vitamins. Partial molting, changes in the cellular structures of musculature and hepatopancreas, blackening of gills, and significant alterations in behaviour and food intake are some of the major deficiency symptoms recorded during the study. Supraoptimal dosages resulted in poor growth and survival, besides alterations in biochemical composition of carcass, behaviour and efficiency of utilization of food and protein. All these results and observations suggest that juvenile P. indicus require vitamins in diets at optimal levels.

Thus, the present investigation in juveniles of P. indicus clearly outlines the dietary requirement of protein and some of the vitamins which significantly influence the growth and survival. The recommended dietary nutrient levels are near optimal levels required by the species under similar experimental conditions. However, the requirement for nutrients can be significantly altered by a variety of endogenous and exogenous factors which affect the metabolism of prawn and needs extensive further study. However, the present study is the first attempt on the vitamin requirements of the prawn P. indicus and the optimum levels suggested would help formulate practical diets for large-scale culture of the species, thereby minimising wastage of nutrients.

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(C. GOPAL)

L I S T O F T A B L E S

CHAPTER - III

CHAPTER - IV

CHAPTER - V

CHAPTER - VI

LIST OF PLATES

LIST OF FIGURES

CHAPTER - I

CHAPTER - VIII

CHAPTER - IX

CHAPTER-I

PROTEIN REQUIREMENT

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

The nutritional requirements of crustaceans have been widely studied and reviewed, from time to time, by a number of research workers (Kanazawa et al., 1970; Subrahmanyam and Oppenheimer, 1970; Cowey and Forster, 1971; Kitabayashi et al., 1971 d; Deshimaru and Shigueno, 1972; Hysmith et al., 1972; Balazs et al., 1973; Forster and Beard, 1973; Shewbart et al., 1973; Sick and Andrews, 1973; Deshimaru and Kuroki, 1974a,b,c; 1975a,b; Regnault et al., 1975; Fenucci and Zein-Eldin, 1976; Forster, 1976; New, 1976; Wickins, 1976; Hanson and Goodwin, 1977; Deshimaru and Yone, 1978; Conklin, 1980; Maguire, 1980). These studies, in general, have shown that crustaceans have all the dietary nutrient requirements usually associated with complex metazoa (Dall and Moriarty, 1983). However, knowledge of essential nutrient requirements of many species of crustaceans still remains incomplete and many of the avenues remain unscanned.

Nutritional studies in crustaceans, as such, is complicated by a number of abiotic and biotic factors which have tremendous influence on growth and utilization of food. Abiotic factors including temperature, pH, salinity, dissolved oxygen, depth, light and many others have been found to directly affect the growth and food utilization of crustaceans (Subrahmanyam, 1962; Teal, 1971; Buikema, 1972; Venkataramiah

et al., 1974; Delistraty et al., 1977; Hu, 1978; Cameron and Magnum, 1983; Vernberg, 1983).

Amongst biotic factors, molting which forms an important event in the life cycle has considerable influence on growth and feed utilization of crustaceans. Wide variations in the biochemical constituents of the body occur during the different phases of the molting cycle and thus, the growth in these forms shows discontinuity. It has also been established that each molting in crustaceans results in considerable energy loss, about 7.3%/molt in Macrobrachium rosenbergii (Nelson et al., 1977b) and potentially an average rate of 0.81%/day is lost. In the case of juvenile prawns, it amounts to a large quantum of energy, since at this stage a prawn molts every 8 to 10 days (Stern, 1976) or even earlier. In Metapenaeus dobsoni, it has been reported that the mean molt weight forms about 7.09% of dry wt. of the whole prawn (Thomas et al.,1984). Thus a crustacean body must efficiently function so as to recoup the lost nutrients, besides synthesizing and mobilising nutrients essential for growth, before the onset of the next molt.

Carr et al. (1977) observed that the nitrogen retention would be maximal in the young animals and tends to be zero in the mature and non-producing animals. Correspondingly, food intake in younger stages is found to be high compared to the adult stages in prawns (Sick et al., 1973; Colvin and Brand, 1977; Clifford and Bricks, 1978) and according to Balazs and Ross (1976) better food conversion efficiency by the young stages results in high food intake.

The intake and utilization of feed not only depends upon the 'physiological state' of the organism, but also depends upon its quality and quantity. Qualitatively, the nutrients composition of the feed ingredients, their cohesive ability on long storage, stability of nutrients in the feed when introduced in the water and the attractability of the feeds are some of the factors which influence the growth performance of the crustaceans (Meyers et al., 1972; Meyers and Zein-Eldin, 1972; New, 1976; Biddle, 1977; Hanson and Goodwin, 1977; Fernandez et al., 1981).

The quality of a feed, in general, is primarily based on the energy nutrients, namely proteins, lipids and carbohydrates and non-energy nutrients comprising of minerals, vitamins, growth factors and binders. Thus, for proper physiological functioning and tissue synthesis, these nutrients should be proportionately added in the diets.

Among the energy nutrients, protein is the most important one, as it forms the major growth nutrient in animal tissues. Protein molecule as such exists in different shapes and these shapes directly reflect on the functional status of the proteins. Globular proteins are relatively soluble and readily go into colloidal suspension, performs all the enzymatic reactions and, transports nutrients and growth promoting factors. On the other hand, fibrous proteins, primarily, form the structural units because of their non-colloidal property.

Besides these functions, proteins serve as a source of energy under acute shortage of other dietary energy components. Various authors, based on the dose-response (growth) curve, have determined the minimal dietary protein level giving maximal weight gain in different species of crustaceans (Provasoli and D'Agostino, 1969; Kanasawa et al., 1970; Andrews and Sick, 1972; Deshimaru and Kuroki, 1975a; Colvin, 1976; New, 1976; Maguire, 1980; Veronica and Lim, 1983). Table I shows the recommended optimum levels of protein for different species and size groups of prawns. The optimum protein requirement for maximum growth varies from 22 to 60% in various prawn species (Venkataramiah et al., 1975; Forster, 1976; New, 1976). These variations can be attributed to both intrinsic and extrinsic factors which effect the organisms.

Protein requirement considerably depends upon the physiological state of the animal. During early growth phase, prawns require more of protein, about 60% in the case of Crangon crangon of size group 19-21mm(Regnault and Luquet, 1974). Regnault and Luquet (1974) reported that with every 3mm increase in length there was a 10% fall in protein requirement as the stage increased, and by the adult stage the requirement of protein was as low as 25-30%. The higher requirement of protein in early stages was attributed to the faster growth rate upto late juvenile stage and thereafter the growth becomes more or less slower, though molting continues.

TABLE 1: PROTEIN REQUIREMENT IN DIFFERENT SPECIES OF PRAWNS

Species	Stage	Optimal level suggested (%)	Reference
Penaeus japonicus		55.0	Kanazawa et al. (1970)
"		53.5	Kitabayashi et al.(1971d)
"		60.0	Deshimaru and Shigueno (1972)
"		40.0	Balazs et al. (1973)
"		50.0	Deshimaru and Kuroki(1975a)
"		52.57	Deshimaru and Yone (1978)
Penaeus monodon	Post-larva	30.0	Khannappa (1978)
"	"	55.0	Bages and Sloane (1981)
"	Juvenile	45.8	Lee (1971)
"	"	40.0	Khannappa (1977)
"	"	35.0	Lin et al. (1981)
"	"	40.0	Veronica and Lim (1983)
Penaeus setiferus		28 to 32	Andrews et al. (1972)
Penaeus azetecus		22 to 30	Shewbart et al. (1973)
"		40.0	Venkataramiah et al.(1975)
"		36.5	Fenucci and Zein-Eldin(1976
"		51.5	Zein-Eldin and Corliss(1976
Penaeus duorarum		28 to 30	Sick and Andrews (1973)

Contd....

Species	Stage	Optimal level suggested(%)	Reference
Macrobrachium rosenbergii	Juvenile	35.0	Balazs and Ross(1976)
"	Larva	15-20	Sick (1976)
Penaeus Indicus	Post-larva	40.0	Bhaskar and Ali (1984)
"	Juvenile	43.0	Colvin (1976)
"	"	42.9	Ali (1982a)
Penaeus merguiensis	Juvenile	43-45	Aquacop (1978)
"	"	34-42	Sedgwick (1979)
Penaeus stylirostris	Post-larva	50.0	Colvin and Brand(1977)
Penaeus californiensis	"	30-35	"
Penaeus vannamei	"	30-35	"
Metapenaeus macleayi		27	Maguire and Hume (1982)

Variations in protein requirement of different species of prawns have also been attributed to the biological value of protein sources,which depends upon the amino acids composition of the protein (Harper, 1981; Kies, 1981). However, some proteins are biologically unavailable for the animals due to alterations in the amino acids composition during processing by combining with other compounds, thereby become resistant to proteolytic enzymes (Cowey and Sargent, 1972).

Protein requirements are also influenced by the composition of other dietary energy components, namely, fats and carbohydrates. Protein-lipid ratio and protein-carbohydrate ratio in the diets also significantly influence the protein requirements of prawns (Andrews et al., 1972; Sick and Andrews, 1973, Abdel-Rahman et al., 1979; Teshima and Kanazawa, 1984). Likewise, protein requirement of crustaceans has been reported to be influenced by the amount of organic salts (Sparks, 1971; Deshimaru and Kuroki, 1974a; New, 1976; Maguire, 1980; Ponat and Adelung, 1980) and composition of vitamin mixture (Adelung and Ponat, 1977).

From the foregoing review, it is clear that prawn species show marked differences in their dietary protein requirements and that protein requirement of a species is significantly influenced by both intrinsic and extrinsic factors. The present study was taken up on juveniles of Indian white prawn P. indicus, since very few studies have been carried out regarding its protein requirement. Earlier studies

on the protein requirement of this species were by Colvin (1976) and Ali (1982a) in juveniles and by Bhaskar and Ali (1984) in post-larvae. The first two works were carried out using compounded diets and therefore may not truly highlight the protein requirement of the species because of the interference of factors other than proteins. Therefore, the present study was undertaken to determine the optimal requirement of protein for juvenile P. indicus using purified diets and thus minimizing the influence of interfering components on growth.

MATERIAL AND METHODS

Experiments were conducted in the laboratory to study the efficiency of different levels of dietary protein and to determine the optimum requirements of protein in the diets of juvenile P. indicus. Data on survival, growth, feed conversion, protein efficiency ratio, and body composition (moisture, ash, protein, carbohydrate, lipid, RNA, DNA, calcium, magnesium and phosphorus) and ammonia excretion rates were obtained from these experiments.

Experimental Aquaria:

Experiments were carried out using plastic tubs of diameter, 54 cm and height, 24 cm. Earlier studies by Bernhard and Zattera (1970) have shown no harmful effect on the animals so held. The tubs were arranged on vertical steel racks and

were randomly distributed. Each of the tubs was provided with two rectangular aerator stones of 3 x 15 mm size, connected to a set of aerators through a plastic tube. The flow of air was maintained uniformly throughout the experimental period. The tubs were covered with nylon screen to prevent the escape of animals. Aeration was suspended for 2 hours every morning, while cleaning the tubs.

Seawater (salinity : 32-35‰) collected from the open sea off Cochin (depth 20-30 m), was transported to the laboratory in plastic jerry cans, filtered thrice using bolting silk (69 µ) and pooled into 500 l plastic pools. The salinity was adjusted to 20 ± 2.5‰ by diluting with tap water, since juvenile P. indicus prefer lower salinities (Colvin, 1976; Paul Raj, 1976; Paul Raj and Sanjeeva Raj, 1980). This water was aerated for 3 to 4 days through a biological filter with sun dried sand and oyster shells. Daily, the water was irradiated for 2 hrs with UV rays using 125 W UV lamp as the bacterial load (Zobell and Feltham, 1938) was lowest in this treatment, when subjected to UV rays for different time periods, as shown in Table 2.

Experimental Animals:

Post-larvae of P. indicus belonging to the same brood-stock were obtained from Narakkal Prawn Culture Laboratory of

TABLE 2: LOG BACTERIAL COUNT IN SEAWATER IRRADIATED AT DIFFERENT TIME PERIODS

Time (mins)	Mean Total Bacterial Number
0	19.95 x 10^1
15	19.51 x 10^1
30	12.75 x 10^1
45	10.85 x 10^1
60	9.50 x 10^1
90	7.75 x 10^1
120	7.25 x 10^1
180	7.11 x 10^1
After 8 hrs of irradiation	9.98 x 10^1

the Central Marine Fisheries Research Institute, Cochin and transported in polythene bags of 10 litre capacity, half-filled with fresh filtered sea water (salinity-25 ppt) and oxygen. These post-larvae were then introduced into 2 x 3 ft. perspex glass tanks, equally distributing about 50 to 60 animals per tank. The animals were then sorted out into different size groups, acclimated to laboratory conditions, and reared for 15-20 days with a compounded pellet diet to obtain the desired early juveniles (total length of about 20 mm) for experimentation.

Juvenile prawns of mean total length 20 ± 5 mm were used for the experiments. The total length of apparently healthy animals were measured to the nearest mm from the tip of the rostrum to the telson. The animals were then blotted dry carefully between the folds of filter paper (Bordner and Conklin, 1978), weighed on a Mettler electronic balance to the nearest mg, and were immediately transferred into the aquaria. The prawns were allowed to starve for 24 hrs to recover from handling stress prior to feeding. Before starting the experiment, about 15 prawns were measured, weighed and left for drying in an oven at 40°C for 48 hrs. The dried prawns were reweighed and the initial dry weight of the prawns were recorded.

Formulation and Preparation of Experimental Feeds:

Formulation and preparation of the feeds were done based on earlier nutritional studies carried out in crustaceans

(Kanazawa et al., 1971, 1977a, b; Deshimaru and Kuroki, 1974a, b, c; Adelung and Ponat, 1977; Conklin et al., 1978; Ponat and Adelung, 1980).

The ingredient composition of the formulated feeds, for determining optimum protein levels for juvenile prawns, is shown in Table 3. Casein has been widely used as a protein source for experimental studies in nutrition as it is the only protein source available in highly purified form (Halver, 1957; Kanazawa et al., 1971, 1976), though it is deficient in some of the amino acids (Halver, 1957; Ponat and Adelung, 1980). In the present study to determine the protein requirement of juvenile P. indicus, purified lipid-free casein was used as the major protein source. Gelatin and egg albumin were also used as protein sources as they supplement some of the deficient amino acids. Egg albumin in the feed is also reported to serve as a feed attractant for juvenile prawns (Clifford and Bricks, 1978). Gelatin, besides being a protein, serves as a binder for feeds (McLaren et al., 1947a).

The energy nutrients namely, proteins, lipids and carbohydrates were adjusted in the diets to obtain approximately a gross energy content of 4.2800 Kcals/g. The energy values for proteins, lipids and carbohydrates were calculated based on their gross calorific values of 5.65, 9.45 and 4.10 Kcals/g, respectively (Halver, 1957).

Test diets with graded levels of protein, ranging from 0 to 60%, were prepared by using casein as the principal protein source. The gross caloric content was adjusted to give approximately isocaloric diets using sucrose and starch as substitutes for protein. Carbohydrates were added both in the form of monosaccharides (glucose), disaccharides (sucrose) and complex polysaccharides (starch). Polysaccharides have been shown to be more efficiently utilized compared to simple sugars (Forster and Gabbott, 1971; Andrews et al., 1972; Sick and Andrews, 1973, Abdel-Rahman et al., 1979) and thus more quantity of starch was included in the diets.

Lipids were added in the form of corn oil (rich in linoleic acid) and cod liver oil (rich in polyunsaturated fatty acids of w3 series) to provide the w6 and w3 fatty acids which are essential for growth in prawns (Shewbart and Mies, 1973; Castell and Cowey, 1976; Colvin, 1976; Guary et al., 1976; Kanazawa et al., 1977a,b; Bottino et al., 1980). Since corn oil contains more of w6 fatty acids, which is detrimental to shrimps when in excess (Castell and Cowey, 1976), a mixture of corn oil and cod liver oil were used in the ratio of 1:2 and a lipid level of 9% was maintained in the diets based on earlier work (Hanson and Goodwin, 1977). Cholesterol was added in the diets, since crustaceans are incapable of sterol synthesis (Van Den Oord, 1964; Zandee, 1964; Whitney, 1970; Kanasawa et al., 1970; Deshimaru and Kuroki, 1974b), but

cholesterol is essential as a precursor for synthesis of steroid hormones, vitamin D and hypodermis pigmentation (New, 1976). Thus, in the diet 0.5% of cholesterol was added based on the recommendations of Kanazawa et al.(1970) for the prawn Penaeus japonicus and Castell et al. (1975) for the lobster Homarus americanus.

Though specific mineral requirements have not been worked out for shrimps, considerable importance has been laid for Ca:P ratio (0.76: 1 to 4:1) as reviewed by New (1976) and Maguire (1980). In the present study, about 7.4% of mineral mixture was added (Table 3) in the diet, based on earlier studies in crustaceans (Kanazawa et al., 1970, 1976, 1977a, b; Adelung and Ponat, 1977; Ponat and Adelung, 1980). Vitamins were added in the diet as non-energy dietary nutrients based on the amounts administered by various earlier workers (Kanazawa et al., 1971, 1977a; Adelung and Ponat, 1977; Watanabe et al., 1977b; Ponat and Adelung, 1980).
Table 3 shows the quantities of different fat and water-souble vitamins used in the experimental diets. Agar, starch and gelatin served as binders.

Finely ground, preweighed ingredients - casein, egg albumin, glucose, sucrose, mineral mixture, cholesterol, additives and agar agar were mixed in a waring blender. Fat soluble vitamins (A,D,E and K) were added into the mixture of codliver oil and corn oil. All the water-soluble vitamins were thoroughly ground and mixed using a mortar and pestle.

TABLE 3: COMPOSITION OF EXPERIMENTAL DIETS

Ingredient g/100 g.	EXPERIMENT - I							EXPERIMENT - II						
	0	10	20	30	40	50	60	32.5	35	37.5	40	42.5	45	47.5
Casein(lipid-free)	-	11	21	31	41	51	61	34	36	39	41	44	46	49
Egg albumin	-	1	1	1	1	1	1	1	1	1	1	1	1	1
Gelatin	-	1	1	1	1	1	1	1	1	1	1	1	1	1
Glucosamine-HCl	-	1	1	1	1	1	1	1	1	1	1	1	1	1
Sucrose	20.11	19.9	15.43	11.68	7.24	4.68	1.18	10.25	9.65	8.28	7.24	6.86	6.65	5.45
Glucose	10.19	7.19	6.53	4.96	3.68	2.32	0.90	4.61	4.41	4.2	3.68	3.33	2.75	2.50
Starch	43.45	32.59	27.39	22.61	18.51	12.98	7.65	21.65	20.58	18.89	18.51	15.61	14.92	13.66
Codliver-Oil	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Corn Oil	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium succinate	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sodium citrate	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	7.41
Mineral mixture*	7.41	7.41	7.41	7.41	7.41	7.41	7.41	7.41	7.41	7.41	7.41	7.41	7.41	3.24
Vitamin mixture**	3.24	3.24	3.24	3.24	3.24	3.24	3.24	3.24	3.24	3.24	3.24	3.24	3.24	2
Agar-agar	2	2	2	2	2	2.	2	2	2	2	2	2	2	4
Cellulose	4	4	4	4	4	4.	4	4	2	4	4	4	4	
TOTAL	100.5	100.42	100.10	100.80	100.18	100.73	100.48	100.26	100.39	100.12	100.18	100.25	100.07	100.36
Proximate Composition %														
Crude Protein	0.0	9.0	19.1	28.9	39.5	48.9	59.4	31.9	34.6	36.2	39.5	41.3	44.8	46.2
Total lipid	12.1	12.2	12.2	13.1	12.6	11.8	11.6	13.2	13.4	13.1	13.8	12.6	12.1	13.9
Ash	18.7	19.1	19.6	19.0	20.1	20.2	19.6	18.1	18.6	19.1	18.5	19.2	18.2	18.6

* $CaHPO_4.2H_2O$ 2.5, $MgSO_4.7H_2O$ 2.0, KH_2PO_4 1.5, $NaH_2PO_4.2H_2O$ 1.0, $MnSO_4.H_2O$ 0.14, $FeSO_4$ 0.10, $ZnSO_4.7H_2O$ 0.10, $C_6H_5O_7Fe.5H_2O$ 0.05, $Co(NO_3)_3$ 0.01, $CuSO_4.5H_2O$ 0.01.

** B-Carotene 0.014, Calciferol 0.002 α-Tocopherol acetate 0.032, Menadione 0.032, Ascorbic acid 2.424, Thiamine hydrochloride 0.01, Riboflavin 0.008, Nicotinic acid 0.032, Pyridoxine hydrochloride 0.016 Calcium pantothenate 0.06, Folic acid 0.001, p-Aminobenzoic acid 0.014, Choline chloride 0.30, Inositol 0.30, Biotin 0.004, Cyanocobalamin 0.001.

Gelatin was dissolved in cold double distilled water (Halver, 1978; 1980) and boiled over a water bath, along with cellulose and starch. After gelatinization, corn oil and cod liver oil containing fat soluble vitamins were added and the heating was continued for another 10 mins. in the water-bath at slightly lower temperature (70°C) and mixed thoroughly. To this mixture, added the powdered protein-mineral-agar mixture, again mixed thoroughly adding slowly double distilled water till the required consistency of moist dough was obtained. The whole mixture was then steamed at 115 lbs pressure for 5 minutes. The steamed feed was allowed to cool to room temperature and the water soluble vitamin mixture was added and mixed throughly. The pH of the diet was adjusted to 6.8 (Kanazawa et al., 1977a) using 0.1 N NaOH and was stored in polyethylene bags in a freezer. The moisture content in the feed was adjusted to about 30%. Each time feed required for 15 days was prepared so as to maintain the quality of the feed. Each day before feeding, the required amount of feed was thawed to room temperature and manually made into small balls, weighed and fed to the prawns.

Feeding Level and Schedule:

The juvenile prawns were fed with the experimental diets at the rate of 10% (by dry weight) of the live body weight/day as suggested by Subramanyam and Oppenheimer (1970). The feeding was done twice a day, in the morning and in the evening.

The amount of feed given was adjusted every 15 days of the experiment based on changes in the body weight.

Collection of Faecal Matter and Left-over Food:

The left-over food and faecal matter were daily collected from the aquaria by slow siphoning of the water through a narrow plastic tube and collected at the other end on a bolting silk. The faecal matter and left-over food, collected separately, were washed in distilled water to remove the adhering salts, transferred to pre-weighed aluminium foils and kept for drying at 70°C for 48 hrs. The dried samples were weighed and the dry weights were recorded. The samples were stored in a desiccator for subsequent analysis.

After 15 days of experiment, the animals were weighed and the tanks were thoroughly washed with detergent, rinsed with tap water and reintroduced the animals in fresh dilute sea water of 20 ppt salinity. The experiment was terminated on the 30th day and the length and weight of the animals were recorded. The dried samples were powdered in a porcelain mortar and pestle and biochemical composition studies were performed.

Monitoring of Physico-Chemical Parameters in Water:

Water temperature was recorded twice a day morning (about 8 a.m.) and evening (about 6 p.m.), using a graduated mercury thermometer with an accuracy of 0.01°C. The dissolved

oxygen content in water samples was determined employing the Winkler's method (Strickland and Parsons, 1972; Spotte, 1979).

Sea water samples for ammonia estimation were collected from the experimental tanks just before and after changing the water and fixed with 4% phenol solution immediately, stored in refrigerator and analysed within 2 hrs. of collection (Spotte, 1979). The ammonia concentration was determined using phenol-sodium hypochlorite method (Solorzano, 1969). Salinity of the water in the experimental tanks was determined thrice a week using argentometric method (Strickland and Parsons, 1972; Riley et al., 1975). Standard sea water was obtained from IAPSO, Institute of Oceanographic Sciences, Surrey, England (chlorinity, 19.37).

pH of the water samples from the experimental tanks was determined thrice a week using Elico pH meter with an accuracy of 0.01. All pH determinations were done at room temperatures. During the experimental study, the prawns were maintained at 12L:12D photoperiod cycle. The mean temperature, salinity, pH, dissolved oxygen and ammonia levels maintained during the experiments are shown in Table 4, which were well within the established tolerance limits of prawns (Wickins, 1973; Colvin, 1976; Delistraty et al., 1977).

Recording of Data:

Survival Rate:

Daily the population of prawns was recorded from each of the experimental treatments and the mean number of surviving

TABLE 4: ENVIRONMENTAL PARAMETERS AND STOCKING SIZE OF JUVENILE PRAWNS

Parameter	Experiment I Meanvalues	Experiment II Meanvalues
Temperature (°C)	27.71 ± 1.94	27.63 ± 2.27
Salinity (ppt)	20.9 ± 2.5	21.23 ± 2.59
pH	8.36 ± 1.011	8.02 ± 0.49
Ammonia concentration in the water (NH_4-N mg/l/d)	0.0352 ± 0.0096	0.0236 ± 0.0032
Initial length (mm)	16.95 ± 1.122	20.06 ± 0738
Initial weight (mg)	30.3 ± 0.0145	44.95 ± 2.193

prawns per week was determined. The final percent survival was determined as follows.

$$\text{Percentage Survival} = \frac{\text{Initial number} - \text{Final number}}{\text{Initial number}} \times 100$$

Growth Rate:

At the end of the experiment, the total length and weight of prawns were measured adopting similar procedures as for initial measurement of these prawns. The prawns were later killed by brief immersion in boiling water (Clifford and Brick, 1983) and left for drying for 48 hrs at 40°C in an oven. The dried samples were weighed and the final dry weight of the prawns was recorded. The mean percent gain in length and weight were determined as follows:

$$\text{Mean percent gain in length/weight} = \frac{\text{Mean final length/weight} - \text{Mean initial length/weight}}{\text{Mean initial length/weight}} \times 100$$

Specific Food Consumption, Food Conversion Ratio and Protein Efficiency Ratio:

The food consumption per body weight (wet weight) per unit time (Bordner and Conklin, 1981) was calculated as follows:

$$\text{Specific food consumption (\%)} = \frac{\text{Total initial dry weight of food fed} - \text{Total final dry weight of food uneaten}}{\text{Number of animals surviving at the end of the experiment} \times \text{Time in days of experimental period} \times \text{Mean animal wet weight (g)}} \times 100$$

Food conversion ratio (FCR) was determined as follows:

$$\text{Food conversion ratio} = \frac{\text{Total dry weight of food fed} - \text{Total dry weight of left-over food}}{\text{Final wet weight of prawns} + \text{Wet weight of dead prawns} - \text{Initial wet weight of prawns}}$$

Protein efficiency ratio was determined as follows:

$$\text{Protein efficiency ratio} = \frac{\text{Final wet weight of prawns} - \text{Initial wet weight of prawns}}{\text{Total protein intake}}$$

All these parameters are apparent, since no correction factor was introduced for the exuviae and dead prawns eaten by the cohabitors during the experimental study.

Chemical Composition of Feed and Carcass:

Moisture content in the feeds and prawns was determined gravimetrically by oven drying the samples at 100°C for feed samples and at 40°C for prawns, till concurrent dry weights were obtained. Percent moisture in the samples was calculated as follows:

$$\text{Percent moisture} = \frac{\text{Wet weight of the sample} - \text{Dry weight of the sample}}{\text{Wet weight of the sample}} \times 100$$

Weighed dried samples of feed and prawn were ashed in silica crucibles at 550°C for 6 hrs in a muffle furnace and the percent ash was determined as follows:

$$\text{Percent ash} = \frac{\text{Weight of the ash}}{\text{Weight of the dried sample taken}} \times 100$$

To determine the crude protein content, weighed samples of feed were digested in tubes with a catalyst mixture (K_2SO_4: $CuSO_4$: SeO_2::8:1:1) and concentrated sulphuric acid (Sp. gr. 1.84) for 3 hrs at 120°C. The total nitrogen content of the digested samples was determined using Kjeldahl method (AOAC, 1975). Crude protein content in the feeds was determined by using the conversion factor of 6.25 per unit of nitrogen.

To determine the protein content in prawns, known quantity of dry samples of prawns were homogenized in chloroform methanol mixture and the supernatant was collected. To the residue added cold 15% TCA, homogenized and kept for 3 hrs in cold chamber (4°C) for complete extraction of carbohydrates. The samples was centrifuged at 1200 rpm for 10 mins and the supernatant was collected. Then washed the sample with cold 5% TCA, centrifuged and the collected supernatant was mixed with the first lot and kept for carbohydrate determination. the residue added 1N NaOH, homogenized and left overnight at 37°C for complete tissue protein dissolution. Tissue protein was determined using Biuret method (Gornall et al., 1949) and the optical density was recorded on ECIL-UV spectrophotometer at 530 nm. The protein content in the sample was determined from standard graph using Bovine albumin serum as standard and the protein content was expressed on percent dry weight.

Lipid content in feeds was determined using Soxhlet extraction method (AOAC, 1970) using petroleum ether (60-80°C) as solvent. Lipid extraction was carried out

for 16 hrs, and the total lipid in feed was determined gravimetrically. Tissue lipid content was determined using Bligh and Dyer (1959) method of chloroform-methanol-water mixture, (2:2:1) modified by Ando et al.,(1977). Weighed, dried samples of whole prawn were homogenized for five minutes with a mixture of choloroform-methanol(1:2). The samples were then kept overnight at 4°C in dark for the complete extraction of total lipids. The extracted lipids in the chloroform-methanol mixture layer was centrifuged at 800 rpm for 10 mins in cold and the supernatant was collected. To the residue added chloroform again, centrifuged for 5 minutes and the supernatant was collected. To the supernatant, added double distilled water and the final solution had chloroform:methanol:water in the ratio 2:2:1. The mixture was thoroughly shaken and allowed to settle. Pipetted out the layer of water:methanol and dried the chloroform-lipid layer in a desiccator with concentrated sulphuric acid as desiccant and the total lipid was estimated gravimetrically.

Tissue carbohydrate was determined from the TCA supernatant extract using modified phenol-sulphuric acid method (Dubois et al., 1956). The optical density (OD) was recorded on ECIL-UV spectrophotometer at 490 nm.

Calcium in the whole prawn was determined by the modified method of Clark and Collip (1925). To the weighed dry samples of prawn about 4% ammonium oxalate was added, mixed thoroughly and allowed it to stand for overnight. The

mixture was centrifuged at 1500 rpm and the supernatant was collected for magnesium determination. The precipitate was washed, centrifuged and washed three times with 2% ammonia solution. To the washed precipitate, added concentrated sulphuric acid (Sp. gr. 1.84) and mixed well. The tube was transfered to a boiling water bath for 1-2 minutes and titrated against 0.01N potassium permanganate to a definite pink color which persists for about a minute. The percent calcium determined in prawns was expressed in percent dry weight basis and was calculated as

$$\text{Calcium (\%)} = \frac{\left\{\begin{matrix}\text{Volume of 0.01N} - \text{Blank} \\ \text{KMnO4 required}\end{matrix}\right\} \times 0.2 \times 100}{\text{Weight of dried sample taken}}$$

where, 0.2 mg of calcium = 1 ml of 0.01N $KMnO_4$

The supernatant collected was used for determination of magnesium employing the modified method of Briggs(1922). Known volume of supernatant was mixed with 5% ammonium phosphate solution and concentrated NH_4OH. The mixture was left overnight for complete precipitation; centrifuged at 1500 rpm and the supernatant was discarded. The precipitate was washed first with 33% ammonium hydroxide solution, two to three times centrifuged and siphoned off the solution and finally washed with alcoholic ammonia solution and decanted the same. Ammonia was completely evaporated from the sample by placing the tube in hot air oven at 50-60°C for 1 hour. The precipitate was dissolved with molybdate solution and to this added amino-naphtholsulphonic acid, allowed to stand for 5 minutes, and the optical density (OD) was measured in a ECIL spectrophotometer

at 680 nm. The magnesium content was expressed as percent dry matter and calculated as follows.

$$\text{Magnesium (\%)} = \frac{\text{OD of unknown}}{\text{OD of standard}} \times \frac{0.03 \times 100}{\text{Weight of dried sample}}$$

Total phosphorus was estimated by the method of Lowry et al. (1954) using phosphomolybdate and ascorbic acid. Weighed samples were added to the ashing mixture containing 70% $HClO_4$ and 20% H_2SO_4. Heated the mixture in an oven at 95°C for 2 hrs, followed by heating at 165°C for another 2 hrs. The mixture was cooled to room temperature and added the mixture of ammonium molybdate and ascorbic acid. Immediately mixed thoroughly and placed the tubes at 37°C for 2 hrs. After cooling, the optical density was recorded in a ECIL-UV spectrophotometer at 820 nm. The phosphorus was expressed on percent dry weight basis and calculated as follows.

$$\text{Phosphorus (\%)} = \frac{\text{OD of sample}}{\text{OD of standard}} \times \frac{\text{Concentration of standard}}{\text{Weight of sample}} \times 10$$

The weighed samples (10 mg dry weight) were individually homogenized in 4 ml ice-cold distilled water with a tissue grinder. The RNA was extracted by the Halliburton and Thompson (1965) method and measured at 260 nm on a ECIL-UV spectrophotometer. RNA obtained from Sigma Chemical Company was used to prepare the RNA standard curve. The DNA content of the sample was determined by the indole method (Ceriotti, 1952, 1955). Highly polymerized calf thymus DNA (Type I, Sigma Chemical Co.) was used to prepare the DNA standard curve. The detailed

procedures are illustrated in Table 5.

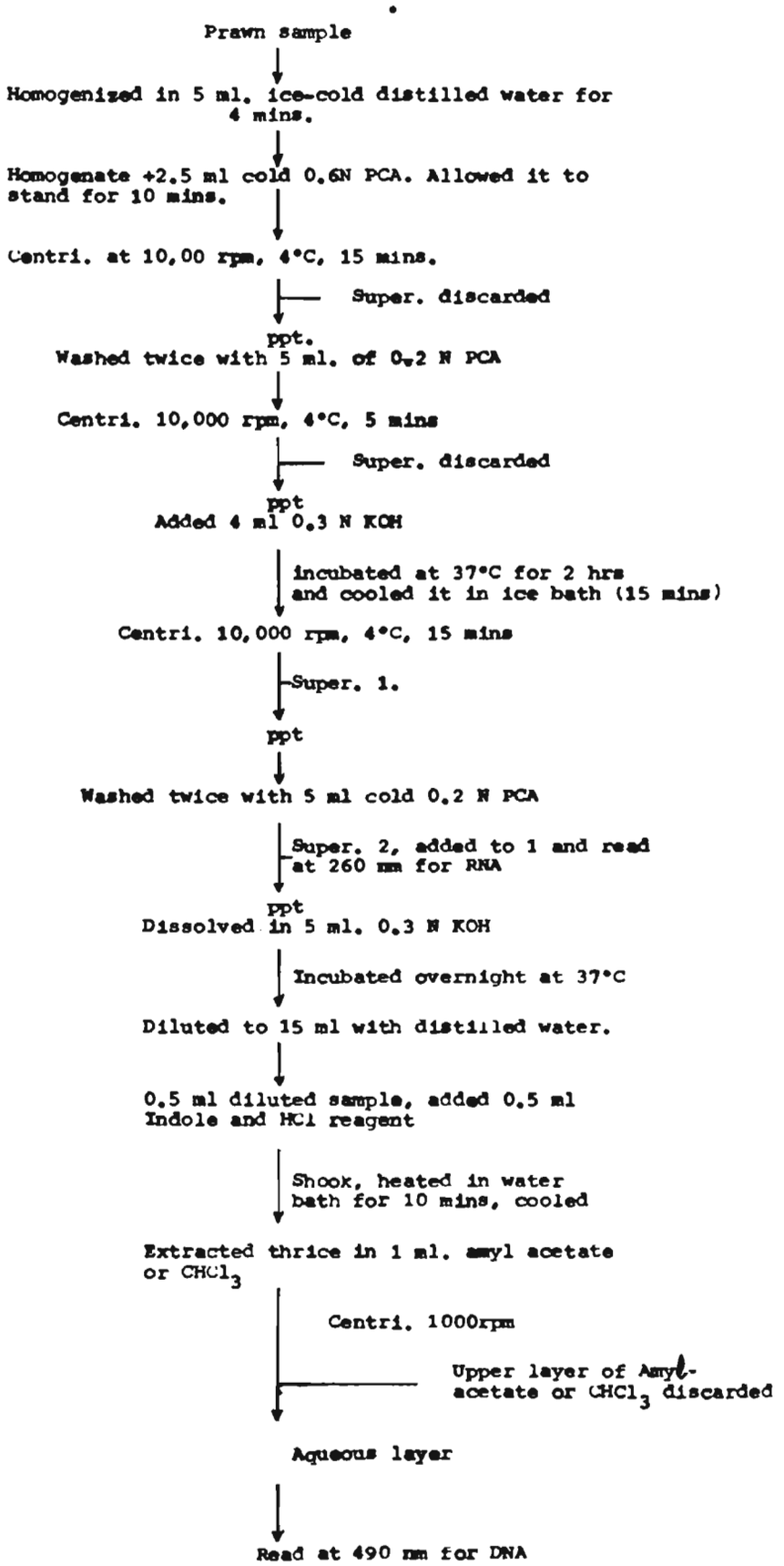
Ammonia Excretion Rates:

Ammonia excretion rates in prawns were studied individually using 3 l conical flask containing 2.1l of fresh diluted irradiated seawater (20 ppt). Each of the conical flasks was provided with an aerator stone (3 x 15 mm size) connected with a plastic tube to the aerator. An additional tube was provided with a stop pinch cork at one end, for collection of water sample. The whole apparatus was plugged with rubber cork and the mouth was covered with aluminium foil with a provision for the two plastic tubes to enter into the conical flasks.

Prior to the introduction of the animals, the sea water was aerated. The volume of sea water taken for the experimental study in each of the conical flask was so adjusted that at the end of 24 hrs, the flask had about 1.5 l of seawater. The temperature, pH, salinity and dissolved oxygen were recorded at the start and end of the experiment. The photoperiod was maintained at 12L:12D and no artificial light was provided other than natural day-light during light period.

Intermolt prawns fed for 30 days on different diets were selected in triplicate from each of the treatment for experimental study. Animals selected for the experimental study were almost of the same size and weight. Prior to the introduction in the experimental flasks, the prawns were fed with respective protein level purified diets. After two hours of

TABLE 5: ANALYTICAL PROCEDURE FOR MEASUREMENT OF RNA AND DNA (FLOW CHART)



ppt - precipitate; Super. - supernatant,
PCA - Perchloric acid; Centri. - centrifuge.

feeding, the prawns were transferred into the individual conical flasks. A control was kept without any prawns.

After introduction, water sample was taken for determination of ammonia concentration. Water samples were collected every four hours from each of the flask and quickly analysed for ammonia. The experiment was carried out for 24 hrs. Ammonia concentration in water was analysed by the method of Solorzano (1969). The ammonia concentration excreted by the prawns was expressed as NH_4-N mg/ day/g prawn.

Statistical Analysis of the Data:

The data obtained on various parameters from the experiment were statistically analysed. Analysis of variance (ANOVA) was carried out to test the difference between the treatments. Least significant difference (LSD) method was followed to compare the means of the treatments (Snedecor and Cochran, 1973).

R E S U L T S A N D O B S E R V A T I O N S

Two sets of experiments were conducted to study the effect of different levels of dietary protein on growth, feed efficiency and body composition and to determine the optimum dietary protein requirement of the juveniles of the Indian white prawn, *P. indicus*.

EXPERIMENT - I

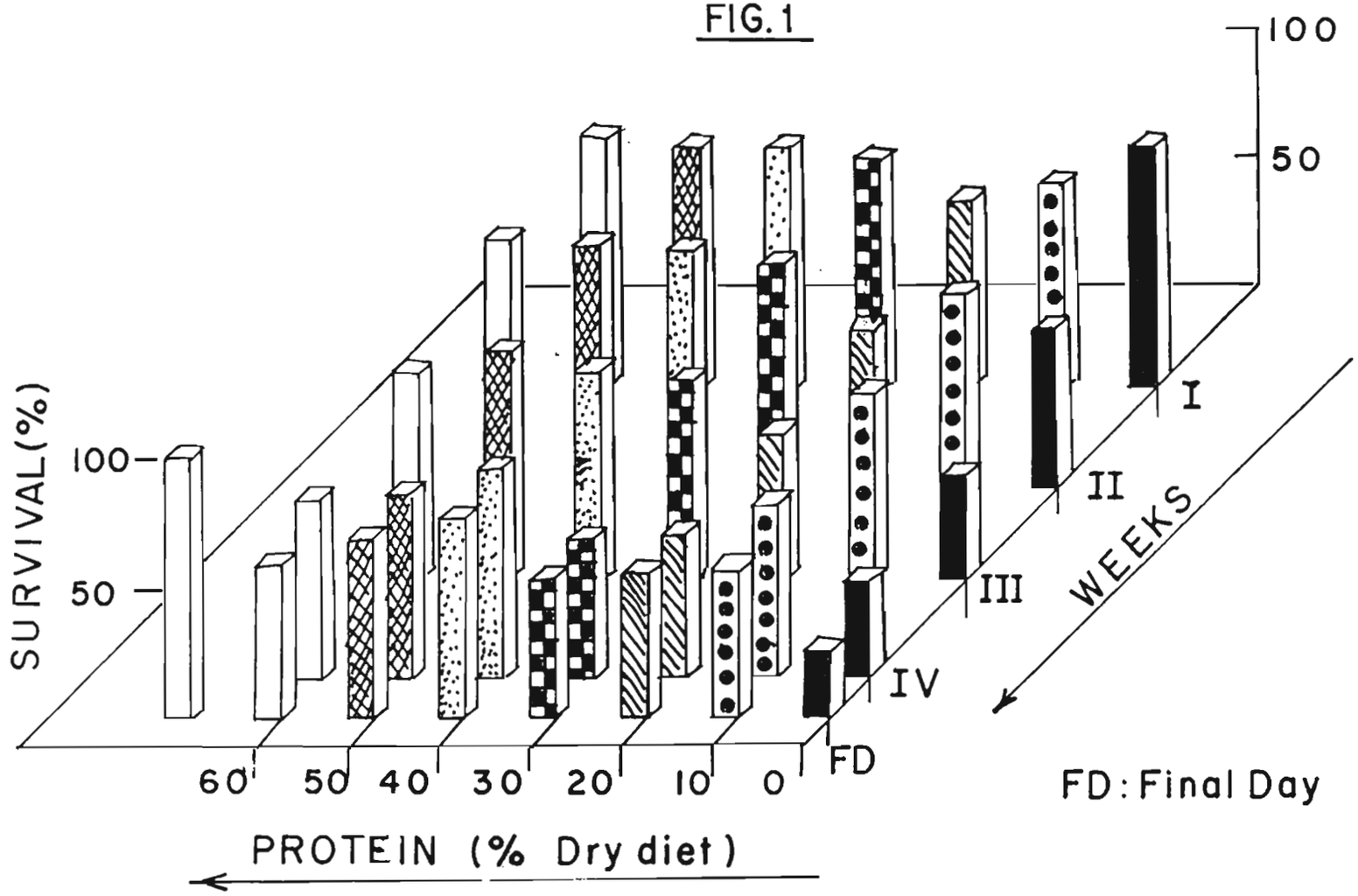
In this experiment, protein levels ranging from 0 to 60%, with an interval of 10% were used for compounding purified diets. The diets were fed to the prawns for a period of 30 days and the results of the experiment are presented here.

Survival:

Data on percent survival recorded from different treatment groups are shown in Fig. 1. Although, the protein content in the diet, had apparent effect on the percent survival, analysis of variance of the data did not show any significant influence of dietary protein level on the survival of prawns. However, the percent survival of prawns increased with protein content in the diet upto 40% and thereafter it showed a gradual decline. The maximum survival (73.3%) was recorded at 40% protein level in the diet and the minimum (26.7%) in the protein-free diet. In all other treatments, the percent survival ranged between 51.1 and 66.7%. While an abrupt decline in survival rate of prawns was observed during the second week in the protein-free dietary treatment (0%); not much variation in the survival rate was observed in the other treatment groups. However, a steady decrease in the survival rate of prawns was observed from the fourth week onwards in treatment groups of prawns fed diets with protein levels ranging from 10 to 30%.

Fig. 1. Weekly percent survival of prawns fed with different levels of protein in the diets(0-60%)

FIG. 1


100
50
SURVIVAL(%)
100
50
I
II
III
IV
WEEKS
FD
60
50
40
30
20
10
0
PROTEIN (% Dry diet)
FD: Final Day

Growth:

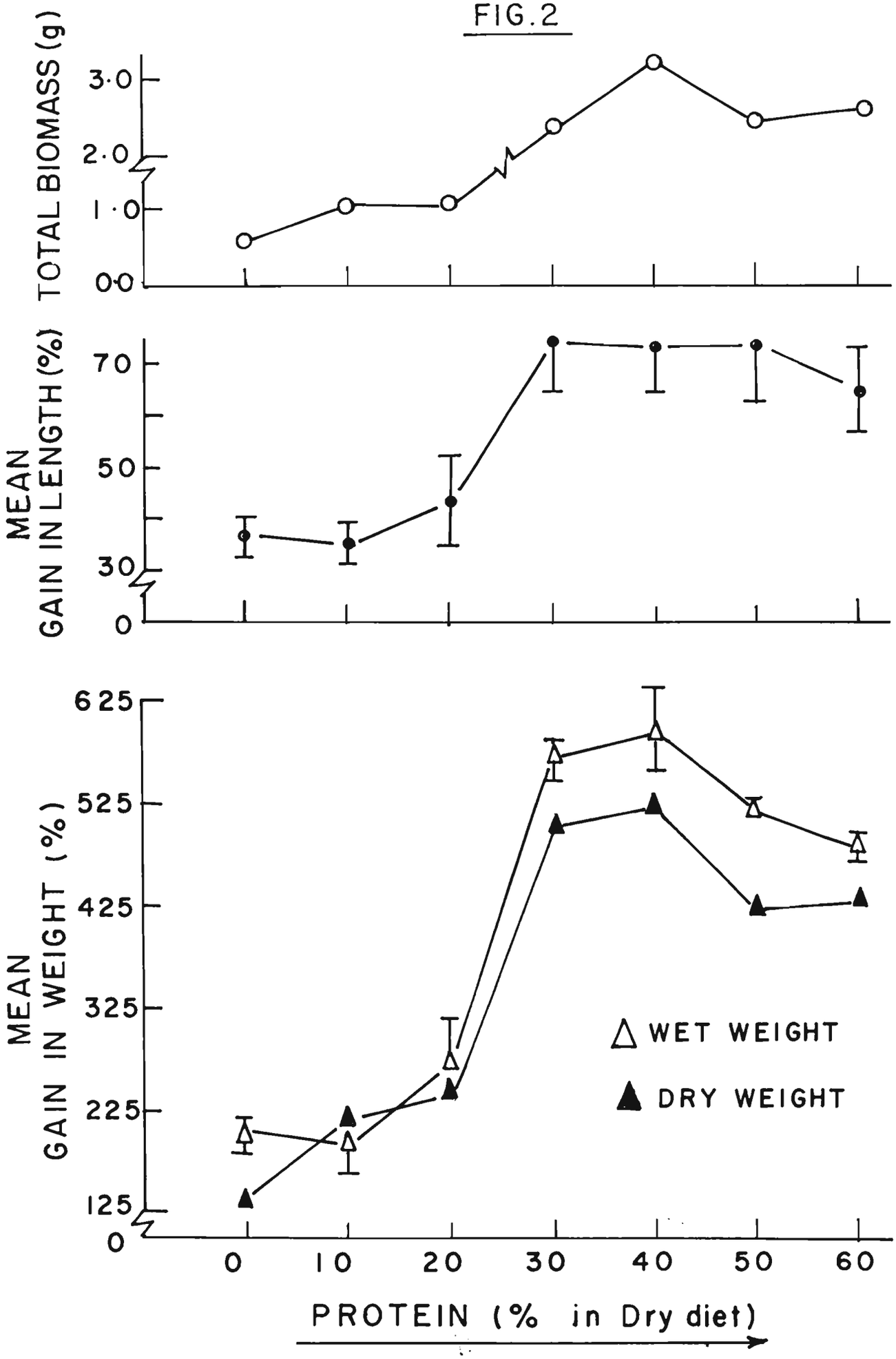
Growth of prawns fed on the protein-free diet (0% protein level) showed marked difference with that of other treatments. Data on mean percent gain in length (37%), wet weight (200%) and dry weight (138%), shown in Fig. 2, indicate that poor growth has occurred in prawns fed on the protein-free diet, and the growth achieved can mainly be attributed to the cannibalism by the cohabiting species on the post-molted and dead prawns, before they were removed from the aquaria.

Fig. 2 shows the mean percent gain in length of prawns fed diets differing in the percent protein. Results of analysis of variance showed that the protein levels in the diets have significant ($P < 0.05$) effect on the mean percent gain in length of prawns. The percent gain in length of prawns increased with increasing protein levels in the diet from 10% upto 30% and showed a gradual decline, thereafter, with further increase in protein level of the diet. The maximum mean percent gain in length was observed at 30% protein level (74%) and the minimum at 10% protein level (35%). There were no significant differences between the mean percent gain in length of prawns fed diet with 30, 40 and 50% protein.

The diets containing various protein levels also had highly significant ($P < 0.01$) effect on the mean percent gains in wet weight and dry weight of prawns. The mean percent wet

Fig. 2. Percent gain in length and weight, and total biomass (g) of prawns fed with different levels of protein in the diets (0-60%).

FIG.2


TOTAL BIOMASS (g)
3·0
2·0
1·0
0·0
MEAN GAIN IN LENGTH (%)
70
50
30
0
MEAN GAIN IN WEIGHT (%)
625
525
425
325
225
125
0
WET WEIGHT
DRY WEIGHT
0
10
20
30
40
50
60
PROTEIN (% in Dry diet)

weight and dry weight increased with increasing protein levels in the diet. However, the increase was observed upto 40% protein in the diet and thereafter a gradual decline was observed as the protein level in the diet increased to 60%. The maximum percent wet weight gain (597%) and dry weight gain (521%) were recorded at 40% protein level and the minimum at 10% protein level (wet weight gain 190% and 213% dry weight gain). There was pronounced increase in the percent gains in wet weight and dry weight of prawns fed diets from 20% protein level upto 40% protein in the diet, indicating the significant influence of protein in the diet on the prawn's growth.

Specific Food Consumption (SFC):

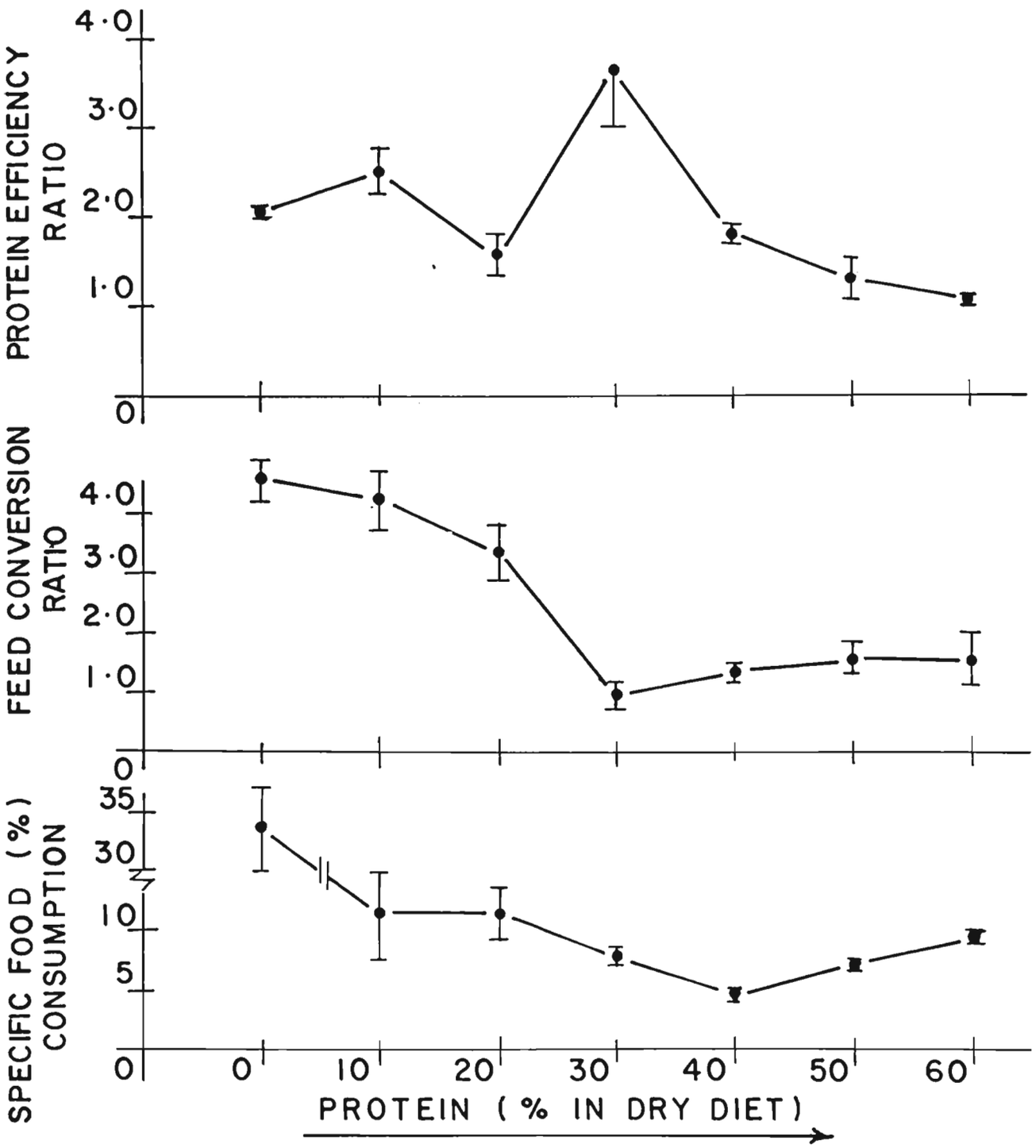
Highly significant ($P < 0.01$) differences were observed among the specific food consumption of prawns fed diets containing different levels of protein. Very high value of SFC (33.7%) was obtained in the case of prawns fed on the protein-free diet (Fig. 3). Between 10% and 60% protein level in the diet, the highest SFC was observed at 10% and 20% protein levels (11.7% and 11.5%, respectively) and the lowest was recorded at 40% protein level (4.3%). In all other treatment groups SFC ranged between 5.5 and 7.3%.

Food Conversion Ratio (FCR):

Food conversion ratios obtained from the experiment are shown in Fig. 3. Highly significant ($P < 0.01$) differences were observed in the FCRs obtained by feeding different protein

Fig. 3. SFC, FCR and PER for different levels of protein in the diets (0-60%).

FIG. 3


PROTEIN EFFICIENCY RATIO
4·0
3·0
2·0
1·0
0
FEED CONVERSION RATIO
4·0
3·0
2·0
1·0
0
SPECIFIC FOOD (%) CONSUMPTION
35
30
10
5
0
0
10
20
30
40
50
60
PROTEIN (% IN DRY DIET)

concentrations in the diet. While the maximum FCR was obtained with 10% protein level (4.2), the minimum was obtained with 30% protein level (0.96). In other treatments (20, 40, 50 and 60%), the FCR values ranged between 1.35 and 3.4.

Protein Efficiency Ratio (PER):

Analysis of the data showed that the protein levels in the diets, significantly ($P < 0.01$) influence the protein efficiency ratio (Fig. 3). The maximum PER was recorded at 30% protein level (3.7) and the minimum at 60% protein level (1.1). There were no significant differences among PER obtained from treatments with protein levels between 40 and 60%. The PER in other treatments ranged between 1.3 and 2.4.

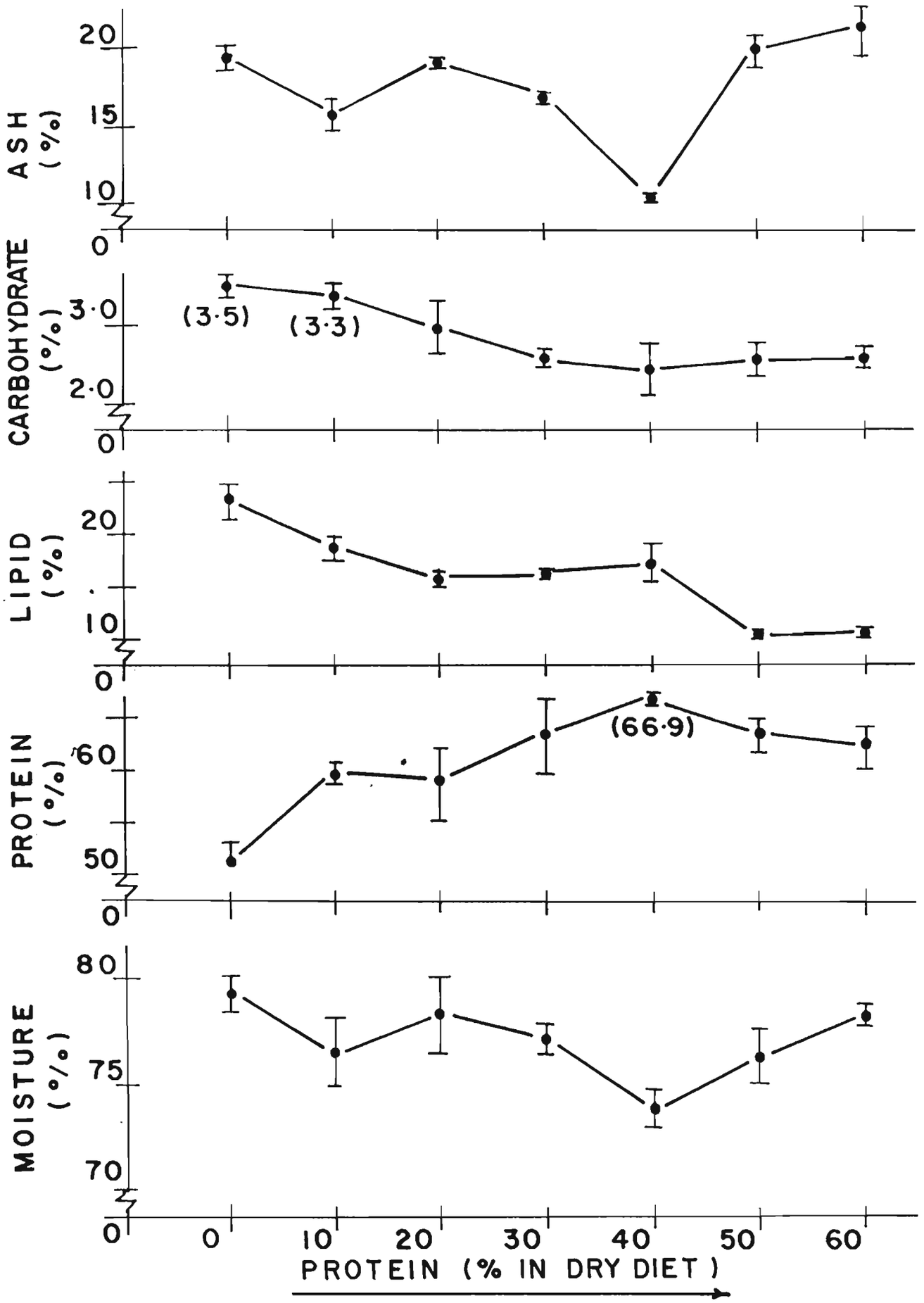
Biochemical Composition:

The moisture, ash, protein, lipid and carbohydrate contents of prawns were also found significantly ($P < 0.01$) affected by the level of protein in the diet. It was observed (Fig. 4) that as the protein level in the diet increased, the moisture content of prawns declined upto 40% protein level and thereafter the moisture content increased with further increase in protein concentration in the diet. The prawns fed diets containing 10% and 20% protein had significantly higher moisture content than most other groups.

The prawns fed diets containing 40% protein had significantly ($P < 0.05$) less ash (10.3%) and more protein (66.9%)

Fig. 4 . Biochemical composition of prawns fed with different levels of protein in the diets (0-60%).

FIG. 4


ASH (%)
20
15
10
0
CARBOHYDRATE (%)
3·0
2·0
0
(3·5)
(3·3)
LIPID (%)
20
10
0
PROTEIN (%)
60
50
0
(66·9)
MOISTURE (%)
80
75
70
0
0
10
20
30
40
50
60
PROTEIN (% IN DRY DIET)

than prawns from other treatments. The highest ash content was recorded at 60% protein level in the diet (21.2%).

Although, the minimum protein content was recorded in prawns fed with the 20% protein diet (58.9%), it was not significantly different from the protein content of prawns fed diet with 10% protein (59.9%). Similarly, there were no significant differences between the protein contents of prawns from treatments with 30, 50 and 60% protein levels in the diet.

The lipid content was significantly higher in prawns fed with 10% protein diet (18.5%). Similarly, lipid contents in prawns fed diets containing 50 and 60% protein (10.4%) were significantly less than the lipid content of prawns from other treatments, which varied between 11.2 and 17.3%. There was a steady decline in the total lipid content of prawns with the increasing protein level in the diets, however, there was a slight increase in the lipid content of prawns fed with 40% protein diet which can be correlated with the low moisture content of prawns recorded at this treatment. An inverse relationship between total lipid and moisture content of the prawns was observed.

Maximum significant ($P < 0.05$) differences in carbohydrate content were observed between 10% and 20% protein levels. The maximum carbohydrate content of prawns was recorded at 10% protein level (3.4%) and minimum at 40% protein level (2.4%). In all other treatments, the carbohydrate content of prawns varied between 2.5 and 2.9%. There were no significant

differences among the carbohydrate content of prawns fed diets containing protein levels ranging from 30 to 60%. However, a declining trend in carbohydrate content of prawns was observed with the increase in dietary protein level upto 40% and thereafter a gradual rise in carbohydrate content occurred as the concentration of protein in the diet increased.

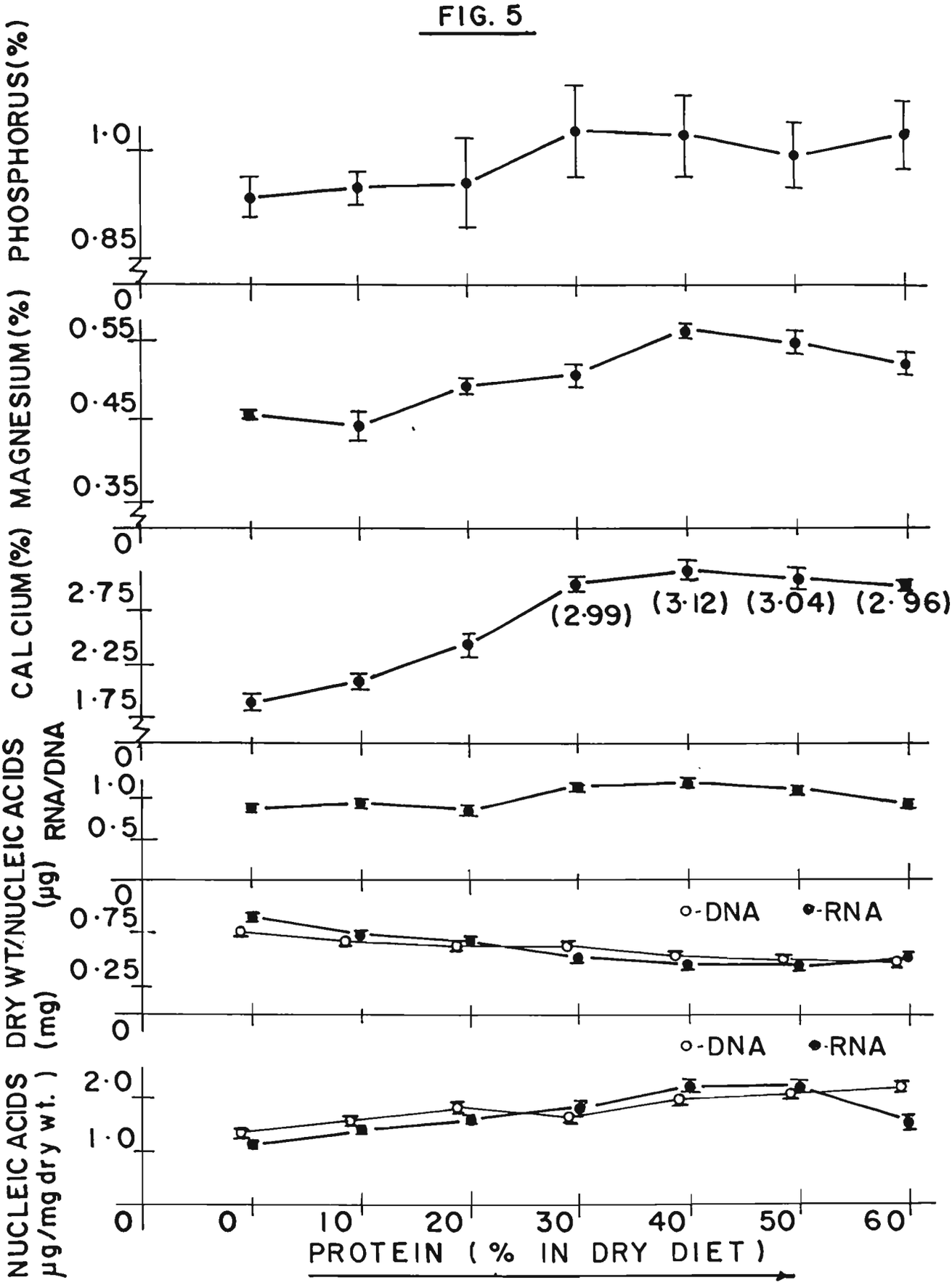
The moisture, ash, protein, lipid and carbohydrate contents of prawns fed the protein-free diet showed significant ($P < 0.05$) differences with that of prawns fed on other diets.

There was high incidence of mortality and cannibalism in this group, which significantly ($P < 0.05$) influenced the composition. It is felt that comparison of the result with that of other treatments may not provide any meaningful conclusions. Despite this, the moisture (79.5%), ash (19.4%), carbohydrate (3.5%) and lipid (23.3%) contents were observed to be high in these groups of prawns with comparatively very low protein content(51%).

The RNA content of prawns (Fig. 5) was also significantly ($P < 0.05$) affected by the protein levels in diets. The prawns fed the protein-free diet and those fed with 10 and 20% protein levels in the diets had significantly ($P < 0.05$) less RNA than those fed on diets with higher protein concentrations. While the maximum RNA content was recorded in prawns fed diets with 40% and 50% protein contents (2.19 µg/mg), the minimum was found in prawns fed on the protein-free diet (1.13 µg/mg). In prawns from all

Fig. 5. Biochemical composition of prawns fed with different levels of protein in the diets (0-60%).

FIG. 5


PHOSPHORUS(%)
1·0
0·85
0
MAGNESIUM(%)
0·55
0·45
0·35
0
CALCIUM(%)
2·75
2·25
1·75
0
(2·99)
(3·12)
(3·04)
(2·96)
RNA/DNA
1·0
0·5
0
DRY WT./NUCLEIC ACIDS (µg) (mg)
0·75
0·25
0
o-DNA
•-RNA
NUCLEIC ACIDS (µg/mg dry wt.)
2·0
1·0
0
o-DNA
•-RNA
0
10
20
30
40
50
60
PROTEIN (% IN DRY DIET)

other treatments, the RNA content varied between 1.39 and 1.96 µg/mg. However, with increasing protein levels in the diet of prawns, the RNA content increased upto 50% protein level, and further increase in the protein content of the diet resulted in decrease of the RNA content.

The DNA content of prawns (Fig. 5) showed a similar trend as that of RNA content, increasing with the protein concentration in the diet of prawns. However, the DNA content did not show any decline beyond 50% level, as observed for RNA, but showed increase upto 60% level. It was also observed that the DNA content of prawns fed the protein-free diet was significantly ($P < 0.05$) less than that of prawns fed diets with different levels of protein. The highest DNA content was recorded in prawns fed on the diet with 60% protein level (2.13 µg/mg) and the lowest in prawns fed the protein free-diet (1.31 µg/mg). In all other treatment groups, the DNA content ranged between 1.5 and 2.05 µg/mg.

Significant ($P < 0.05$) variations were also observed in the dry weight/total RNA ratio in prawns (Fig. 5) fed diets containing different levels of protein. However, prawns fed the protein free diet and those fed diets with 10% protein had significantly ($P < 0.05$) higher ratios compared to prawns from other treatments. The dry weight/RNA ratio showed a decreasing trend with increasing protein content of the diet upto 50%, and thereafter a slight increase was observed at 60% protein diet.

Similarly, the dry weight/total DNA ratio (Fig. 5) decreased with increase in protein content of the diet of prawns and the highest was recorded in prawns fed on the protein-free diet (0.79) and lowest in prawns fed with 60% protein (0.47) in the diet. For all other treatment groups, the ratio ranged between 0.44 and 0.67. The ratio was significantly (P 0.05) higher for prawns fed the protein-free diet as well as those fed with 10% protein level that for other treatments.

The RNA/DNA ratio increased with the protein concentration in the diet upto 40% and thereafter it showed a decline. Though the RNA/DNA ratio was significantly ($P < 0.05$) influenced by the protein level in the diets, only prawns fed diets with 30 and 40% protein levels had significantly higher RNA/DNA ratios, than prawns from other treatments.

The dietary protein concentrations also had highly significant ($P < 0.01$) effect on the calcium content (Fig. 5) of prawns. The prawns fed on the protein-free diet had significantly ($P < 0.05$) lower calcium content (1.87%) than that of prawns from other dietary protein treatments. The highest calcium content was recorded in prawns fed 40% protein diet (3.13%) followed by those fed 50% protein in the diet (3.05%).

The magnesium and phosphorus contents (Fig. 5) of prawns were not significantly affected by the levels of protein in the diet. While the magnesium content ranged from 0.44 to 0.55%, the phosphorus content varied between 0.95 and 1.02% in prawns from various dietary treatments.

Ammonia Concentration in Water:

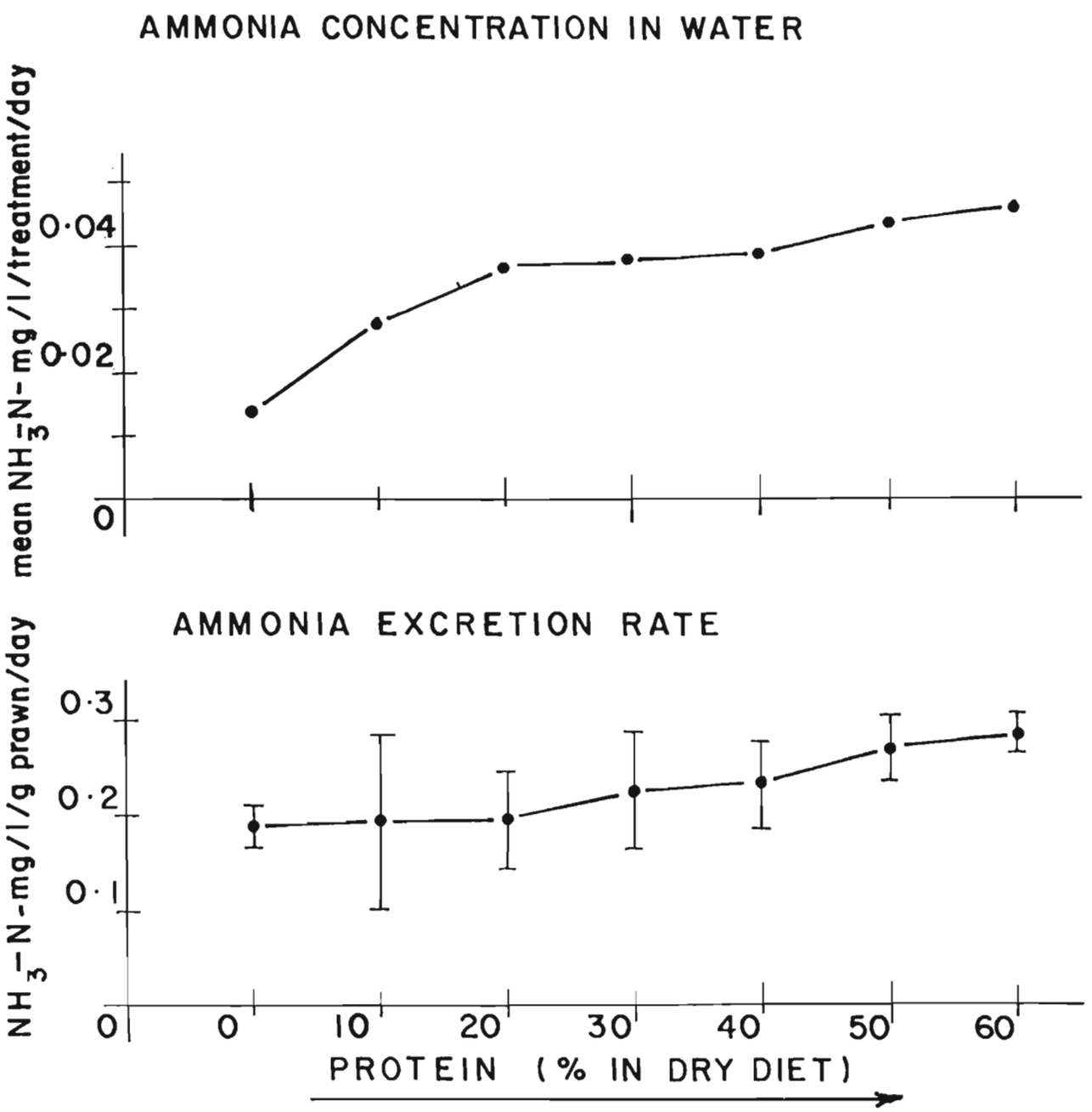
The ammonia concentration in water was significantly ($P < 0.05$) influenced by the dietary protein levels (Fig. 6). With increasing protein level in the diet, the prawns showed an increasing trend in ammonia concentration in water. The highest ammonia concentration in water was recorded at 60% protein (0.47 mg/l) and lowest in prawns fed on the protein free diet (0.43 mg/l). However prawns fed between 20% and 40% protein in the diet showed almost the same ammonia concentration in the water.

Ammonia Excretion Rates:

The ammonia excretion rates showed significant ($P < 0.05$) influence of dietary levels of protein (Fig. 6) with increasing concentration of protein in the diet, the prawns excreted higher concentration of ammonia. The highest ammonia excretion rate was recorded in prawns fed on 60% protein (0.28 mg/g prawn/d) and lowest in prawns fed on protein free diet (0.19 mg/g prawn/day). Prawns fed on 30% and 40% protein did not show much variation in ammonia excretion rates.

Fig. 6. Ammonia concentration in seawater and ammonia excretion rate in prawns fed on different levels of protein in the diets (0-60%)

FIG. 6


AMMONIA CONCENTRATION IN WATER
mean NH3-N-mg/l/treatment/day
0·04
0·02
0
AMMONIA EXCRETION RATE
NH3-N-mg/l/g prawn/day
0·3
0·2
0·1
0
0
10
20
30
40
50
60
PROTEIN (% IN DRY DIET)

DISCUSSIONS

Moulting:

Observations made on the basis of exuviae collected (Table 6), indicate that moulting in prawns is affected by the protein levels in the diet. Greater numbers of exuviae were collected from treatments with 30%, 40% and 50% protein levels (32 nos) compared to that of other treatments. The number of exuviae recorded were not absolute figures since, moulting occurs during night and the exuviae at times were eaten by cohabiting prawns. Post-moult deaths were relatively more in the treatment fed protein-free diet, after 15 days from start of experiment, suggesting that post-molt deaths occur due to the deficiency of dietary protein in the diet. Similar, observations were also made in the case of prawn groups fed diets containing 10% and 20% protein after the third week, indicating inadequacy of the protein in the diet for normal physiological processes.

Food Intake:

Food intake in prawns was not affected during the first two weeks in all the treatment groups. However, from the second week onwards, food intake was reduced in the prawns fed on the protein free diet. From the end of the third week, food intake was greatly reduced and the prawns responded passively when the feed was introduced in the aquaria. There was not much variation

TABLE 6A: OBSERVATIONS IN PRAWNS FED WITH DIFFERENT EXPERIMENTAL DIETS

Protein in the diet	Mean nos. of molts recovered	Mean nos. of post-molt deaths	Texture of the body
0	19	25	SO
10	21	16	SO
20	25	16	SO
30	31	8	H
40	32	8	H
50	32	13	SO
60	31	16	SO

H - Hard, SC - soft

in the food intake of prawns from various treatments, though minor variations were observed during molting.

Behaviour Towards Light:

When table lamp light (1625 x 10^2 lux) was shown, prawns from the various dietary treatments, responded differently. Normally prawns preferred low intensity lights and evaded brightly lighted regions. During the first two weeks, no striking differences in response to photostimuli occurred in prawns from various treatments; but in subsequent weeks at 0%, 10% and 60% protein levels, languid response of prawns was observed. However, in other treatments, the response was spontaneous and the prawns moved away to shaded regions in the experimental tanks.

External Morphology:

The prawns subjected to different levels of protein in the diet, showed distinct variability in their activity. While the prawns fed diets with 0%, 10% and 60% protein levels showed hypoactivity, those fed diets containing 20%, 30% and 40% protein in the diet showed normal activity and prawns fed with 50% protein in the diet showed hyper-activity. The hepatopancreas of prawns fed with diets containing 10%, 20% and 50% protein levels showed diffused appearance and brownish color. However, in all other treatments, the hepatopancreas of prawns appeared compact, brownish in color underlined by a whitish mass. No other significant charges in the morphology was observed in the different treatment groups.

EXPERIMENT II

The second experiment was conducted based on the results of the first experiment in which the diet containing a protein level of 40% produced maximum growth. In this experiment, protein levels ranging from 32.5 to 47.5%, with an interval of 2.5%, were selected with a view to determining near optimum levels of protein, which produce maximum growth.

Survival:

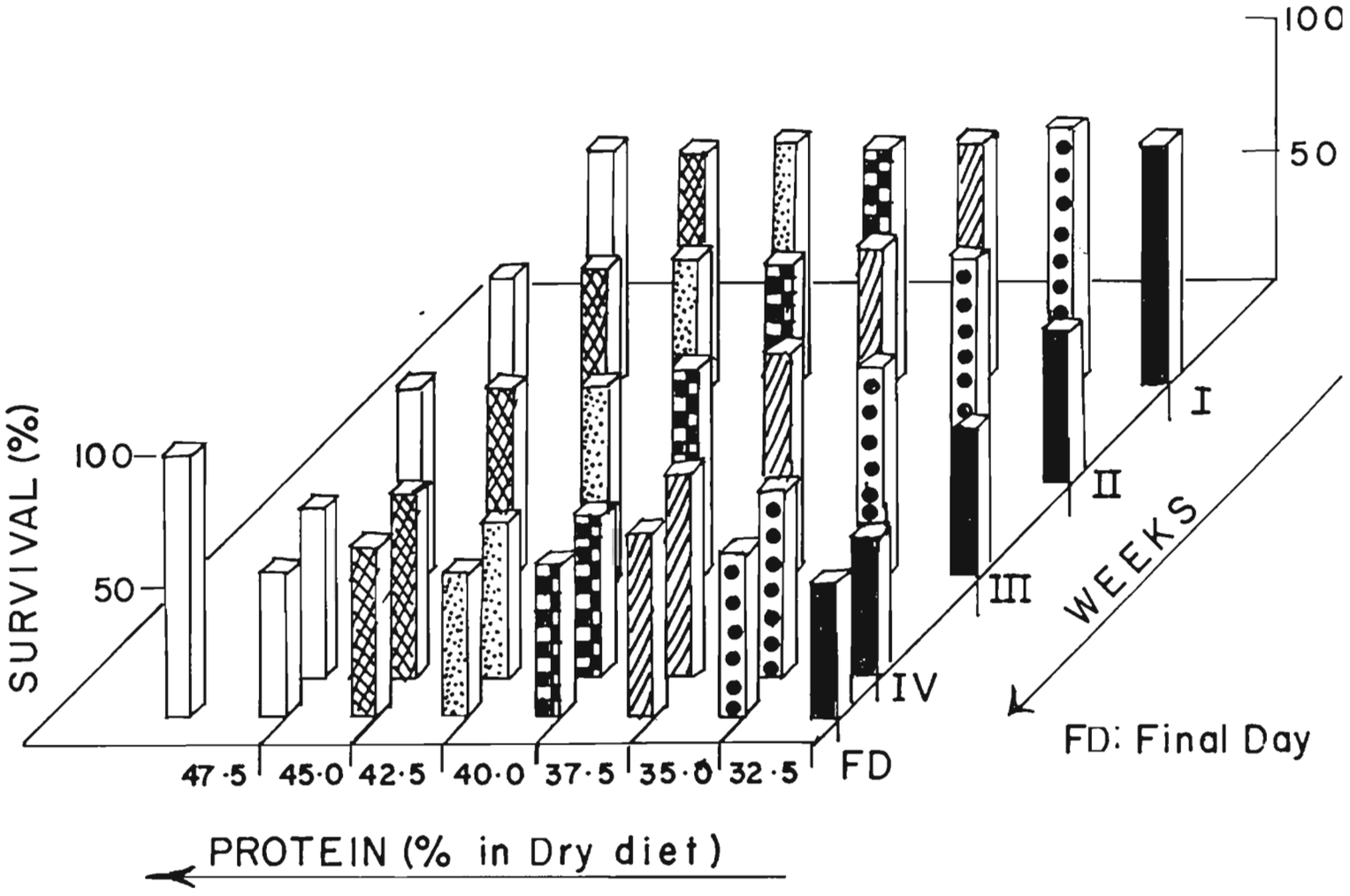
The survival rate of prawns (Fig. 7) showed an increasing trend upto 37.5% protein level in the diet, and thereafter showed a decreasing trend as the concentration of protein in the diet increased further. Analysis of variance performed on the survival rate data failed to give any significant differences between treatments. While, the highest percent survival (70%) was recorded in groups of prawns fed diet with 37.5% protein level, the lowest (52%) was recorded in the prawn groups fed diet with 32.5% protein level. In all other treatments, the percent survival varied from 55.5% to 67.5%. There was also no significant treatment to treatment difference in the weekly survival of prawns.

Growth:

Significant ($P < 0.05$) differences in the mean percent gain in length of prawns were observed (Fig. 8) between the

Fig. 7. Weekly percent survival of prawns fed with different levels of protein (32.5-47.5%) in the diets.

FIG. 7


100
50
SURVIVAL (%)
100
50
I
II
III
IV
WEEKS
FD: Final Day
47·5
45·0
42·5
40·0
37·5
35·0
32·5
FD
PROTEIN (% in Dry diet)

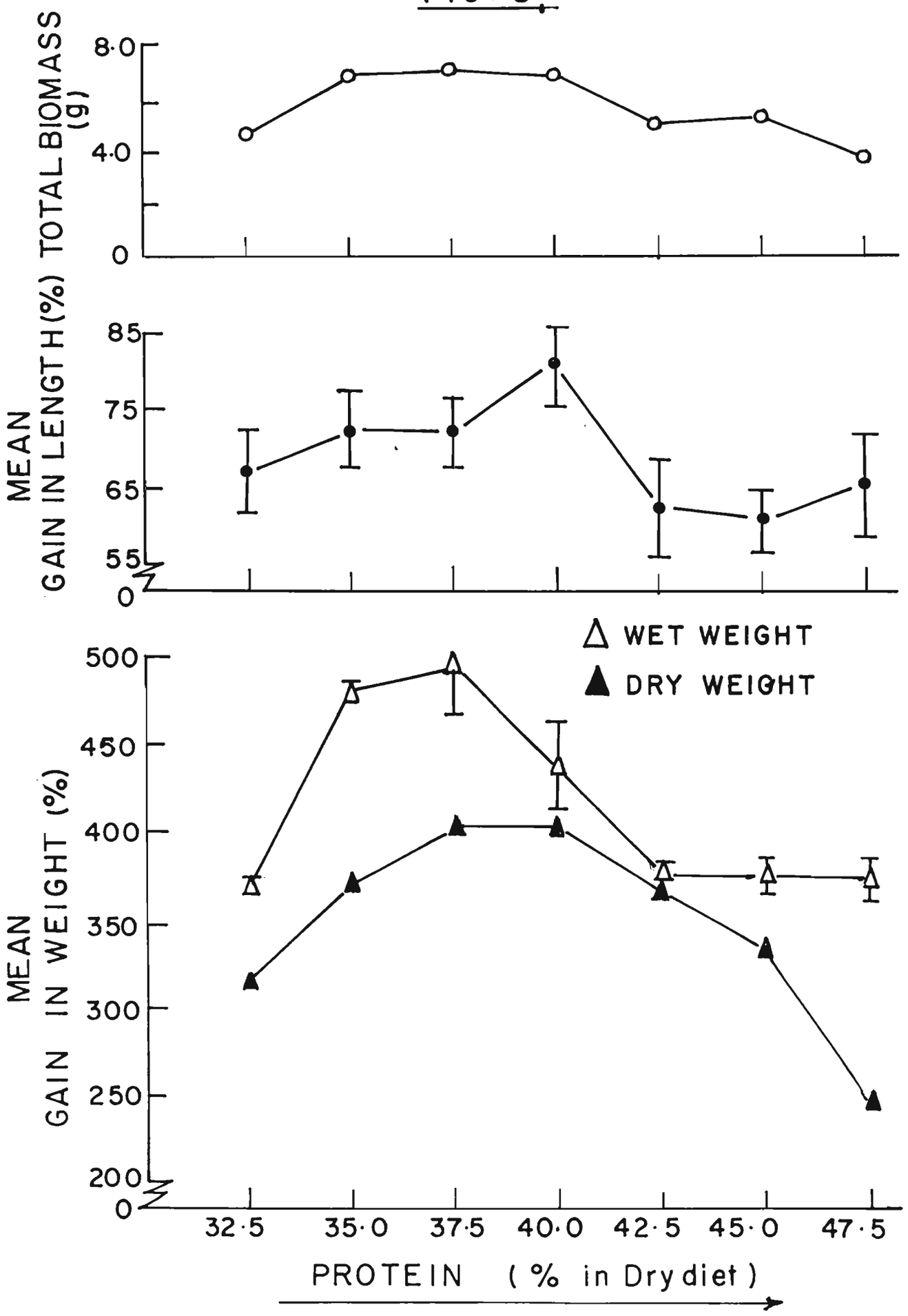
treatments. The mean percent gain in length of prawns increased with the protein level in the diet upto 40% and thereafter declined sharply with further increase in the dietary protein level. The maximum mean percent gain in length was recorded in the prawns fed with diet containing 40% protein (80%); whereas the minimum was recorded at 45% protein (61%) in the diet. The mean percent gain in length of prawns recorded in diets with 35 and 37.5% protein level were almost equal (72%).

The mean percent gains in wet weight and dry weight of prawns recorded from the various treatments are shown in Fig. 8. The protein levels in diets had highly significant ($P < 0.01$) influence on the mean percent gains in wet weight and dry weight of prawns. The mean percent gain in wet weight increased with the protein level in the diet upto 37.5% and thereafter it declined as the protein level in the diets increased to 42.5% and beyond this protein level, it remained more or less steady. The mean percent gain in wet weight obtained at 35% protein level (480%) and 40% protein level (445%) were not significantly different from the maximum recorded at 37.5% protein level (494%).

The maximum mean dry weight gain of prawns was recorded at 40% protein level (405%) and minimum at 47.5% (251%) protein level and these results are different from that observed for mean percent wet weight gains. The mean dry weight gain of prawns also increased with the protein level in the diet upto 40% and thereafter a steady decline was observed with further increase

Fig. 8. Percent gain in length and weight, and total biomass (g) of prawns fed with different levels of protein (32.5-47.5%) in the diets.

FIG. 8


MEAN GAIN IN LENGTH (%)
MEAN GAIN IN WEIGHT (%)
TOTAL BIOMASS (g)
8·0
4·0
0
85
75
65
55
0
500
450
400
350
300
250
200
0
WET WEIGHT
DRY WEIGHT
32·5
35·0
37·5
40·0
42·5
45·0
47·5
PROTEIN (% in Dry diet)

in protein level in the diet. There was no significant difference between 37.5 and 40% protein, in the percent gain in dry weight.

Specific Food Consumption (SFC):

Data on specific food consumption recorded from the treatments are shown in Fig. 9. Significant ($P < 0.05$) differences were observed in between the specific food consumption of prawns fed on diets with different protein levels. The SFC was highest in 42.5% protein level (7.26%) and lowest in 37.5% protein level (3.68%). However, the SFC recorded at 35% protein level (3.71%) was slightly higher than that recorded at 37.5% protein level. In all other treatments the SFC ranged between 4.8 and 6.2%.

Food Conversion Ratio (FCR):

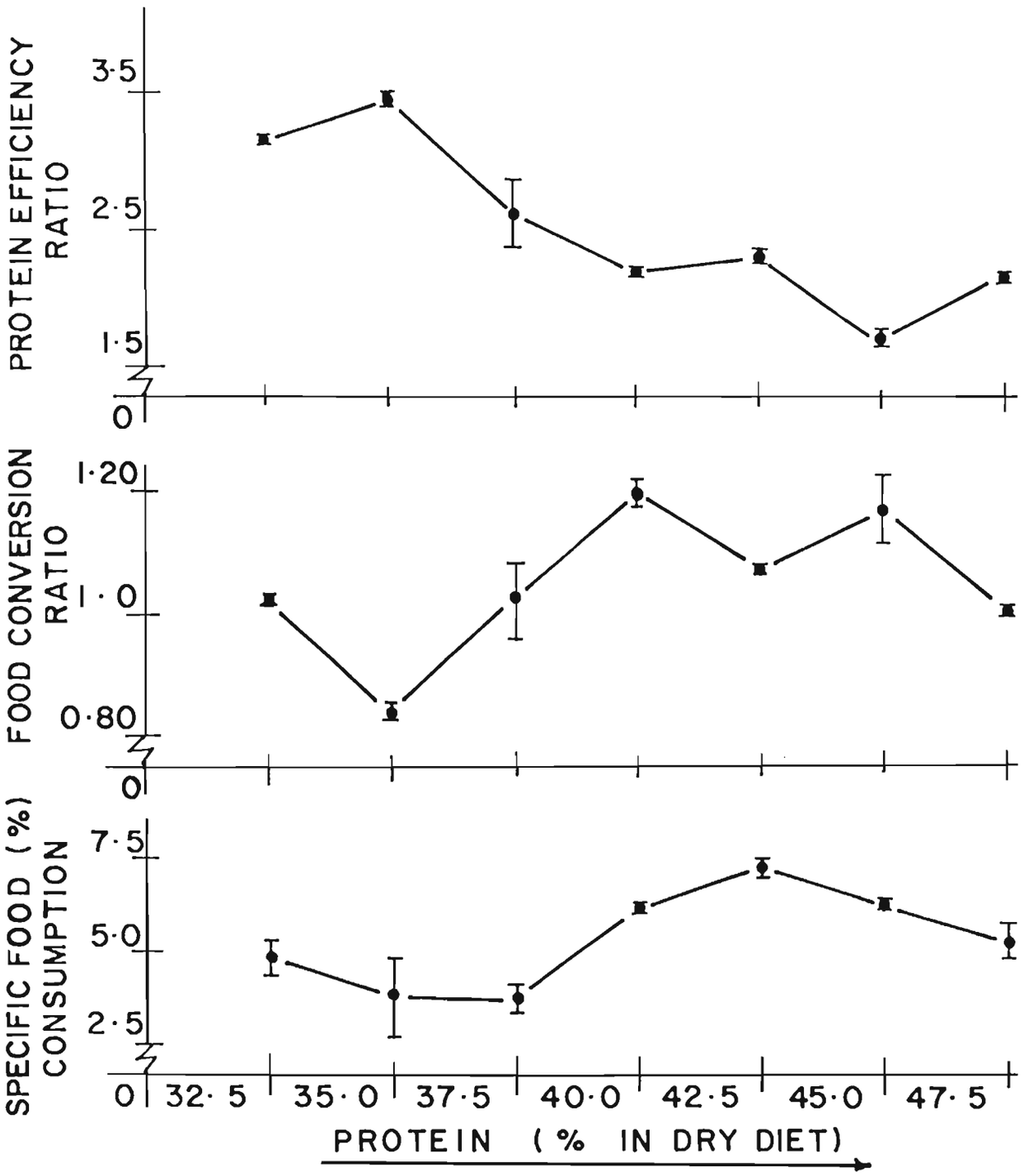
Food conversion ratios obtained from the different treatments are shown in Fig. 9. There were no significant differences among the FCRs recorded from the different treatments, though the FCRs recorded from treatments with 32.5% and 35% protein levels were relatively less than that of other treatments. In all other treatments FCR ranged between 0.99 and 1.02.

Protein Efficiency Ratio (PER):

Protein efficiency ratio showed (Fig. 9) an inverse relationship with that of food conversion ratio. There were statistically no significant differences in between the PER

Fig.8. SFC, FCR and PER for different levels of protein (32.5-47.5%) in the diets.

FIG. 9


PROTEIN EFFICIENCY RATIO
3·5
2·5
1·5
0
FOOD CONVERSION RATIO
1·20
1·0
0·80
0
SPECIFIC FOOD (%) CONSUMPTION
7·5
5·0
2·5
0
32·5
35·0
37·5
40·0
42·5
45·0
47·5
PROTEIN (% IN DRY DIET)

from the treatments. The maximum PER was recorded at 35% protein level(3.4) and minimum at 45% protein level (1.97). The PER did not vary markedly between 40 and 47.5% protein levels.

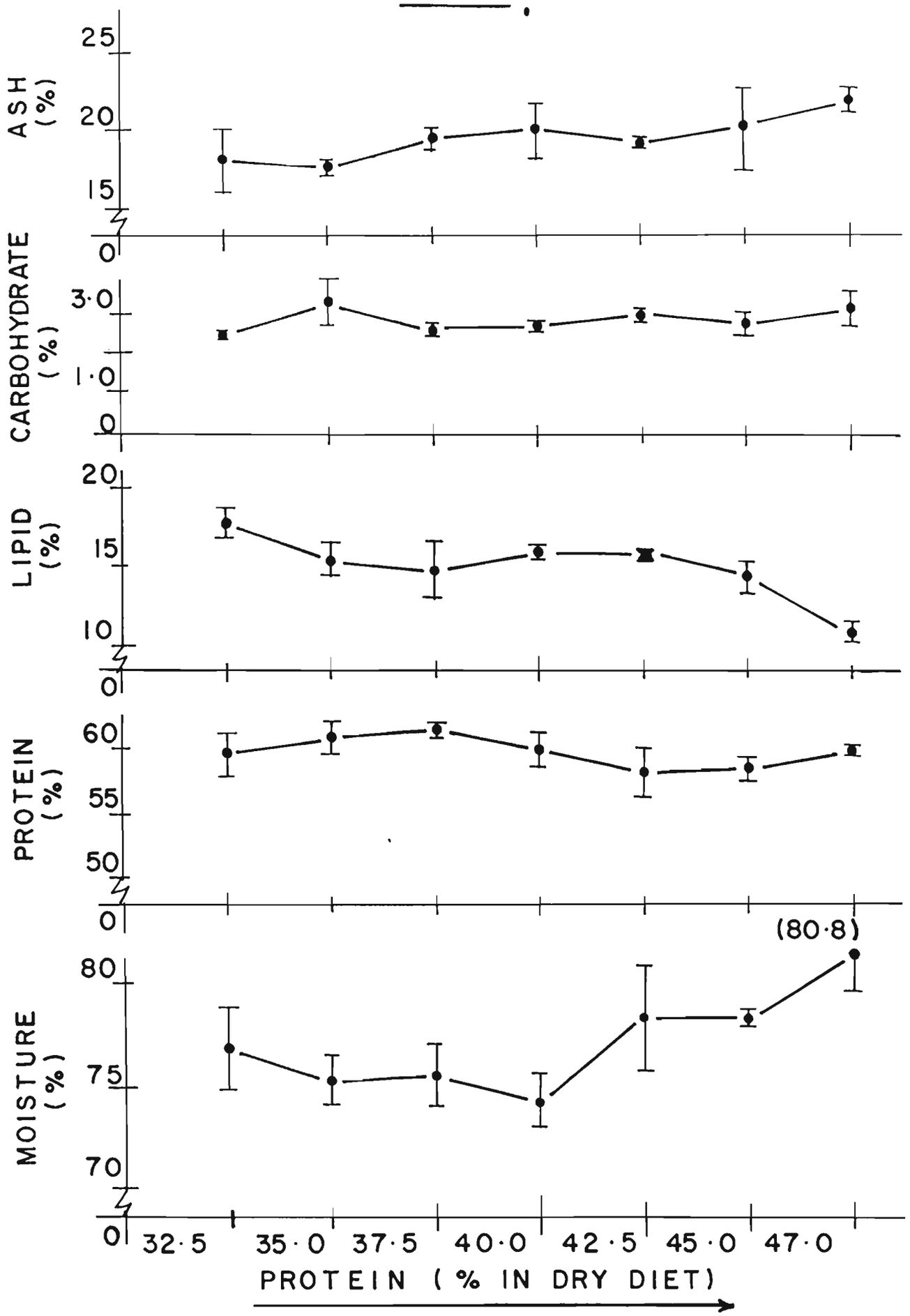
Biochemical Composition:

The moisture, ash, protein, lipids and carbohydrate contents of prawns from different dietary treatments are shown in Fig. 10. While the dietary protein level had significant ($P < 0.05$) influence on the moisture, ash and lipid contents, the protein and carbohydrate contents were not significantly affected. The prawns fed on the diet containing a protein level of 47.5%had the maximum moisture (80.9%) and ash contents (21.8%). There was not much variation in the moisture content of prawns fed diets containing protein levels between 35% and 40%. There was also a decrease in the moisture content with the increase in protein level in the diets upto 40% and thereafter the moisture content showed a steady increase as the protein level in the diet increased to 47.5%. The ash content of prawns fed on diets containing 32.5% protein level (18.2%) did not differ significantly from the minimum (17.7%) recorded at 35% protein level. However, an increasing trend was observed in the ash content with increasing protein level in the diet above 35% protein.

No significant differences were observed (Fig. 10) between the protein content of prawns fed diets containing different protein levels. The prawns fed the diet with 37.5%

Fig. 10. Biochemical composition of prawns fed with different levels of protein (32.5-47.5%) in the diet.

FIG. 10


ASH (%)
25
20
15
0
CARBOHYDRATE (%)
3·0
1·0
0
LIPID (%)
20
15
10
0
PROTEIN (%)
60
55
50
0
(80·8)
MOISTURE (%)
80
75
70
0
32·5
35·0
37·5
40·0
42·5
45·0
47·0
PROTEIN (% IN DRY DIET)

protein had the highest protein content (61.9%); whereas the lowest protein content was observed in prawns fed diets containing 32.5% and 42.5% protein (59.3%).

Significant ($P < 0.05$) differences were also observed between the total lipid content of prawns from the various treatments. The highest lipid content was recorded at 42.5% protein level (18.5%) and the lowest at 47.5% protein level (10.9%). However, no specific trend was observed between the lipid and moisture contents of prawns fed diets with different protein levels.

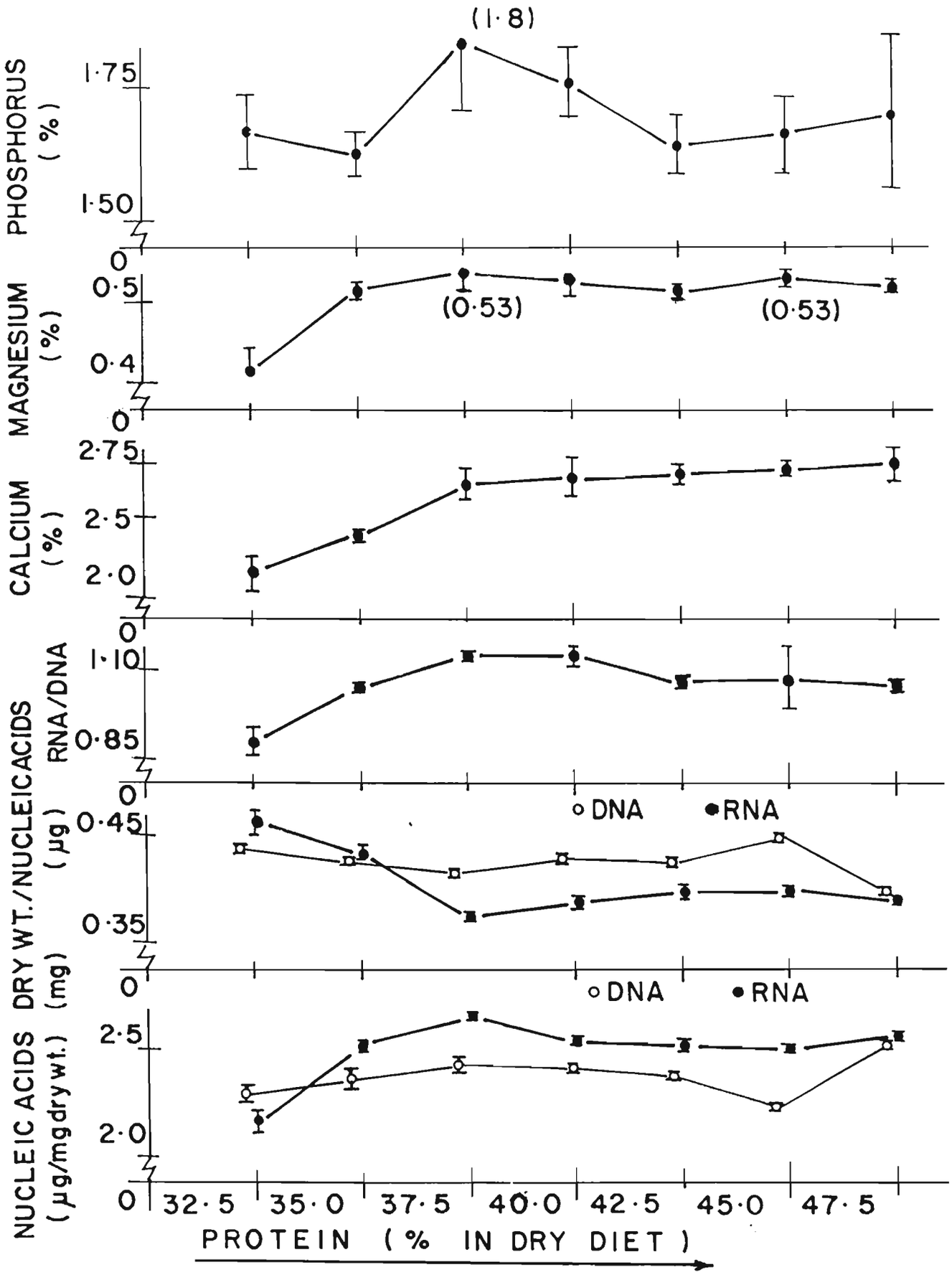
The carbohydrate content in prawns fed with various protein diet also did not show any specific trend, though the prawns fed diet with 47.5% protein level (3.5%) and 35% protein level (3.4%) had relatively higher carbohydrate contents.

No significant variations were observed in the RNA and in the DNA contents in between treatments (Fig. 10). The highest RNA and DNA contents were recorded in prawns fed diets containing 37.5% protein (2.67 µg/mg) and 47.5% protein (2.50 µg/mg), respectively. In all other treatment groups, the RNA content ranged between 2.16 and 2.56 µg/mg, the lowest RNA being at 32.5%.

The dry weight/total RNA ratio of prawns did not show any significant variation with reference to the levels of protein in the diet. However, prawns fed with lower protein levels (< 35%) had relatively higher ratios compared to prawns fed with higher protein levels in the diet. Similarly,

Fig. 11. Biochemical composition of prawns fed with different levels of protein (32.5-47.5%) in the diet.

FIG. 11


(1·8)
PHOSPHORUS (%)
1·75
1·50
0
0·5
0·4
(0·53)
(0·53)
MAGNESIUM (%)
0
2·75
2·5
2·0
CALCIUM (%)
0
1·10
0·85
RNA/DNA
0
0·45
0·35
DNA
RNA
(µg)
0
DNA
RNA
2·5
2·0
NUCLEIC ACIDS (µg/mg dry wt.)
DRY WT./NUCLEIC ACIDS (mg)
0
32·5
35·0
37·5
40·0
42·5
45·0
47·5
PROTEIN (% IN DRY DIET)

dry weight/total DNA ratio was also not significantly influenced by the different dietary levels of protein, though the ratios varied between 0.39 and 0.45.

The RNA/DNA ratios of prawns were significantly ($P < 0.05$) influenced by the dietary protein level. However, the prawns fed with the 32.5% protein diet had significantly ($P < 0.05$) lower RNA/DNA ratio than that of prawns fed diets containing other dietary protein levels. The highest RNA/DNA ratio was observed in prawns fed diets with 37.5%, 40% and 45% protein (1.13) and lowest ratio in prawns fed the diet containing 32.5% protein (0.89). A declining trend was observed in the RNA/DNA ratio in prawns fed beyond 40% protein level, excepting an unexpected rise at 45% protein level in the diet.

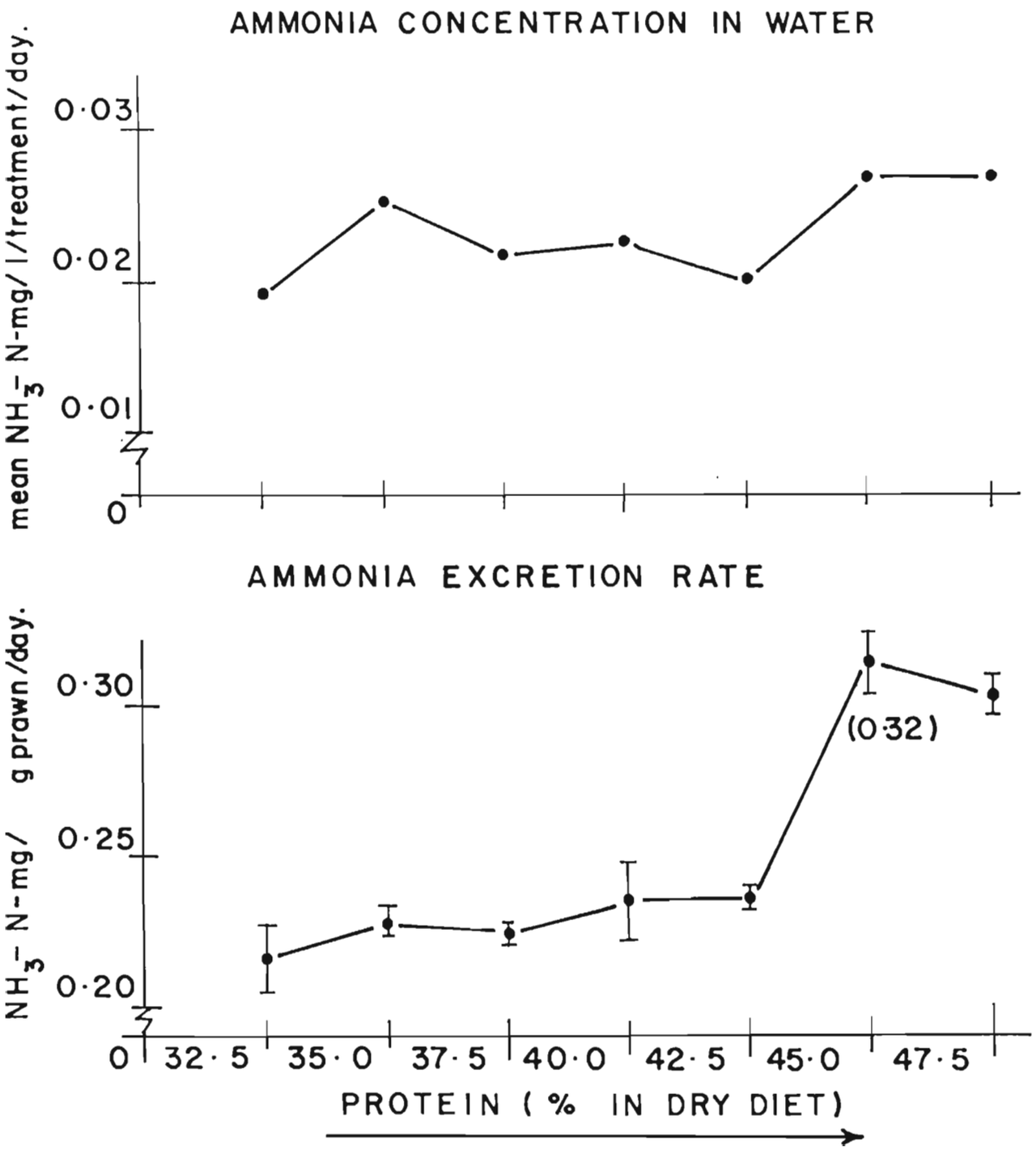
The calcium, magnesium and phosphorus contents of prawns, expressed as percentages, from various dietary treatments are shown in Fig. 10. Analysis of variance of the data showed that the protein levels in the diet do not significantly influence these parameters. While the calcium contents varied between 2.13 and 2.7%, the magnesium contents varied between 0.41 and 0.57% and the phosphorus contents ranged from 1.62 to 1.83%.

Ammonia Excretion Rates:

Ammonia excretion rates of prawns (Fig. 12) was also significantly ($P < 0.05$) influenced by the dietary protein levels. Significant treatment differences were observed in the ammonia

Fig. 12. Ammonia concentration in seawater and ammonia excretion rate in prawns fed on 32.5–47.5%.

FIG. 12


AMMONIA CONCENTRATION IN WATER
mean NH_3- N-mg/ l/treatment/day.
0·03
0·02
0·01
0
AMMONIA EXCRETION RATE
NH_3- N-mg/ g prawn/day.
0·30
0·25
0·20
0
(0·32)
32·5
35·0
37·5
40·0
42·5
45·0
47·5
PROTEIN (% IN DRY DIET)

excretion rates between prawns fed on diets with less than 42.5% to that of prawns fed on diets more than 42.5% protein. The highest was recorded in prawns fed with 45% (0.32 mg/g prawn/d) and lowest in prawns fed with 32.5% (0.21 mg/g prawn/d).

O B S E R V A T I O N S

Molting:

The number of exuviae collected from the various treatments (Table 6b) did not show much variation. Relatively few numbers of exuviae were collected from treatments with diets containing 32.5, 35 and 47.5% protein, when compared to other treatments.

The number of post-molt deaths (Table 6b) recorded were invariably similar for all the treatments, excepting in the case of treatment with 32.5% protein level, where a slight increase in the number of post-molt deaths was observed.

Food Intake:

Although not much variations were observed in the amount of left-over feed in different treatments, slightly reduced feed intake was observed in prawns fed diets containing protein levels greater than 45%, from the fourth week onwards.

Behaviour Towards Light:

When a light source from a table lamp (1625×10^2 lux) was suddenly flashed into the experimental tanks, almost in all the treatment groups, similar active response was observed during the first two weeks. However from the third week onwards,

TABLE 6b: OBSERVATIONS IN PRAWNS FED WITH DIFFERENT EXPERIMENTAL DIETS

Protein in the diet %	Mean nos. of molts recovered	Mean nos. of post molt deaths	Texture of the body
32.5	22	24	SO
35.0	22	18	H
37.5	24	18	H
40.0	29	18	H
42.5	29	22	H
45.0	28	18	H
47.5	21	23	H

H - hard, SO - soft

the prawns fed diets containing 32.5, 42.5 and 47.5% protein showed quite passive response. In all other treatment groups, active response was observed throughout the experimental period and the prawns moved away from the lighted area in response to light.

External Morphology:

No specific changes in the external morphology were observed in prawns from different treatments, after 30 days of feeding with the test diets. However, few prawns fed diets containing 32.5 and 47.5% protein were observed to have few scattered brown spots in the abdominal region. These prawns, however, grew as well as those without these brown spots.

D I S C U S S I O N

Dietary protein requirements of a number of crustaceans, especially the penaeid prawns, have been widely studied and optimal protein levels in diets for different developmental stages have been recommended (New, 1976; Biddle, 1977; Maguire, 1980; Millikin *et al.*, 1980). In most of these studies, protein requirements have been reported based on experimental trials using semi-purified or compounded diets and very few studies have been conducted with

purified diets using casein as a protein source (Kanazawa *et al.*, 1970, 1976; Deshimaru and Kuroki, 1974a; 1975a; Boghen and Castell, 1980; Bhasker and Ali, 1984). Since casein is the only protein source available in highly purified form, its use as a protein source reduces considerably extraneous nutritional factors, which markedly alter the protein requirement of prawn species. Thus, purified diets allow precise nutritional studies, whereby relationships between particular dietary ingredients and physiological indices can be observed (D'Abramo *et al.*, 1982). In the present study, to define the protein requirement of juvenile *P. indicus*, a purified diet, with casein as the primary protein source, was used.

The present findings clearly show that protein level in the diet significantly influence the survival and growth of prawns, which is in accordance to earlier observations in crustaceans (New, 1976; Maguire, 1980). In the first experiment, using wide range of protein intervals, survival rate was found to increase with the protein level in the diet upto 40% and thereafter it declined. In the subsequent experimental study using narrower ranges of protein level, survival rates increased with increasing protein level upto 37.5% and thereafter a declining trend was observed. The survival rate of prawns at 40% protein level differed insignificantly between the two experiments. The variations may be due to minor changes in the experimental conditions as well as due to genetical variations in the broodstock, since the juvenile prawns used for the two

experiments came from different parentage.

The results also indicate that protein levels below 32.5% and above 45% have detrimental effect on survival and growth. In the case of prawns fed on the protein free-diet, almost the whole population was wiped off with very low survival, largely due to their cannibalistic behaviour, as well as due to devouring of freshly molted prawns by the cohabitors. This severe effect of protein deficiency in prawn's diet suggests that prawns have a minimum requirement for protein to meet their basal metabolic needs. However, the highest survival in prawns fed the 37.5% protein in the diet, signifies that the juvenile prawns may have a requirement around this level for normal metabolism. According to Colvin and Brand (1977), protein requirement decreases with the increase in size and the post-larvae of P. indicus have been reported to require about 40% protein in the diet (Bhaskar and Ali, 1984). All these observations indicate, the significant variations in the dietary protein requirement of various growth stages of prawns.

Like the survival rate, significant effect of protein levels was observed on growth. In the first experiment, growth of prawns was found to be relatively high between 30% and 40% protein. But the subsequent experimental study, significantly high growth was obtained with 35 to 40% protein in the diet. While P. japonicus (Kanazawa et al., 1970), Palaemon serratus

(Forster and Beard, 1973) and Homarus americanus (Castell and Budson, 1974) have been found to require less than 50% protein in the diet, P. azetecus (Shewbart et al., 1973), P. setiferus (Andrews et al., 1972) and P. duorarum (Sick and Andrews, 1973) seems to require relatively lower protein levels ranging from 20-30%, and in Procambarus clarkii (Huner and Meyers, 1979) and P. mercuiensis(Sedgewick, 1979), the protein requirement is found to be about 34 to 42%. Thus, it is evident that the present experimental species is having a relatively lower protein requirement than many of the other species so far studied. However, Colvin (1976) and Ali (1982a) found that P. indicus require around 43% protein in the diet for optimal growth. These values are significantly higher than what has been observed in the present study and can be accounted for the type of protein source used, as the above researchers used compounded diets as against the purified diet in the present case. Probably, compounded feeds might have had growth promoters which could have influenced the growth. However, use of purified diet in the present study seems to have removed the effect of growth promoters (Kanazawa et al., 1970; New, 1976a; Conklin et al., 1980).

Although, the growth and survival in prawns showed slight difference between 35%, 37.5% and 40% protein levels; the observed differences were not statistically significant. So it is evident that dietary protein levels ranging from 35 to 40% can be used for formulation of complete practical diets for

juvenile P. indicus without affecting survival and growth significantly. On the other hand, considerably reduced growth attained by prawns fed diets with 32.5% or less of protein or above 40% protein level, indicate that underfeeding and overfeeding of protein, significantly affect growth probably due to alterations in the metabolism. As in the protein deficient diet, poor growth and relatively poor survival were observed at 10% and 20% protein levels, which clearly indicate that at these protein levels and restricted feeding, the prawns are unable to meet their dietary protein requirements for proper growth.

The protein level in the diet also had significant effect on the specific food consumption (SFC), with the values increasing with levels of protein from 0 to 30% and above these levels, no significant variation was observed between the treatment groups. These results indicate that the prawns tend to reach a satiation level for protein requirement, resulting in optimum food consumption, maximum growth, and high survival rate, above 30% protein. In comparison, the food consumption was relatively poor in prawns fed with less than 30% protein in the diet and in prawns fed without protein, the food consumption was very low leading to poor growth and survival. Similarly, high values of SFC against poor growth in prawns fed with high protein ($\geq$ 50%)diets, indicate the interference of excess dietary protein on the growth of the prawns. Following the second set of experimental results, it is evident that there

is no significant variation in SFC values between 35 and 37.5% protein levels and so the optimal protein level could be well within this range. The values recorded for SFC in these levels of protein fed prawns are almost same to that recorded in juvenile H. americanus which were fed on a natural diet(Bordner and Colvin, 1981).

The FCR and PER values are also significantly influenced by the protein levels in the diet. Protein levels less than 20% in the diet, gave significantly higher FCR and PER values than higher levels of protein. However, the lowest FCR and highest PER at 35% protein level in the diet indicate that food as well as protein are efficiently utilized. But considering the growth and survival, 37.5% protein seems to be better than that of 35% protein. However, since the FCR and PER were not significantly different between 35% and 37.5% protein, it is apparent that the optimal protein requirement may fall within the range of 35% to 37.5%. On the other hand, high FCR and low PER values recorded at protein levels below 32.5% and above 40%, indicate that dietary protein inadequacy or excess, affect food conversion and dietary protein utilization.

The biochemical composition of carcass of prawns further provide evidences in support of the above suggestions relating to the optimal requirements in the prawns. Amongst, the various biochemical parameters determined moisture, protein, lipid, ash

and calcium are the most prominent to be affected by the dietary levels of protein. It is evident that prawns fed below 35% or above 40% protein levels, tend to have more moisture than prawns fed diets containing protein between 35% and 40%. This signifies that when prawns are fed with supra-optimal or sub-optimal protein levels, the organic matter accumulation is reduced considerably. However, it was observed that the moisture content in prawns fed with 35-40% protein was low, indicating that at near optimal protein requirement, maximum nutrient deposition occurs.

From the results it is evident that the ash content of prawns is significantly affected by the dietary protein level with the prawns fed below 35% protein in the diet having significantly lower ash content than those fed diets containing above 35% protein. However, in the first experiment, prawns fed diet containing 40% protein level recorded the lowest ash content for unknown reasons, but in the subsequent experiment, the ash content recorded at 40% protein level was almost as high as in other protein level fed prawns.

Ash from prawns fed with experimental diets when analysed for calcium, magnesium and phosphorus, showed significant variations only in the calcium content. The prawns fed with the protein deficient diet had significantly lower calcium content than those fed with more than 30% protein level; however, at higher protein levels ($\geq$50%), the calcium content showed a gradual decline. Prawns fed diets with protein

ranging between 32.5% and 47.5% showed an increasing trend in calcium content but plateauing beyond 37.5% protein level. This suggests that possibly calcium contents is not influenced by protein levels between 37.5 and 47.5%. No significant variations were observed in magnesium and phosphorus content of these prawns fed diets containing increasing protein levels though in higher vertebrates protein level in the diet has been shown to influence the degree of utilization of phosphorus and magnesium (Georgievskii et al., 1979). The variations in the present observation with that of the above workers may be due to the differences in the physiological processes taking place in these forms.

So it appears, from the results obtained on calcium content, that possibly the uptake, repletion and depletion of calcium in prawns is influenced by the protein levels, since calcium in crustaceans forms an important major inorganic constituent. On the other hand, magnesium and phosphorus are relatively minor components (Richards, 1951, Huner et al.,1978) and are probably not influenced by dietary protein levels, so insignificant variations were observed in the different treatment groups.

The protein content in prawns, helps interpret the effect of dietary protein level, as well as other metabolic changes associated with the dietary treatment. Protein content in prawns was significantly influenced by the protein level in

the diet. The protein content increased with the protein level upto 40% and thereafter declined. On the other hand, the prawns fed the protein deficient diet had significantly low protein content, indicating that under dietary protein deficiency, the tissue proteins are catabolised leading to depletion in tissue protein levels. The ammonia excretion rate was also found to be significantly low in prawns from this treatment on prolonged deprivation of protein. Further, Mendes and Waterlow (1958), demonstrated that protein malnutrition results in the loss of cellular protein fraction, and thus, the low protein content in these prawns are quite expected.

The prawns fed with more than 30% protein in the diet had higher protein content than prawns fed with less than 30% protein. This indicates that since dietary proteins availability was limited, both due to the low protein level in the diet and restricted feeding, protein deposition in the body is greatly affected. However, insignificant variations in protein content of prawns was observed with further increase in concentration of protein in the diet. Besides, the protein contents recorded in the first experiment are not exactly comparable to that of the second experiment. Probably, differences in brood stock from which prawn juveniles were obtained could have contributed to this variation in protein content. Even then, it appears that protein level in the diet does not significantly affect the protein content in prawns fed on diets having protein levels ranging from 32.5% to 47.5%. The experimental study, however,

shows that when threshold levels of protein are added in the diet to meet the minimum protein requirements of the animal, the protein deposition is not significantly affected with any further increase in the levels of dietary protein.

The RNA and DNA content in prawns are important parameters since the ratio of these explains the state of metabolic activity undergoing in the tissues (Hotchkiss, 1955; Buckley, 1979a) and in many cases, the RNA content in organisms has been related to their growth rates (Leick, 1968, Sutcliffe, 1970; Dagg and Littlepage, 1972). However, the changes in RNA-DNA ratios are primarily due to changes in RNA-P rather than DNA-P which remains constant (Bulow, 1970). According to Bulow (1971) and Buckley (1979a, b), RNA-DNA ratios are very sensitive to changes in feeding levels and could be used as indicators of growth. The present study also shows the significant influence of dietary protein levels on the RNA and DNA content in prawns. The increase in the concentration of RNA upto 40% protein level in the diet shows the increased rate of protein synthesis with the increase in protein level in the diet. The highest RNA content at 40% protein level, indicate active efficient protein synthesis, which was also reflected in the highest growth achieved at this level. The RNA-DNA values recorded in the prawns during the present study are within the limits reported by Bulow (1970) and Buckley (1979b) in fishes and Sutcliffe (1970) in amphipods.

Since the RNA-DNA ratio as well as the growth of prawns was observed to be highest at 40% protein level, the suggestion by Bulow (1971) and Buckley (1979a, b) for fishes that RNA-DNA ratio can be used as growth indicator may also hold true for prawns. Similar, observations were also made in the second set of experiment where the highest RNA-DNA ratio was recorded at 37.5% and 40% protein levels. These results indicate that the high growth attained at 37.5% protein level is justifiable, since the protein synthesis was perhaps most efficiently functioning, resulting in high protein deposition in the tissues for growth. This is in conformity with the observations of Leslie (1955) and Brachet (1955) who postulated that although DNA content of the fish tissue will vary little, RNA content will vary much more and be highest in those fishes undergoing fastest growth or protein synthesis.

Lipid content in prawns decreased with the increase in protein level in the diet and prawns fed with protein-free diet had significantly high lipid content compared to prawns fed with more than 45% protein which had significantly low lipid content. These results clearly indicate the effect of dietary protein on lipid metabolism. As observed in the present study, high lipid content was also obtained in the fish, sea bass, fed on a protein-deficient diet (Metailler et al., 1973). On the other hand, prawns fed protein diets ranging from 20 to 40%, did not show any significant variation in the lipid content, though there was variation in

growth. The relatively low lipid content in prawns fed with high protein diets indicate that probably energy derived from lipid is used for deamination associated with protein catabolism which requires energy. The process of active deamination of proteins in these prawns is evident from the high ammonia excretion rates. The carbohydrate content also shows similar trends like lipid content in these prawns decreasing with increasing protein level, though not prominent as the lipid content.

From the ammonia concentration in water, it is evident that prolonged protein deficiency leads to low ammonia excretion indicating reduced metabolic activity (Harper, 1971; Clifford and Bricks, 1978). The passive responses observed in these prawns when disturbed, further support the above observations of low metabolic rate.

In the case of prawns fed with the high protein diets (more than 42.5%), the carbohydrate content was high and the ammonia excretion rates were significantly higher than that of prawns fed diets with lower (less than 42.5%) protein levels. This suggests that in this case, the excess proteins are catabolised and probably part of the energy liberated is converted into carbohydrate and lipid, releasing ammonia in the process.

CONCLUSIONS

The present study indicates that juvenile prawns, *P. indicus* require proteins for normal growth, survival and general maintenance of body functions. Deficiency of proteins in diets for longer duration results in near complete mortality of prawn population. From the second week onwards these prawns show increasing cannibalistic behaviour. Prolonged deficiency results in alterations in metabolic activities, which is evident from the carcass composition and general declining activity.

Sub-optimal levels of proteins in diets are unable to sustain growth for long; and to meet their energy and protein requirements, the cohabiting prawns resort to feeding on freshly molted prawns and thereby lower survival was recorded.

The perferable levels of protein in diets of juvenile *P. indicus* seems to be within the range 35 to 40%. These results are sufficiently supported by other parameters studied and are well within the protein levels suggested for this species using compounded diets (Colvin, 1976). However, it is difficult to point out optimal protein requirement specifically for a species as multivariate factors are involved in determining the nutritional requirements for a species which are difficult to control at a time (Goodwin and Hanson, 1977).

Also, Colvin and Brand (1977) observed that protein requirement decreases as the size increases and thus the developmental stage of prawn has significant influence on protein requirements.

Protein diets above 40% results in reduced growth, increased catabolism of protein, increased ammonia excretion rates and affects the deposition of inorganic and organic nutrients, finally resulting to lower survival rate. Thus, for proper maintenance of body metabolism which reflects on the growth and survival, prawns like any organism needs optimal protein concentration in their diets.

CHAPTER-II

NUTRITIVE VALUE OF NATURAL PROTEIN SOURCES

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

It is now well established that the growth of prawns and the cost of feed are significantly affected by the quality and quantity of protein in the feeds. Therefore, it is essential to identify protein sources, which can promote maximum growth and protein retention in the body, by comparing the biological value of easily available and aesthetically non-usable protein sources for human consumption.

Since amino acids constitute the proteins, a comparison of the amino acids profile of the protein source with that of the amino acids profile of the cultivable prawn species, to a greater extent, would indicate the nutritive value of the protein source for the species (Wolvekamp and Waterman, 1960; Cowey and Sargent 1972). The protein quality can also be assessed based on its influence on nitrogen retention (Carr et al., 1977). Protein sources with any of the deficient essential amino acids will show poor nitrogen retention and the best output can be achieved only by adding more of this protein source in the diet, which will enhance the cost of feed formulation. In this case, nitrogen retention per unit intake of protein will be less than that of a protein source with balanced amino acids profile (Boorman, 1979). Thus, for

economically viable feed formulation, dietary protein sources which satisfy the amino acid needs of the animal are essential (Deshimaru and Shigueno, 1972; Hanson and Goodwin, 1977; Shang and Fugimura, 1977; Aquacop, 1978; Boghen and Castell, 1980).

Protein sources can be obtained from either plant or animal products, provided the amino acids profile of these satisfy the requirement of the animal under study. In juvenile M. rosenbergii (Nelson et al., 1977b) highest assimilation rates were found using a diet containing animal protein (249.41 Cal/g/day) followed by a mixed diet (plant - animal source) (218.04 Cal/g/day) and plant diet (183.72 Cal/g/day). However, the animal protein diet produced poor growth rates compared to the mixed diet, apparently due to the higher metabolic rate in the prawns fed the animal diet. In addition to this, the caloric cost of food utilization was reported to be high in the case of animal protein diets compared to the mixed diet (Nelson et al., 1977b).

However, soybean meal has been found to be a superior ingredient among all the plant protein sources tested for many crustaceans (Kanazawa et al., 1970; Balazs et al., 1973; Sick and Andrews, 1973; Balazs et al., 1974b; Pascual and Destajo, 1978). While Kanazawa et al. (1970) agreed that soybean meal is the best protein source for P. japonicus, Deshimaru and Shigueno (1972) reported poor growth rates with soybean meal compared to animal protein sources. Similarly, Ponat and

Adelung (1980) reported poor growth rates in crabs fed on soybean meal diets compared to animal protein sources and purified diets (casein).

Thus, other than few plant protein sources, animal protein sources always proved best for formulation of diets for prawn. Among the animal protein sources, proteins of marine origin are found to be superior because of relatively high ash content, which influences the growth significantly (Colvin and Brand, 1977; Boghen and Castell, 1980). Shigueno et al. (1972) reported that diets containing high levels of protein from different sources including squid meal, white fish meal, dried euphausia and active sludge were more efficient than diets containing low protein levels for P. japonicus. In many studies, squid was used as protein source because of its high content of arginine (Subrahmanyam and Oppenheimer 1969; Kitabayashi et al., 1971c; Deshimaru and Shigueno, 1972; Kittaka, 1976, Femucci and Zein-Eldin 1976). However, diets based on blood meal produced poor growth rates in P. aztecus (Colvin and Brand, 1977).

A mixture of two or more protein sources, invariably show better growth than a single protein source (Deshimaru and Shigueno, 1972). Compounded diets with a mixture of shrimp meal and fish meal (marine protein sources) were found to cover the protein needs of juvenile lobsters (Boghen and Castell, 1980). A mixture of two or more protein sources in the diets showed better growth performance in prawns,

M. rosenbergii than when used individually (New, 1976). Balazs et al. (1974 b) reported better growth rate in M. rosenbergii with a mixture of soybean meal and tuna meal compared to soy-tuna or shrimp meal used individually. Similarly, Venkataramaiah et al. (1978) reported better growth performance by shrimps feeding on a diet with shrimp shell waste and grass, owing to the high percentage of protein. Sandifer and Joseph (1976) reported enhanced levels of free amino acids and pigmentation of the body, when shrimp head meal was used in diets. Joseph and Meyers (1975) also advocated the use of crustacean wastes, such as shell, heads or any other waste products for formulation of practical diets as these are rich in sulphur amino acids which influence growth in shrimps.

As such no protein is complete in itself, so as to provide all the essential amino acids in proportions required by all the species. Therefore, supplementation of deficient amino acids to diets should improve growth rate. However, conflicting results have been obtained by supplementing amino acids to crustacean diets. While Boghen and Castell (1980), in Homarus americanus, and Stahl and Ahearn (1978) in juvenile M. rosenbergii, found better growth response in diets lacking individual indispensable amino acids, Kanazawa et al., (1970); Sick et al. (1972); Deshimaru and Kuroki (1974c, 1975a, b); Deshimaru (1976a); Colvin (1976) and Ponat and Adelung (1980) found pure amino acids and peptides resembling most other protein sources in amino acids composition, unsuitable in the

diets for crustaceans. However, attempts were made in balancing dietary amino acid composition by manipulating intact ingredients (Deshimaru and Shigueno, 1972), but conclusive results are still at large. It is unlikely that any multi-ingredient rations for shrimp will be qualitatively deficient in any of the ten indispensable amino acids; yet, it is still impossible to forumulate rations with a balanced amino acids profile (New, 1976) until the quantitative amino acids requirements are established. Current diets are certain to be qualitatively deficient in some amino acids, while providing excess of others. Thus, the formulation becomes inefficient, uneconomical and excess of amino acids in diets may induce inhibition due to toxicity.

Eventhough many studies have shown that purified diets are as such not successful as those made from whole protein sources, such as fishmeal, soybean meal, whole egg, house-fly meal and chicken feather (Kanazawa et al., 1970; Sick et al., 1972; Deshimaru and Kuroki, 1974c, 1975a, b; Stahl and Ahearn, 1978; Tinsley et al., 1984), purified diets are used extensively to determine optimal requirements of protein and other nutrients for crustaceans in many laboratories. The better growth in the latter is quite probable since the use of natural ingredients in compounded feeds for determining optimum requirements of specific nutrients will affect the results due to the presence of nutrients already present in the ingredients. However, poor growth with purified diets (compared to natural protein sources) has been

attributed to the poor gut bacteria, low pH of the diet (Nose et al., 1974), more gastric evacuation time and deficiency of some of the amino acids in casein, etc.

Although numerous studies have been carried out to evaluate the nutritive value of various sources of protein for crustaceans, as evident from the foregoing review, studies on the nutritive value of various protein sources for the Indian prawns are very few. The existing few studies (Colvin, 1976; Ali, 1982a) on Penaeus indicus deal with the nutritive value of few protein sources based mainly on growth and conversion efficiency. Therefore, in the present study, nutritive value of a variety of protein sources have been evaluated by gathering data on survival, growth, food conversion, protein efficiency ratio, biochemical composition of the body and ammonia excretion.

M A T E R I A L A N D M E T H O D S

The plant and animal protein sources used for the study are as follows:

Plant origin	:	Ground nut oil-cake, soybean meal, coconut oil-cake and gingely oil-cake.
Animal origin	:	Fish meal (Nemipterus japonicus) Crab meal (Scylla serrata), Prawn meal (P. indicus and Metapenaeus dobsoni) Clam meal (Meretrix casta)

These protein sources were taken individually and in combination for formulation of diets. Along with these, purified diet using casein (control) was also formulated for comparison purpose.

The protein sources were initially oven-dried, powdered in a electrically run kitchen grinder and then seived through a sieve of mesh size 200 μ and stored in polythene bags in a desiccator. Prior to formulation of experimental diets, proximate analysis of the protein sources was done and the results are shown in Table 7. Based on their crude protein content, diets were formulated to have about 25% crude protein level, except for the purified diet. The ingredient composition of the diet are shown in Table 8. In the present study, lecithin (phospholipid) was used at a level of 3% in the diet as it improves survival rate in crustaceans (Boghen and Castell, 1980; Conklin et al. 1980). The total lipid content was however maintained same as that used for the first experimental study.

Experimental procedures adopted for rearing of prawns were same as described in Chapter 1. Table 9 shows the mean environmental conditions maintained, initial size and weight of the animals taken for the study.

The parameters considered to evaluate the nutritive value of protein sources are similar as that adopted for the experiment in Chapter I. These include survival, growth, food consumption and conversion, protein efficiency ratio, body chemical composition, and ammonia excretion rates after feeding

TABLE 7: PROXIMATE COMPOSITION OF PROTEIN SOURCES

Ingredient	%			
	Ash	Protein	Lipid	Carbo-hydrate
Ground nut oil-cake	14.32	38.0%	6.98	40.61
Soybean meal (BDH Chemicals)	8.56	45.6	5.32	38.32
Coconut oil-cake	22.48	27.8	4.18	44.36
Gingely Oil-cake	19.26	32.6	5.72	41.64
Fish meal (*Nemipterus japonicus*)	24.8	59.7	10.8	3.69
Crabmeal (*Scylla serrata*)	22.6	60.2	10.2	3.10
Prawn meal (*P. indicus*; *M. dobsoni*)	19.2	65.6	9.7	2.58
Clam meal (*Meretrix casta*)	11.2	56.6	8.2	20.8
Casein (Vitamin-free) (ICN-Bio-chemicals)	1.2	96.2	-	-

TABLE 8: COMPOSITION OF FEED USING DIFFERENT PROTEIN SOURCES

Ingredient g/100 g	Groundnut Oil-cake	Soybean meal	Coconut oil-cake	Gingely oil-cake	All plant proteins	Fish meal	Crab meal	Prawn meal	Clam meal	All animal proteins	All plant animal protein	Purified diet
Protein source	63.7	52.5	89.1	73.9	69.8	9.5	43.8	41.4	50.2	48.6	66.7	39.0
Gelatin	-	-	-	-	-	1	1	1	1	1	1	1
Egg albumin	-	-	-	-	-	1	1	1	1	1	1	1
Glucosamine-Hcl	1.0	1	1	1	1	1	1	1	1	1	1	1
Sucrose	0.5	3	0.5	2	1.5	4	5.0	7.5	3	5	3	6.28
Glucose	0.5	2	0.5	1	1	2	4.5	5	3	2	1	4.2
Starch	2	8	4	5	3	8	8	8	8	8	6	18.6
Fish Oil	6.7	6.7	6.7	6.77	6.77	5.27	5.27	5.77	5.77	4.77	5	6.77
Corn Oil	3.3	3.3	3.3	3.38	3.38	2.38	2.68	2.38	2.38	2.38	2.38	3.38
Lecithin	3.3	3.3	3.3	3.38	3.38	3.38	3.38	3.38	3.38	3.38	3.38	3.38
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium succinate	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sodium citrate	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Mineral mixture*	7.4	7.4	7.4	7.41	7.41	7.41	7.41	7.41	7.41	7.41	7.41	7.41
Vitamin mixture**	3.2	3.2	3.2	3.25	3.25	3.25	3.25	3.25	3.25	3.25	3.25	3.25
Agar-agar+	2	2	2	2	2	2	2	2	2	2	2	2
Additives	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Cellulose	1.5	2	2	4	4	2	4	4	2	2	4	4
TOTAL	100.7	100.00	100.09	100.99	100.69	100.29	100.09	100.79	100.49	100.49	100.02	100.97
Proximate Composition %												
Crude protein	24.2	25.5	24.4	24.1	24.5	25.3	25.5	26.1	25.9	25.6	25.2	36.4
Crude lipid	12.8	13.4	11.6	11.1	11.2	16.2	17.9	16.9	15.2	16.8	15.4	14.2
Ash	19.9	18.8	22.6	19.0	20.8	19.9	19.6	17.7	19.9	20.1	21.2	18.8

* Same as in Chapter 1, Table 3.

** B-Carotene 0.0016, Calciferol 0.0015, -Tocopherol acetate 0.032, Menadione 0.004, -Ascorbic acid 2.00, Thiamine-Hcl 0.01 Riboflavin 0.008, Nicotinic acid 0.032, Pyridoxine-Hcl 0.020, Calcium pantothenate 0.075, Folic acid 0.001, Cyanocobalamine 0.001 Biotin 0.004, P-PABA 0.014, Choline chloride 0.75, Inositol 0.30.

\+ Arginine 3.00, Glycine 0.60, Lysine 0.40, Glutathione 0.16, l-Proline 0.14, Taurine 0.10, Phenylalanine 0.10

TABLE 9: ENVIRONMENTAL PARAMETERS AND STOCKING SIZE OF JUVENILE PRAWNS

Parameter	Mean values
Temperature (°C)	26.3 ± 0.71
Salinity (ppt)	20.3 ± 2.8
Ammonia concentration in the water (NH_4-N mg/l/d)	0.061 ± 0.011
pH	7.719 ± 0.37
Initial length (mm)	23.47 ± 1.14
Initial weight (mg)	85.31 ± 0.041

for 30 days with the diets. The procedures adopted were similar to the earlier experiment, as described in Chapter 1. The data obtained for various parameters were statistically analysed as described in Chapter 1.

RESULTS AND OBSERVATIONS

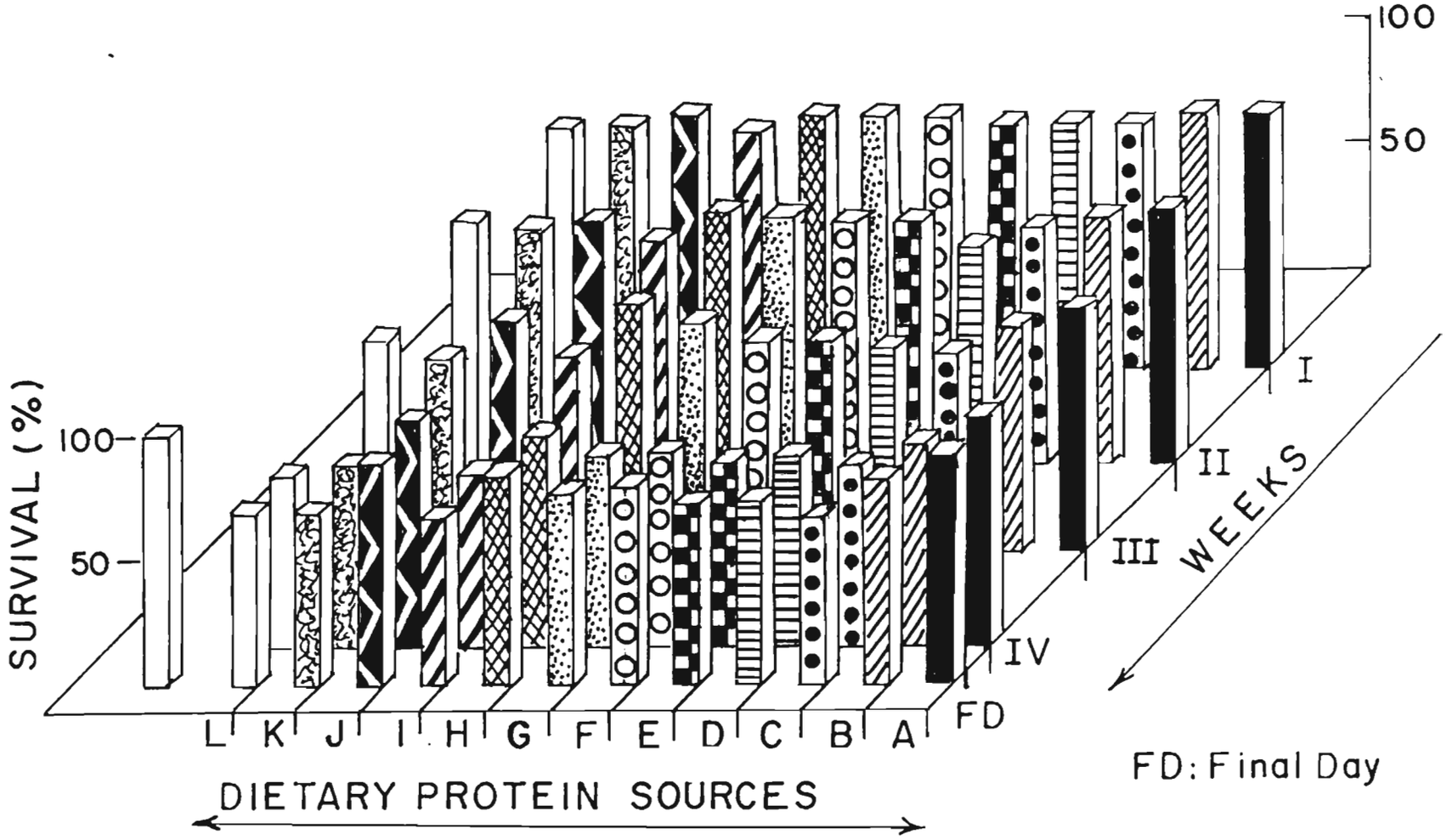
Survival:

The survival rates (%) of prawns recorded from the treatments are shown in Fig. 13. Analysis of variance of the data showed that the dietary protein sources significantly ($P < 0.05$) influence the survival of prawns. Among the diets compounded with plant protein sources the highest survival (86%) was recorded in the prawn groups fed on the diet containing ground nut oil-cake (A) and the lowest (67%) in prawn groups fed on the diets with coconut oil-cake (C) and a combination of plant protein sources (E) at the end of 30 days. The prawn groups fed with soybean meal (B) in the diet recorded relatively higher survival (80%) than those fed on gingely oil-cake (D) (73%).

Among animal protein sources, the diet with a mixture of animal protein sources (J) produced the highest (93%) survival, whereas the diet with clam meal produced the lowest survival (67%). Diets containing fish meal (F) (78%), crab meal (G) (76%) and prawn meal (H) (84%) also produced relatively higher

Fig. 13. Weekly percent survival of prawns fed with different protein sources A-groundnut oil-cake, B-soybean meal, C-coconut oil-cake, D-gingely oil-cake, E-all plant proteins, F-fish meal, G-crabmeal, H-prawn meal, I-clam meal, J-all animal proteins, K-combination of all plant-animal proteins, L-purified diet.

FIG. 13


SURVIVAL (%)
100
50
100
50
I
II
III
IV
WEEKS
FD
L K J I H G F E D C B A
DIETARY PROTEIN SOURCES
FD: Final Day

survival than clam meal diet. Diet with a combination of animal and plant protein sources (K) and purified diet (Control-L) gave relatively low survival rates (69%), which was almost similar to the survival obtained in prawns fed diets compounded with a combination of plant protein sources (E) and coconut oil-cake (C) (67%).

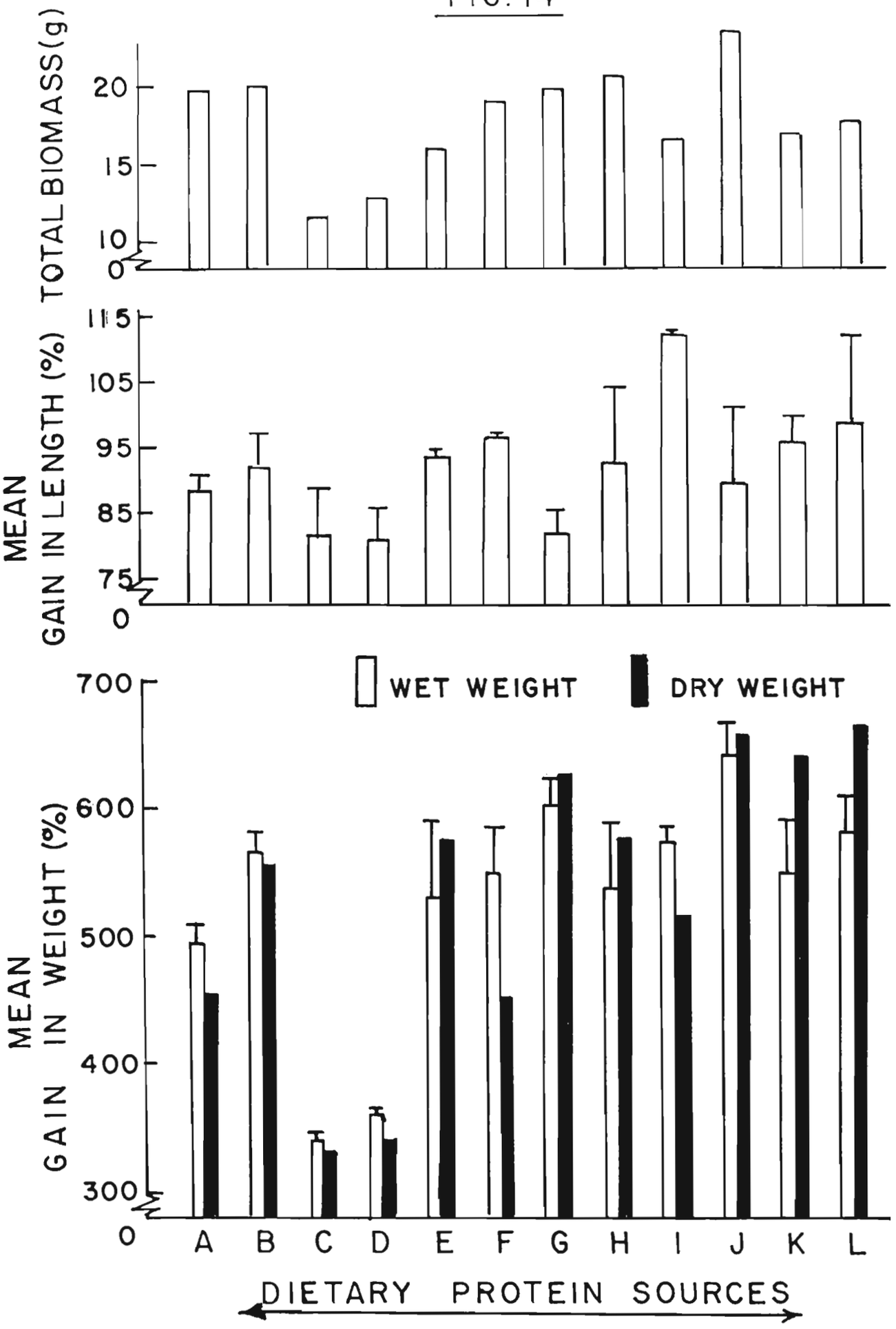
Growth:

The mean percent gain in length, wet weight and dry weight of prawns fed on the experimental diets are shown in Fig. 14. Analysis of variance of the data showed that the dietary protein sources significantly ($P<0.05$) influence all the above three growth parameters. The prawns fed on the diet containing soybean meal had the highest mean percent gain in length (92%) among the tested plant protein sources, which was however not significantly different from that obtained with a combination of plant protein sources (91%). Similarly, there was no significant difference between the percent gain in lengths of prawns fed diets containing gingely oil-cake (81%) and coconut oil-cake (82%). The mean percent gain in length of prawns fed with the diet containing ground nut oil-cake was significantly ($P<0.05$) higher than those fed with diets containing gingely oil-cake and coconut oil-cake.

In the case of animal protein sources, clam meal produced the highest mean percent gain in length (112%) and the lowest mean percent gain in length was observed in prawns fed diet containing crab meal (82%). There were no

Fig. 14. Percent gain in length and weight, and total biomass (g) of prawns fed with different protein sources A-groundnut oil-cake, B-soybean meal, C-coconut oil-cake, D-gingely oil-cake, E-all plant proteins, F-fish meal, G-crabmeal, H-prawn meal, I-clam meal, J-all animal proteins, K-combination of all plant-animal proteins, L-purified diet.

FIG. 14


MEAN TOTAL BIOMASS(g)
20
15
10
0
MEAN GAIN IN LENGTH (%)
115
105
95
85
75
0
WET WEIGHT
DRY WEIGHT
MEAN GAIN IN WEIGHT (%)
700
600
500
400
300
0
A
B
C
D
E
F
G
H
I
J
K
L
DIETARY PROTEIN SOURCES

significant differences among the mean percent gain in lengths of prawns fed diets with fish meal (96%), prawn meal (92%) and mixture of animal protein sources (96%) in the diets. The prawns fed diets with a mixture of plant-animal protein sources and purified diet recorded mean percent gain in lengths of 96 and 99%, respectively, which are comparable to that recorded with most of the other individual animal protein sources.

The mean percent gain in wet weight of prawns (Fig. 14) fed diets with plant protein sources were found to be significantly ($P < 0.05$) less than those fed diets containing animal protein sources. Among the plant protein sources tested, soybean meal gave the highest mean percent gain in wet weight (568%), which was followed by diets with a mixture of all plant protein sources (529%) and ground nut oil-cake (496%). Diets compounded with gingely oil-cake and coconut oil-cake gave significantly ($P < 0.05$) lower percent gains, 361% and 341%, respectively, than that of other plant protein sources.

In the case of animal protein sources, the highest mean percent gain in wet weight was recorded with a mixture of animal protein sources (644%) and the lowest (537%) with prawn meal. The wet weight gains recorded in prawns fed with fish meal (552%), crab meal (576%) and clam meal (575%) were not significantly different from each other.

Diet with a mixture of plant and animal protein sources produced a mean percent wet weight gain of 551%, which was not different from that observed in prawns fed with fish meal in the diet. However, purified diet fed prawns recorded relatively high percent wet weight gain (581%).

The protein source used in the diets also had highly significant ($P < 0.05$) effect on the percent dry weight gain of prawns. Among plant protein sources, the highest percent gain in dry weight was recorded with a mixture of plant protein sources (575%), but the lowest was obtained with coconut oil-cake (332%) closely followed by gingely oil-cake (341%). Although, the highest mean percent wet weight gain was obtained with soybean meal in the diet, the percent dry weight (558%) gain was relatively less than that obtained with a mixture of plant protein sources. Similarly, among the animal protein sources tested, diet with a mixture of animal protein sources produced highest percent dry weight gain (697%); whereas, fish meal diet produced the lowest dry weight gain (453%). Among the other individual animal protein sources, crab meal (627%), and prawn meal (580%) produced relatively higher percent gain in dry weight of prawns when compared to clam meal (517%). Statistically significant differences ($P < 0.05$) were however, observed only between the mean dry weight gains of prawns fed on the different diets containing plant protein sources. Also, the diets containing animal protein sources gave

significantly ($P < 0.05$) higher dry weight gains than diets containing plant protein sources excepting, the diet with soybean meal.

Specific Food Consumption (SFC):

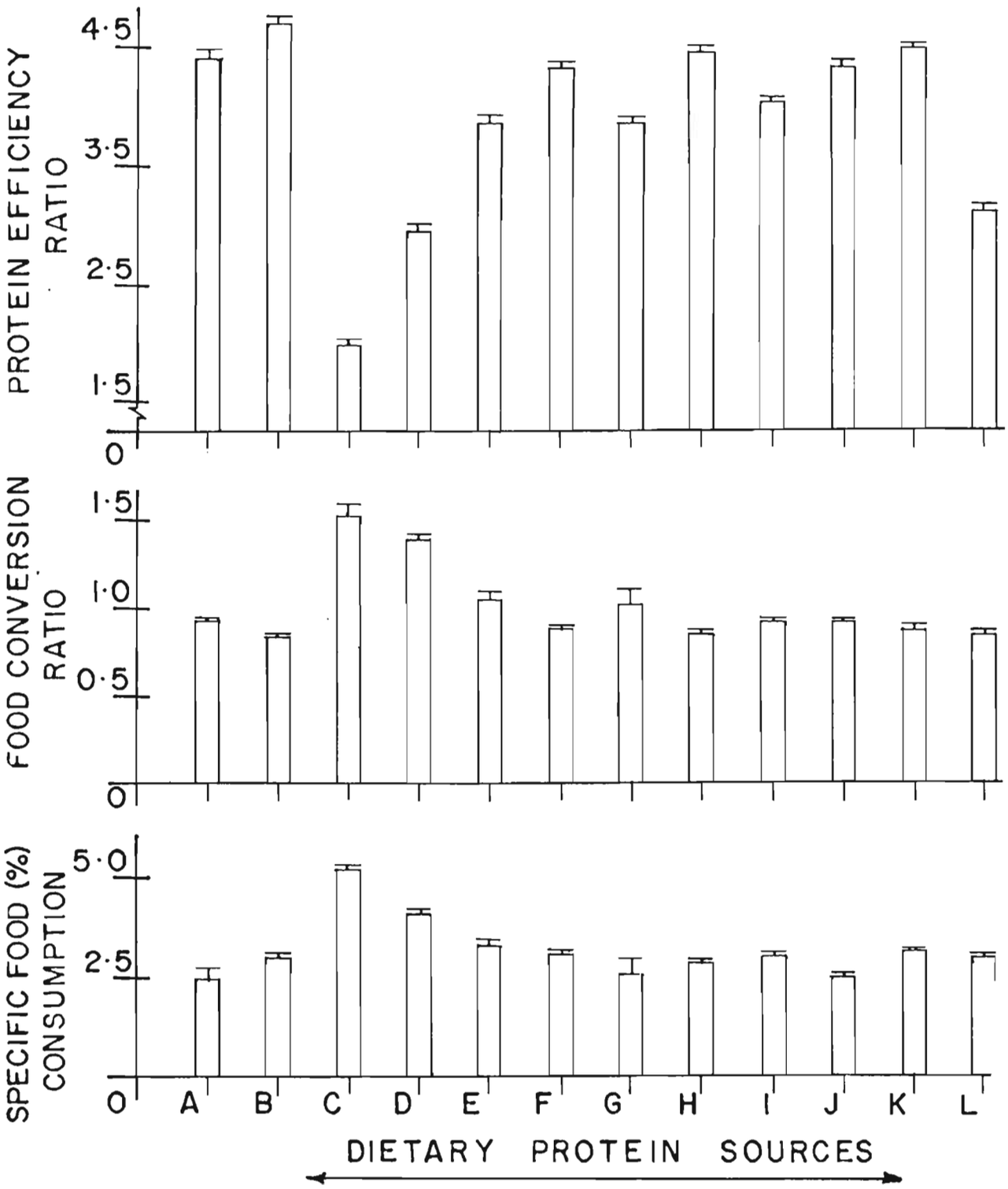
Significant ($P < 0.05$) influence of dietary protein sources was also observed on the specific food consumption of prawns (Fig. 15). Prawns fed on diets with coconut oil-cake and gingely oil-cake showed significantly ($P < 0.05$) higher SFC than those fed diets with other dietary protein sources. There were no significant differences in the SFCs between prawn groups fed on various diets with animal protein sources.

Food Conversion Ratio (FCR):

Food conversion ratio (FCR), as shown in Fig. 15, did not show much variation between the protein sources; however, diets with plant protein sources gave relatively higher values than those fed diets with animal protein sources. Among, plant protein sources, diets with coconut oil-cake (1.44) and gingely oil-cake (1.38) gave relatively higher FCRs compared to soybean meal, ground nut oil-cake and mixture of plant protein sources. The FCRs obtained for diets with different animal protein sources, did not vary markedly from each other and it ranged from 0.92 for clam meal to 0.81 for prawn meal. Among, the plant protein sources, soybean meal gave FCR value (0.88), almost equivalent to that of most of the individual animal protein sources. Also, the diet with a mixture of plant and

Fig. 15. SFC, FCR and PER for different protein sources. A-groundnut oil-cake, B-soybean meal, C-coconut oil-cake, D-gingely oil-cake, E-all plant proteins, F-fish meal, G-crabmeal, H-prawn meal, I-clam meal, G-crabmeal proteins, K-combination of all plant-animal proteins, L-purified diet.

FIG. 15


PROTEIN EFFICIENCY RATIO
4·5
3·5
2·5
1·5
0
FOOD CONVERSION RATIO
1·5
1·0
0·5
0
SPECIFIC FOOD (%) CONSUMPTION
5·0
2·5
0
A
B
C
D
E
F
G
H
I
J
K
L
DIETARY PROTEIN SOURCES

animal protein sources (0.89) and purified diet (0.85), gave FCR values similar to that recorded for soybean meal. However analysis of variance of the data did not give any significant F value showing that all the observed differences were statistically not-significant.

Protein Efficiency Ratio (PER):

Protein efficiency ratios recorded from different treatments are shown in Fig. 15. Among the diets, ground nut oil-cake, soybean meal, fish meal, prawn meal, mixture of animal protein sources and mixture of plant and animal protein sources gave significantly ($P < 0.05$) higher PER compared to that of prawns fed with coconut oil-cake and ground nut oil-cake. The highest PER was recorded with Soybean meal (4.72) amongst plant protein sources and lowest with coconut oil-cake (1.98). Amongst animal protein sources, the PER was highest for prawn meal (4.47) and lowest for crab meal. However, amongst all diets, mixture of plant-animal protein sources recorded the highest PER (4.73). Purified diet fed prawns showed significantly ($P < 0.05$) lower PER (3.13) than most other protein sources.

Biochemical Composition:

With a view to ascertaining if there was any significant influence of the diets, containing the various protein sources, on the body composition, biochemical composition of the whole prawns were determined after the experiments and the results are presented below. The percentages of moisture, ash, protein,

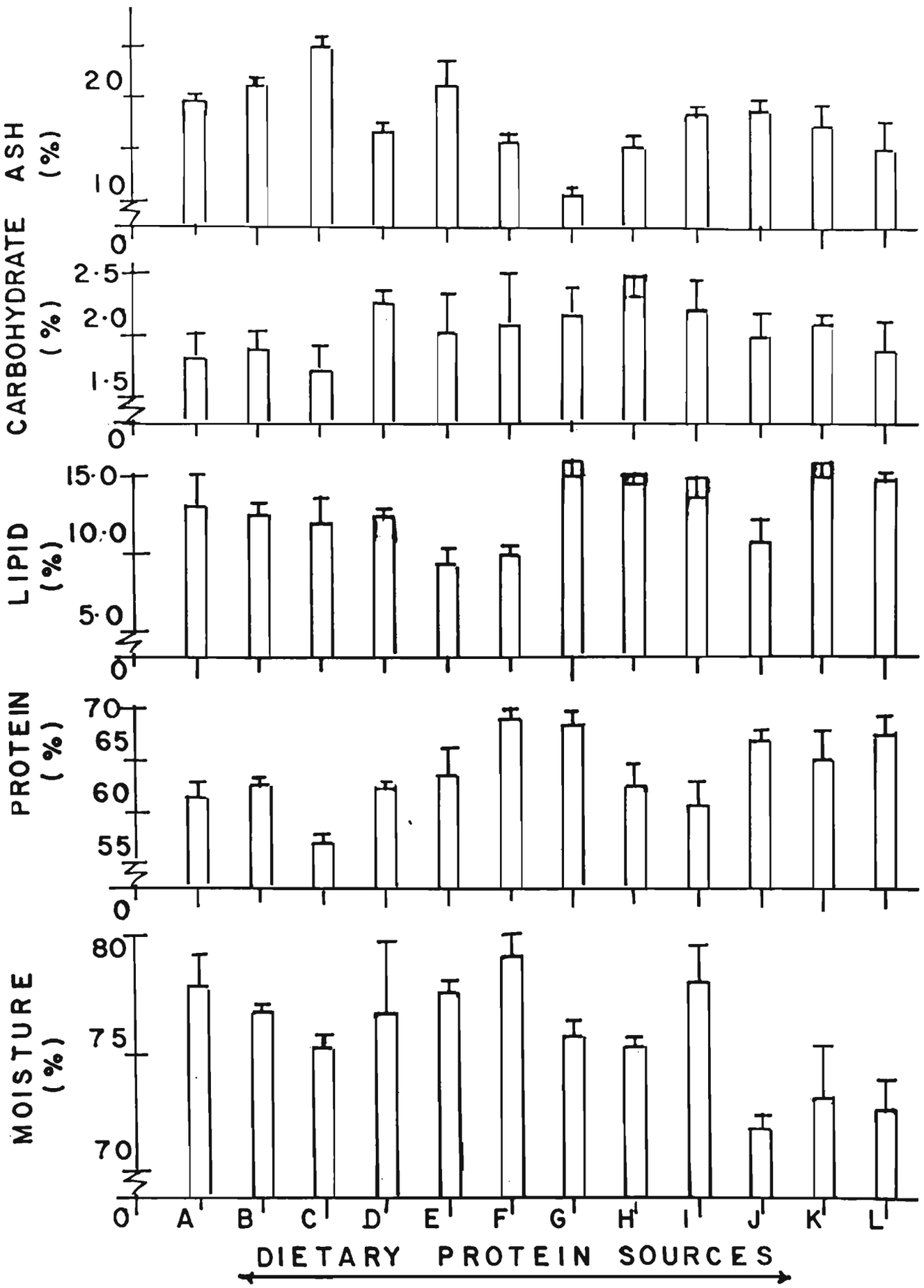
lipids and carbohydrate in prawns from different dietary treatment are shown in Fig. 16.

Analysis of variance of the data showed that the protein sources used in the diets significantly ($P < 0.01$) influence the moisture, protein, lipid and ash content of prawns. The moisture content of prawns fed diets with plant protein sources was relatively higher than those fed diets with animal protein sources. However, there were no significant differences between prawn groups fed diets containing the various plant protein sources, as the moisture content ranged from 76.8% to 77.9%. On the other hand, prawns fed on diets with animal protein sources showed significant ($P < 0.05$) differences in the moisture contents. The prawns fed on diets with fish meal (79.2%) and clam meal (78.3%) had relatively higher moisture contents than prawns from other treatments. The moisture content of prawns fed on diets with a mixture of plant-animal protein sources (73.1%) and the purified diets (72.8%) were also not significantly different from that observed in prawns fed on the diet with a mixture of animal protein sources (72.4%).

Total ash content was relatively higher in prawns fed on diets with plant protein sources than those fed on diets with animal protein sources. Among the plant protein sources, coconut oil-cake diet fed prawns had the highest ash content (25.3%); whereas prawns fed on the diet with gingely oil-cake had the lowest ash content (16.9%). There were no significant differences between the ash content of prawns fed on diets with

Fig. 16. Biochemical composition of prawns fed with different protein sources. A-ground nut oil-cake, B-soybean meal C-coconut oil-cake, D-gingely oil-cake, E-all plant proteins, F-fish meal, G-crabmeal, H-prawn meal, I-clam meal, J-all animal proteins, K-combination of all plant-animal proteins, L-purified diet.

FIG. 16


ASH (%)
20
10
0
CARBOHYDRATE (%)
2·5
2·0
1·5
0
LIPID (%)
15·0
10·0
5·0
0
PROTEIN (%)
70
65
60
55
0
MOISTURE (%)
80
75
70
0
A
B
C
D
E
F
G
H
I
J
K
L
DIETARY PROTEIN SOURCES

soybean meal (21.3%), mixture of plant protein sources (21.5%) and ground nut oil-cake (19.7%). In the case of prawns fed on diets with animal protein sources, the highest ash content was recorded with a mixture of animal protein sources in the diets (18.9%), which was closely followed by clam meal (18.2%) diet fed prawns. The prawns fed on diets with crab meal had the lowest (10.9%) ash. The ash content of prawns fed on the fish meal diet (15.4%) was not significantly different from that fed with prawn meal (15.3%) diet. Prawns fed on the diet with a mixture of plant and animal protein sources had ash 17.3% which was not significantly different from that observed in prawns fed on diets with most of the animal protein sources. Purified diet fed prawns had lower ash content (14.5%) than the prawns fed on diets with all other dietary protein sources, excepting crab meal. The ash content of prawns fed on diets with groundnut oil-cake, coconut oil-cake, gingely oil-cake was significantly ($P < 0.05$) different from that found in prawns from all other treatments.

The protein content of prawns showed distinct variability in relation to the dietary protein sources. Diets based on plant protein sources produced significantly ($P < 0.05$) lower protein contents than animal protein sources. Among, the plant protein sources treatments, diets with gingely oil-cake (63.77%) and a mixture of plant protein sources (63.7%) produced relatively higher protein contents. Whereas, the prawns fed with coconut

oil-cake in the diet had significantly ($P < 0.05$) lower protein content (57.2%) than those fed on diets containing other plant protein sources. Similarly, the prawns fed on diet with fish-meal had the highest protein content (69.4%) and those fed with clam meal (60.8%) had the lowest protein content, among prawns fed on animal protein diets. Prawns fed diets with a mixture of animal protein sources (67.5%) and crab meal (69.6%) had significantly ($P < 0.05$) higher protein content than those fed on diets with clam meal. No significant variation in the protein content was observed between the groups of prawns fed on diets with fish meal, crab meal and mixture of animal protein sources. The prawns fed with a mixture of plant and animal protein sources had a protein content (65.2%), which was relatively higher than prawns from most other treatments. Similarly, prawns fed on the purified diet had high protein content (67.6%), which was almost equivalent to the protein content of prawns fed with diets containing fish meal or crab meal.

It was also observed that the prawns fed on diets containing animal protein sources had higher lipid contents, when compared to the prawns fed on diets with plant protein sources. Amongst prawns fed on diets containing plant protein sources, soybean meal (14.3%) fed prawns had relatively higher lipid content, whereas prawns fed on the diet containing a mixture of plant protein sources (9.4%) had the lowest lipid content . There were not much differences between the lipid

content of prawns fed diets with groundnut oil-cake (13.1%), coconut oil-cake (12.1%) and gingely oil-cake (11.2%). Among the animal protein sources, prawns fed on crab meal (16.9%) diet had significantly ($P < 0.05$) higher lipid than all other treatments. Whereas, prawns fed on diets with fish meal (10.0%) and mixture of animal protein sources (10.9%) had low lipid content. The lipid content of prawns grown on diets with crab meal, prawn meal, clam meal and mixture of plant and animal protein sources was significantly ($P < 0.05$) different from that of prawns from most other treatments.

Analysis of variance of the data showed that the protein sources used in the diets significantly ($P < 0.05$) influence the carbohydrate content of prawns. Amongst prawns fed on diets compounded with plant protein sources, those fed on coconut oil-cake (1.7%) diet had significantly ($P < 0.05$) lower carbohydrate content and those fed on diets with gingely oil-cake (2.3%) had the highest total carbohydrate content. However, there were no significant differences between carbohydrate contents of prawns fed on other protein sources diets which ranged between 1.89 and 2.13%. There was also not much difference in the carbohydrate content of prawns fed on diets containing various animal protein sources, as well as a mixture of plant and animal protein sources.

The RNA/DNA ratios for prawns from various treatments are shown in Fig. 17. There were significant differences

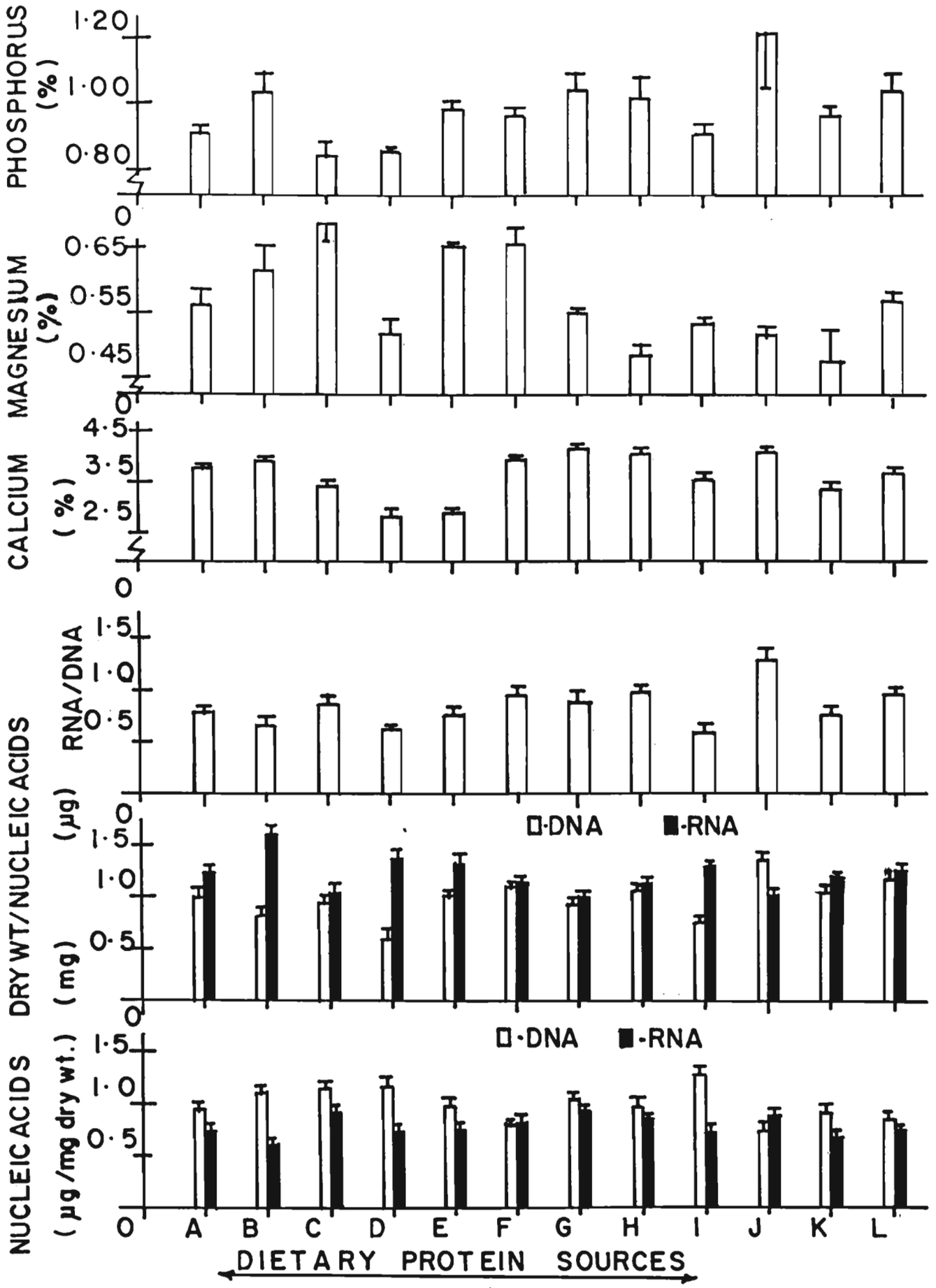
($P < 0.05$) between the RNA/DNA ratios for prawns from various treatments. The prawns fed on the diet with a mixture of animal protein sources had significantly ($P < 0.05$) higher (1.13) RNA/DNA ratio compared to prawns fed on clam meal (0.61) diet. However, RNA/DNA ratios of prawns fed on diets with fish meal (0.96), crab meal (0.9) and prawn meal (0.99) did not differ significantly from that of purified diet (0.98). The RNA/DNA ratios were relatively low in prawns fed on the plant protein diets and it ranged from 0.62 to 0.88.

Among, the various treatments, soybean meal (1.61) diet fed prawns had significantly ($P < 0.05$) high dry weight/total RNA ratio. It was also observed that the dry weight/total RNA ratio (Fig. 17) was relatively higher in prawns fed on diets with plant protein sources, when compared to those fed on diets with animal protein sources, except clam meal.

The dry weight/total DNA ratio did not show any significant variation in relation to dietary protein sources (Fig.17). However, the ratio of dry weight/total DNA was relatively higher in prawns fed diets with groundnut oil-cake coconut oil-cake and mixture of plant protein sources (1.03) when compared to diets with other plant protein sources. Amongst animal protein sources, the highest ratio was recorded in prawns fed on the diet containing a mixture of animal protein sources (1.38) and the lowest ratio in prawns fed on diet with clam meal (0.78). The dry weight/total DNA ratios observed in prawns fed diets with fish meal (1.1), crab meal

Fig. 17. Biochemical composition of prawns fed with different protein sources. A-groundnut oil-cake, B-soybean meal, C-coconut oil-cake, D-gingely oil-cake, E-all plant proteins, F-fish meal, G-crab meal, H-prawn meal, I-clam meal, J-all animal proteins, K-combination of all plant-animal proteins, L-purified diet.

FIG. 17


PHOSPHORUS (%)
1·20
1·00
0·80
0
MAGNESIUM (%)
0·65
0·55
0·45
0
CALCIUM (%)
4·5
3·5
2·5
0
RNA/DNA
1·5
1·0
0·5
0
□-DNA ■-RNA
DRY WT/NUCLEIC ACIDS (µg)
1·5
1·0
0·5
0
(mg)
□-DNA ■-RNA
NUCLEIC ACIDS (µg/mg dry wt.)
1·5
1·0
0·5
0
A B C D E F G H I J K L
DIETARY PROTEIN SOURCES

(0.97), prawn meal (1.06), a mixture of plant and animal protein sources (1.08) and purified diet (1.18) were not significantly different from each other.

The calcium, magnesium, and phosphorus contents of prawns from various treatments are shown in Fig. 17. Analysis of variance of the data showed that the calcium, magnesium and phosphorus content of prawns are significantly ($P < 0.05$) influenced by the dietary protein sources.

The calcium content was significantly ($P < 0.05$) less in prawns fed with gingely oil-cake and mixture of plant protein sources among plant protein sources. The highest calcium content was recorded in prawns fed with soybean meal (3.9%) diet, which was closely followed by prawns fed with groundnut oil-cake (3.8%) and coconut oil-cake (3.5%) in the diets.

In the case of animal protein sources, no significant variation was observed between treatments, excepting prawns fed with clam meal diet which had significantly ($P < 0.05$) lower calcium content than prawns fed with other animal protein sources in diets. Calcium content in prawns fed on diets with animal proteins sources was relatively higher than those fed on plant protein sources in the diets. Prawns fed on diet with a mixture of plant and animal protein sources had significantly ($P < 0.05$) lower calcium content (3.4%) than prawns from most other treatments. In the case of

purified diet fed prawns, the calcium content (3.7%) was significantly ($P < 0.05$) higher than their counterparts fed on diets containing most plant protein sources and clam meal.

Prawns fed on diets with gingely oil-cake, crab meal, mixture of animal protein sources and mixture of plant and animal protein sources showed significant ($P < 0.05$) variations in the magnesium content with that of prawns fed on diets with other protein sources. In the case of animal protein sources, only prawns fed with fish meal (0.65%) in the diet had significantly ($P < 0.05$) higher magnesium than prawns from other treatments.

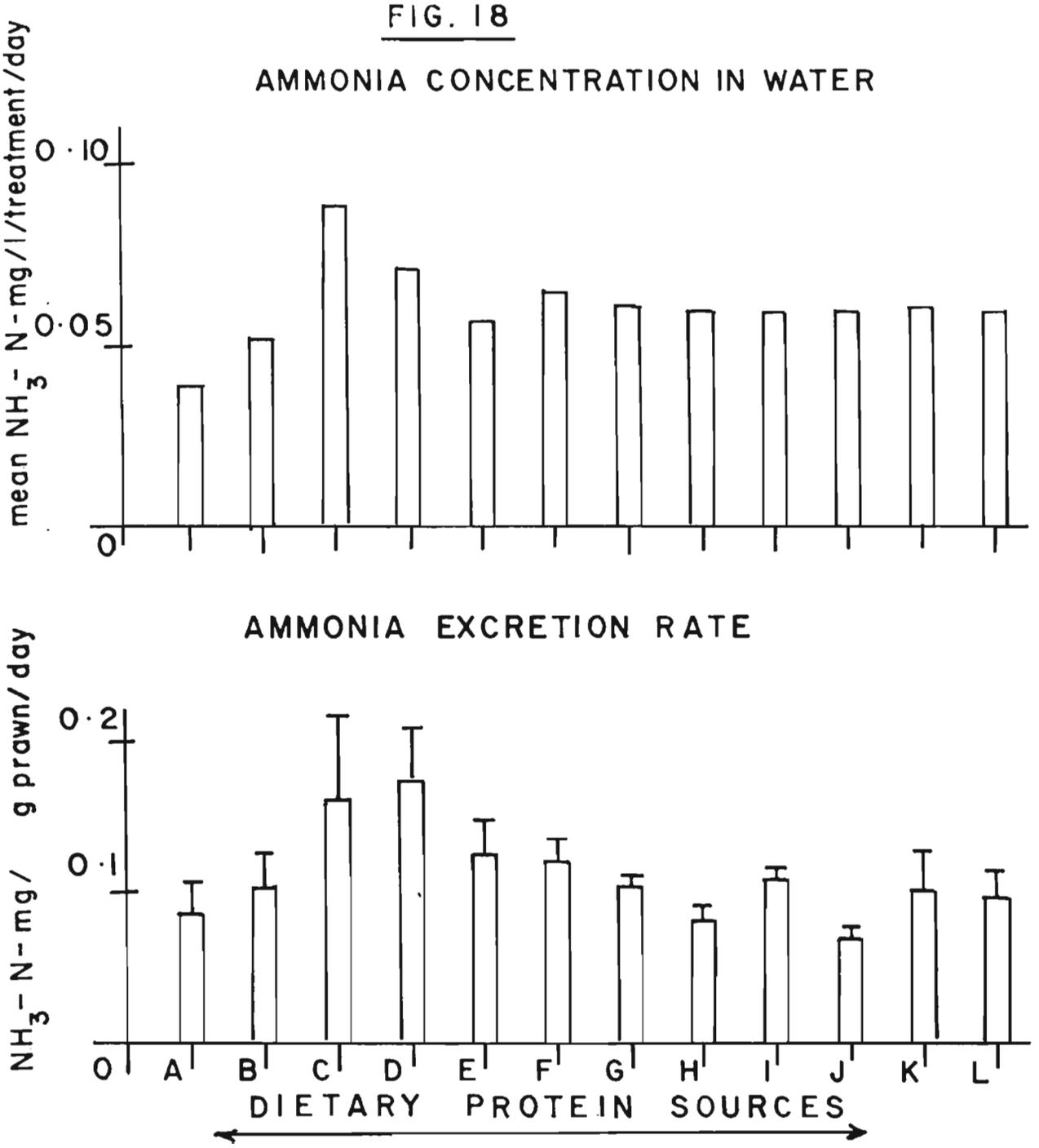
The phosphorus content of prawns fed with coconut oil-cake (0.84%) and gingely oil-cake (0.85%) in the diets were significantly ($P < 0.05$) lower than that in prawns fed with other dietary protein sources in which the phosphorus content varied between 0.91 and 1.04%. Amongst, the animal protein sources diets fed prawns, the phosphorus content was relatively higher in prawns fed on the diet containing a mixture of animal protein sources (1.22%). In all other dietary protein sources fed prawns, the phosphorus content ranged between 0.91 and 1.04%.

Ammonia Excretion Rates:

Ammonia excretion rates of prawns (Fig. 18) was also significantly ($P < 0.01$) influenced by the dietary protein

Fig. 18. Ammonia concentration in seawater and ammonia excretion rate in prawns fed different protein sources. A-groundnut oil-cake, B-soybean meal, C-coconut oil-cake, D-gingely oil-cake, E-all plant proteins, F-fish meal, G-crab meal, H-prawn meal, I-clam meal, J-all animal proteins, K-combination of all plant-animal proteins, L-purified diet.

FIG. 18


AMMONIA CONCENTRATION IN WATER
mean NH3 - N - mg/l/treatment/day
0·10
0·05
0
AMMONIA EXCRETION RATE
NH3 - N - mg/ g prawn/ day
0·2
0·1
0
A
B
C
D
E
F
G
H
I
J
K
L
DIETARY PROTEIN SOURCES

source. Significant ($P < 0.05$) treatment differences were observed in the ammonia excretion rates between prawns fed diets with coconut oil-cake, gingely oil-cake, mixture of plant protein sources and fish meal with that of prawns fed diets with other dietary protein sources. Among prawns fed diets with plant protein sources, the ammonia excretion rate was highest with gingely oilcake (0.148 mg/ g prawn/day) and lowest with ground nut oil-cake (0.0697 mg/ g prawn/day).

The prawns fed on diets with animal protein sources excreted less ammonia when compared to many of the prawn groups fed on diets with plant protein sources with the exception of fish meal diet. The lowest rate of ammonia excretion (0.061 mg/ g prawn/day) was recorded in prawns fed on the diet with a mixture of animal protein sources. On the other hand, ammonia excretion rates observed in prawns fed on diets with a combination of plant and animal protein sources (0.081 mg/ g prawn/day) and purified diets (0.082 mg/ g pfawn/day) were almost equal.

O B S E R V A T I O N S

During the 30 days of experimentation, observations were also made on molting, food intake, behaviour towards light and external morphology of prawns.

Molting:

Observations were made on the incidence of occurrence of exuviae and post-molt deaths during the experimental study and the data are shown in Table 10.

The number of exuviae collected in 30 days, from treatments with soybean meal, prawn meal, crab meal and a mixture of animal protein sources (31-32 nos) were higher than that of other treatments. Treatments with coconut oil-cake, gingely oil-cake, and mixture of plant protein sources had relatively less number of exuviae (18-19 nos). The number of exuviae given in the Table 10 are apparent, because at times fresh exuviae were devoured by cohabiting non-molted prawns in the night, before the collection could be done on the following morning.

Post-molt deaths (Table 10) usually occurred due to a number of factors, but primarily as a result of devouring of freshly molted animals by cohabitors. Post-molt deaths were significantly higher in the case of prawn groups fed on diets with plant protein sources, with the exception of soybean meal, than prawn groups fed on diets with animal protein sources or the purified diet. During the first two weeks there was not much variation in the occurrence of post-molt deaths between

TABLE 10: OBSERVATIONS IN PRAWNS FED WITH DIFFERENT EXPERIMENTAL DIETS

Diet	Protein Source	Mean nos of molts recovered	Mean nos.of post-molt deaths	Texture of the body
A	Ground nut Oil-cake	26	4	H
B	Soyabean Meal	30	6	H
C	Coconut Oil-cake	18	14	SO
D	Gingely Oil-cake	19	10	SO
E	Mixture of Plant protein source	18	10	H
F	Fish Meal	23	8	H
G	Crab Meal	31	8	H
H	Prawn Meal	31	5	H
I	Clam Meal	27	11	H
J	Mixture of Animal protein source	32	4	H
K	Mixture of Plant-Animal protein sources	22	10	H
L	Purified Diets	27	8	H

H - hard, SO - soft

the treatments. However, from the third week onwards post-molt deaths were higher in the case of prawn groups fed on diets with gingely oil-cake, mixture of plant and animal proteins sources, coconut oil-cake and clam meal. In all other treatments, post-molt deaths were relatively less.

Food Intake:

During the first week of the experiment, no variation in the feed intake was observed between the treatments and the food offered was almost completely ingested by the animals. From the 2nd week onwards, food intake was observed to be less in the prawn groups fed on diets with coconutoil-cake. A reduction in food intake was also observed in the third week in the case of prawn groups fed on diets with ground nut oil-cake, gingely oil-cake and mixture of plant protein sources. During the final week, before the termination of the experiment, food intake was severely affected in the case of prawn groups fed on diets with groundnut oil-cake, coconut oil-cake and gingely oil-cake and appreciable amounts were seen strewn all over the bottom of the experimental tanks. Amongst the prawn groups fed on diets with animal protein sources, reduced food intake was observed only in clam meal fed prawns. Differences in attractability to feed on introduction in the experimental tanks could be seen clearly between prawn group fed with animal protein sources and that of coconut oil-cake and gingely oil-cake

treatments. While active response of prawns was observed towards diets with animal protein sources, the prawns fed with coconut oil-cake and gingely oil-cake, responded passively to the introduced feed.

Behaviour Towards Light:

When table lamp light (1925×10^2 lux) was suddenly directed into the experimental tanks, the prawns from various treatments showed variation in responses. While, the prawns in the treatments fed with animal protein sources responded actively to the light, prawns fed with plant protein sources, with the exception of soybean meal, showed passive response. The most active and agitated movements were shown by prawns fed on the diet with crab meal. With purified diet, the responses of prawns towards light were normal.

External Morphology:

The prawns fed on soybean meal, crab meal, clam meal and purified diets had dense brownish-black spots on the rostrum and abdominal region. However, in prawns from other treatments the spots were distributed sparsely on the body and the rostrum.

The prawns fed on clam meal and mixture of animal protein sources had greenish color gut. This green coloration of the gut may be due to the presence of algal cells that was eaten by the molluscs earlier. The hepatopancreas were found

to be diffused in form in most of the prawns fed on diets with most of the plant protein sources and a mixture of plant and animal protein sources; whereas the hepatopancreas was compact in all the prawns fed on diets with animal protein sources and soybean meal.

D I S C U S S I O N

Different sundry protein sources of plant and animal origin have been widely tested for their biological values, to identify cheap protein sources for formulation of practical diets for prawn culture (New, 1976a). In the present study also few locally available protein sources were tested for their nutritional quality for early juveniles of Penaeus indicus based on the survival, growth, food conversion ratio (FCR), protein efficiency ratio (PER), specific food consumption(SFC), body composition and ammonia excretion rates in prawns. These parameters were also employed by few other authors to test the biological value of protein sources, when used in the diets of various penaeids and palaemonids (Balazs and Ross, 1976; New,1976).

However, results from different experimental studies are not easily comparable, since the percentages of various protein sources added to the diets varied from species to species (Andrews et al., 1972). Also,other factors such as environmental parameters used for the study, type of species selected, its age, size and its dietary protein requirements

significantly influence the results (New, 1976a; Maguire, 1980).

Most plant proteins have been shown to yield poor growth rates in prawns, when used individually, excepting a few like soybean meal (Kanazawa *et al.*, 1970; Sick and Andrews, 1973; Millikin *et al.*, 1980; Maguire and Hume, 1982), wheat gluten (Forster and Gabbott, 1971; Deshimaru and Shigueno, 1972) and peanut meal (Lee, 1970; Sick *et al.*, 1972; Forster and Beard, 1973; Balazs and Ross, 1976; Balazs *et al.*, 1973). The improved growth rates produced by some of the plant protein sources have been attributed to their higher polysaccharide contents compared to monosaccharides (Forster and Gabbot, 1971; Kitabayashi *et al.*, 1971a; Andrews *et al.*, 1972; Sick and Andrews, 1973). These plant protein sources have also been observed to promote superior growth rates when mixed with animal protein sources (Lee, 1970).

In the present study with early juveniles of *P. indicus*, soybean meal diet fed prawns were found to grow best, and convert the ingested food and protein more efficiently into tissues; amongst the plant protein sources tested. Though, the survival rate was only 80%, compared to ground nut oil-cake, which gave a survival rate of 86.7%, the difference observed in the survival rate was not significant. Studies of Kanazawa *et al.*, (1970) in *P. japonicus* and Sick and Andrews (1973) in *P. duorarum* have also shown that soybean meal is nutritionally superior among plant protein sources. These results indicate that the amino acids profile of soybean meal may almost satisfy

the requirement of prawns. This is further supported by the observations of Deshimaru and Shigueno (1972) that the amino acids profile of soybean meal resembles that of prawns to a large degree. Although ground nut oil-cake gave the highest survival, the growth, food conversion and protein efficiency ratios were relatively less than that of soybean meal. The differences between these two protein sources can be primarily attributed to the differences in their amino acids pattern. Sure (1948) reported relatively higher levels of lysine and threonine in the soybean meal compared to groundnut oil-cake. But, these two amino acids have been shown to be essential for growth in prawns (Cowey and Forster, 1971; Shewbart et al.,1972).

Similarly, poor growth in prawns fed on diets with coconut oil-cake and gingely oil-cake can be attributed to the poor amino acids profile in these ingredients, and according to Swaminathan (1967) partial deficiency of any one EAA affects adversely the utilization of proteins for the maintenance of nitrogen equilibrium and growth.

Besides the amino acids pattern, poor growth obtained in prawns fed with coconut oil-cake, gingely oil-cake and also to some extent in ground nut oil-cake, compared to soybean meal, could be because of relatively higher levels of unavailable carbohydrates, such as cellulose, hemi-cellulose, gums and pectins in the cell walls in these sources, which are not acted upon by the digestive enzymes and excreted mainly through the

faeces (Swaminathan, 1967). Besides, certain EAA present in the protein may not be fully released after digestion, as well as the rate of release of different amino acids during digestion varies from protein to protein (Swaminathan, 1967). All these conditional availability of dietary nutrients might have influenced the growth and utilization of food in prawns.

Amongst, the biochemical parameters studied, the moisture and lipid content in prawns fed on diets containing different plant protein sources, did not show any significant variation between themselves, which suggests that possibly these dietary protein sources do not have any significant influence on these parameters. On the other hand, major differences were observed in the ash, protein and to some extent in the carbohydrate content of prawns. Prawns fed on the diet containing coconut oil-cake had higher levels of ash, probably due to the higher levels of calcium and magnesium, especially the latter, which was highest, among the four protein sources tested. Prawns from other treatments had almost same ash content (excepting gingely oil-cake fed prawns) and correspondingly the calcium, magnesium and phosphorus contents did not vary significantly, indicating that the utilization of these minerals are not affected by the dietary protein sources. However, when compared to animal protein sources, prawns fed on diets with plant protein sources, recorded lower phosphoyus, levels. This can be expected since, major part of phosphorus in plant products is bound to phytic acid (Lall, 1978) which

is usually found unavailable to animals due to the absence of the enzyme phytase (Muir and Roberts, 1982).

In the coconut oil-cake fed prawns, the protein content was significantly lower than prawns fed on diets containing the other three protein sources. This together with the low PER values indicates that dietary protein is poorly converted into tissue protein in prawns. In all other protein sources fed prawns, no significant variation was observed in the protein content and the PER values were found to be higher, indicating that dietary proteins are efficiently converted into tissue proteins.

The RNA content of a wide variety or organisms has been related to growth rate (Sutcliffe, 1970) and the RNA/DNA ratio has been widely used as an indicator of the potential future growth rate of a population (Buckley, 1979a). While, higher RNA-DNA ratio suggests faster growth rate, lower RNA-DNA ratio indicates slower growth rate (Buckley, 1979b). Correlations between RNA concentrations or RNA-DNA ratios and growth rate for a wide variety of organisms (Kennell and Magasanik, 1962; Bulow, 1970; Sutcliffe, 1970) have shown that RNA concentration will vary much in fast growing individuals and those undergoing high protein synthesis (Brachet, 1955; Leslie, 1955). However, in the present study by comparing the protein content and the RNA-DNA ratio in these plant protein sources diet fed prawns, no specific conclusions could be drawn because of the fluctuating values in the

protein content and the RNA-DNA ratios in various treatments. Similar observations were also reported in Crangon crangon (Regnault, 1977). However, from the ammonia excretion rates, it is evident that soybean meal and ground nut oil-cake are more efficiently utilized for tissue growth with relatively low rates of catabolism of protein. On the contrary, coconut oil-cake and gingely oil-cake are poorly utilized for tissue synthesis, since the ammonia excretion rates were high due to catabolism of the proteins. Thus, groundnut oil-cake and soybean meal seem to be better protein sources for prawns when compared to other plant protein sources.

One of the earliest compounded rations for shrimps was that of Kanazawa et al. (1970) for P. japonicus using silkworm, chinook salmon, and brine shrimp as protein sources. Subsequently, Shigueno et al. (1972) have shown that high levels of protein obtained from several protein sources such as squid meal, white fish meal, dried euphausia and active sludge are more efficient than low protein levels. Thus, these studies led to the identification of animal protein sources that can be efficiently used for formulation of feeds for prawns. Among animal protein ingredient sources, proteins of marine origin are preferred to freshwater origin not only due to their amino acids profile but also due to their better composition of unsaturated fatty acids (Cowey and Sargent, 1972) essential for prawns, as well as higher ash content (Boghen and Castell, 1981).

Amongst the animal dietary protein sources tested in the present study, shrimp meal fed prawns showed highest survival rate and gain in dry weight. Sick and Andrews (1973) also obtained higher survival in P. duorarum fed on a diet with shrimp meal. Comparatively, on percent gain in wet weight basis, prawns fed with fish meal and crab meal in the diets showed higher percent gain than those fed on diet with shrimp meal. However on dry weight basis fish meal diet fed prawns recorded lower percent gain than those fed on diets with crab meal and shrimp meal. These variations in percent gain in dry weight and wet weight in prawns could be well correlated with the significant variations in the moisture content of prawns. The improved growth (dry weight basis) in prawn meal diet fed prawns indicates the superiority of the prawn meal as a protein source for juvenile P. indicus. Earlier studies by Forster and Beard (1973) also indicated that shrimp meal is nutritionally superior to fish meal in the diet of P. serratus. The superiority of prawn meal may be because of its amino acids profile, which may satisfy the nutritional demands of the juvenile prawn. According to Deshimaru and Shigueno (1972) only diets with an amino acids profile similar to shrimp and rich in basic amino acids would produce relatively good growth.

Comparatively, clam meal fed prawns, showed poor survival and gain in dry weight, but on wet weight basis, the growth was highest compared to all other animal protein sources. The

variation in dry weight and wet weight gain could, however, be due to high moisture content in these prawns fed with clam meal. However, Forster and Beard (1973) in Palaemon serratus, and Kanazawa et al. (1970) in P. japonicus have obtained highest growth rates, with fresh mussel mantle or fresh short-necked clam, respectively. The significant differences observed between the present study as well as those of earlier authors may be due to the form of molluscs used, since during the present study dried and powdered clam meal was used. Probably, during drying, certain essential amino acids are lost as a result of reaction with reducing sugars and carbonyl compounds present in the diet (Swaminathan, 1967). It is also not known whether the clam meal utilised in the present study had any anti-nutritional factors. Bivalves, especially clams have been generally known to accumulate toxic components (Bayne, 1975), as well as algae ingested by bivalves also may contain compounds which inhibit growth and affect survival. However, no attempt has been made in this study to examine the above possibilities.

Sick and Andrews (1973) have shown high survival and growth in P. setiferus fed on a diet with menhaden meal compared to that of shrimp meal where the survival was low but growth was high. However, in the present study, it was observed that crustacean meals had better influence on growth and survival of prawns compared to fish meal and other animal protein source.

Significant differences observed in the SFC, FCR and PER between the various animal protein sources could be accounted for the variations in acceptability of feed, digestion and assimilation rates, depending upon the protein make up of the source. Shrimp meal may be accepted better than the other three diets because of the amino acids profile which may, to a greater extent, meet the requirements of prawns (Deshimaru and Shigeuno, 1972).

The biochemical composition of the prawns showed significant differences between treatments in many of the parameters considered. Significantly, prawns fed on the diet with crab meal had very low ash content, inspite of relatively high percentage of ash in the crab meal. Probably, ash from crab meal is not efficiently assimilated, and deposited in tissues of the prawn. On the other hand, clam meal fed prawns had significantly higher ash content than prawns fed on other animal protein sources fed prawns, may be due to delayed molting, which was similar to the observations in crabs fed on diets with higher salt content (Ponat and Adelung, 1980). These differences were, however, found to be reversed, when the calcium and phosphorus contents in prawns were considered. Prawns fed with clam meal had significantly lower calcium and phosphorus levels compared to those fed on diets with fish meal, crab meal and shrimp meal which indicates that calcium and phosphorus metabolism are perhaps affected in these prawns,

resulting in high mortality rate (New, 1976a), especially during post-molt stages.

Prawns fed on diets with fish meal and crab meal had significantly higher protein content compared to those fed on diets containing shrimp meal or clam meal. However, these variations in the protein content of prawns could be due to the variation existing in the levels of other organic and inorganic matter present in the tissues. It is also possible that the presence of certain amino acids in higher dosages in the fish protein might have influenced the protein content in these prawns as suggested by Cowey and Forster (1971). In Calanus finmarchicus, Cowey and Corner (1963) reported that dietary concentration of non-essential as well as essential amino acids affect metabolic performances. So possibly, variations in the concentration of non-EAA and EAA in different animal protein sources could have brought about metabolic changes resulting in variability in the digestion of various constituents.

The prawns fed with crustacean meals in diets accumulated high levels of lipids, indicating that the nutritional demands for various unsaturated fatty acids are met by feeding crustacean meal, as they contain the same profile of unsaturated fatty acids which the fish meal and clam meal may be lacking (Sick and Andrews, 1973; New, 1976a). However, the carbohydrate content in prawns was observed to be not significantly influenced by the different animal protein sources.

The RNA/DNA ratio shows some correlation with the protein content of prawns. Fish meal diet fed prawns had relatively high protein content and RNA-DNA ratio, indicating efficient protein synthesis in these prawns. Conversely, clam meal diet fed prawns had low protein content and RNA-DNA ratio, which are reflected on poor growth and survival.

The efficiency of protein metabolism in prawns is evident from the ammonia excretion rates. The high level of protein metabolism as observed in fish meal fed prawns was reflected in the high ammonia excretion rates indicating the catabolism of excess dietary protein. However, the ammonia excretion rates observed in prawns fed with crustacean meals, suggests that the feed is efficiently utilized for maximum growth with the minimum catabolism.

Earlier studies in crustaceans (Shewbart et al., 1972; Shigueno et al., 1972; Conklin et al., 1978; Ponat and Adelung, 1980) have shown that diets containing a mixture of protein sources, significantly influence growth in prawns. Invariably, a mixture of plant and animal protein sources are used in the diets (New, 1976a), with relatively lesser quantities of plant protein sources than animal protein sources. Nelson et al., (1977b) showed that the assimilation rates of juvenile prawns, M. rosenbergii fed on mixed diet were low compared to the animal diet fed prawns, but higher than prawns fed on only plant diets.

According to Deshimaru and Shigueno (1972), only diets with an essential amino acids profile similar to shrimp and rich in basic amino acids would produce relatively good growth. However, mixing two or more protein sources in the diets, should enhance the growth in prawns, since deficient amino acids in one protein may be compensated through the amino acids present in the other protein.

Thus, inspite of these conflicting results about the nutritive value of animal and plant protein sources in the diet, Fenucci and Zein-Eldin (1976) speculated that a number of other nutritional factors synergistically influence the results in the same species itself. These interactions of various dietary components, sometimes effect the quality of the feed as a result of toxicity or unpalatability (Zein-Eldin and Corliss, 1976). Thus, various ingredients should be included in correct proportions so as to obtain better growth rate and survival of penaeid prawns.

In the present study in P. indicus, the diet containing mixture of animal protein sources gave significantly higher survival rate and growth than all other protein sources indicating that the mixture of animal protein sources is better suited for the diet of juvenile prawns.

However, data indicates that prawns can be fed on a diet containing a mixture of animal and plant protein sources without adversely affecting growth; but diets containing only plant

protein sources induce growth retardation. Besides the imbalance of essential aminoacids, the low survival and retarded growth in prawns fed exclusively on plant proteins, can be due to the presence of antinutritional factors or excess use of some impalatable ingredients in the diet resulting in reduced food intake. In P. azatecus, when different levels of rice bran, in place of soybean meal, was used (Zein-Eldin and Corliss 1976), the food intake was greatly reduced resulting in reduced growth rate.

Even though mixed plant-animal protein sources diet produced lower survival and growth rates, the efficiency of conversion of food and protein was superior to that of diet containing mixture of all animal protein sources. However, if only plant protein and no animal protein source was included in the diet, the SFC, FCR and PER values were significantly affected suggesting that plant proteins sources are nutritionally inadequate for prawns. Similar observations were made in fishes where complete replacement of fish meal with soybean meal resulted in poor growth and higher cost of feed as a result of supplementation of amino acids and oil (Viola et al., 1981/ 1982; Kim et al. 1984). Thus, partial replacement of animal protein sources with plant protein sources gives better growth than only plant protein sources diets.

Significant variations were observed for all the biochemica parameters studied, except for carbohydrate content in prawns fed with the three types of diet containing a mixture of protein sources. This clearly suggests that the prawns show variation

in their body composition depending on the quality of protein sources in the diets. The low lipid content in prawns fed with animal protein sources may be due to faster mobilisation of lipid for energy purpose, sparing protein resulting in higher growth and survival in these prawns. On the other hand, lower lipid deposition in plant proteins fed prawns may be as a result of inhibitory effect of higher levels of fatty acids of W6 (linoleic) series which have inhibitory effect on growth of animals when in excess(Cowey and Sargent, 1972).

The moisture, ash and magnesium contents were relatively higher in prawns fed on diet with a mixture of plant protein sources than the other groups. However, inspite of high ash content in these plant protein fed prawns, the calcium content was relatively low. This suggests that the high incidence of post-molt deaths could have occurred due to some changes in calcium metabolism in these prawns.

Data for protein content shows that prawns show preference to animal protein sources compared to plant protein sources for maximum protein synthesis. Since the quality of protein and its amino acids profile determine the growth and deposition of protein (Cowey and Forster, 1971; Deshimaru and Shigueno,1972), it can be expected that the animal protein sources, when included in the diet, promote higher growth rate and protein deposition, because of their superior biological value. The increased rate of protein synthesis was also evident from the higher RNA-DNA ratio in prawns fed with animal protein sources.

However, prawns fed diets with a mixture of plant and animal protein sources showed higher protein content and lower ammonia excretion rates, than other treatments, indicating that this protein source was not indiscriminately catabolized by the prawns unlike those fed on diets containing plant protein sources which had lower protein content and higher ammonia excretion rates.

In recent years, casein is widely used, as a reference protein source in the diets, (Castell et al., 1975, 1976) as it is the only highly purified protein source available in enough quantities to carry out nutritional studies. Inspite of its deficiency in some EAA(Palakas et al., 1979, Mason and Castell, 1980), nutritional studies in prawn are still designed mainly using casein (Castell et al., 1975, 1976). Deshimaru and Kuroki (1974c, 1975a, b) showed that casein and egg-albumin mixture of 10:1 yields good growth in P. japonicus. Similarly, casein when supplemented with mineral mixture, gave good growth in P. azetecus (Sick, et al., 1972). Kanazawa et al. (1970) showed that supplementation of cholesterol and vitamin mixture influence growth when casein was solo protein source in the diet. In the present study too, casein based protein diet was formulated, fortified with ingredients such as cholesterol, lecithin, mineral mixture and vitamin mixture as suggested by other authors and fed to juveniles of P. indicus. However, the purified diet containing casein produced relatively low survival

rate, which was comparable to that recorded for diets with coconut oil-cake, mixture of plant protein sources, clam meal and mixture of plant-animal protein sources; eventhough the crude protein content was high in the purified diet. Sick and Andrews (1973) also reported relatively lower survival rates in P. duorarum with casein based diet. But on the other hand, mixture of animal protein sources showed superior growth to that of purified diet inspite of lower crude protein content in the diet. However, this superior growth in these prawns could have been due to other nutritional factors present in the natural feed ingredients which influence the growth significantly.

Despite the low survival, the purified diet was efficiently assimilated by the prawns. However, the survival can be improved through inclusion of more quantity of lecithin in the diet as suggested by Boghen and Castell (1980). According to Kanasawa et al. (1970), Sick et al. (1972), Deshimaru and Kuroki (1974a), Ponat and Adelung (1980), supplementation of casein with certain other ingredients in small amounts is necessary for better growth of prawns. Thus, the efficient assimilation rate in these purified diet fed prawns, as indicated by the low SFC, FCR and high PER values, suggests that the purified diet has high palatability which resulted in efficient conversion of dietary proteins for tissue growth and protein and lipid synthesis. Earlier studies also demonstrated, the accumulation of high levels of lipid in prawns and lobsters fed on purified diets

(Brockernoff and Hoyle, 1967; Stewart et al., 1972; Mauviot and Castell, 1976; Ando et al., 1977).

CONCLUSIONS

In the present study, different plant and animal protein ingredient sources were tested for their nutritional qualities for juvenile P. indicus. Among the plant protein sources, soybean meal and ground nut oil-cake and among animal protein sources, a mixture of animal protein sources, shrimp meal and crab meal were found to be better for feed formulation than other protein sources. However, by manipulating the ratio of ingredients it may be possible to obtain better growth with other combinations.

Fish meal was found to be not very suitable for juvenile prawns, even when the protein congent in prawns was highest. Clam meal in the present study was also less successful because of poor survival, low growth and protein content in prawns. The poor performance can be attributed to the reduction in food value due to drying.

Among plant protein sources, soybean meal and groundnut oil-cake served efficiently as protein source by promoting growth of prawn. Between the two, soybean meal gave high survival and growth rate equivalent to the animal protein sources tested showing that the nutritional demands of prawns

for amino acids were mostly met, even though lysine and methionine content are low (Swaminathan, 1967). Thus, in commercial dietary formulation soybean meal can successfully replace fish meal (Viola *et al.*, 1981/82). Besides, the content of arginine, one of the essential amino acids, is very high in soybean meal (Swaminathan, 1967). Ground nut oil-cake can also be used in lieu of soybean meal because the results show that it has good potential as that of soybean meal to produce better growth rates. Though the levels of lysine, threonine and methionine in ground nut oil-cake(Swaminathan, 1967) are low, it can be used as an ingredient for formulation of practical diets along with animal protein sources.

In the present study on *P. indicus* juveniles, there was no significant difference in survival, gain in weight, protein content, lipid, ash, calcium and phosphorus contents in prawns between shrimp meal diet and soybean meal diet as protein sources. Thus, soybean meal is as effective as shrimp meal, as protein source for prawns. So, soybean meal can effectively replace shrimp meal or can be used in proportions with shrimp meal.

As the present study highlights, though the casein based purified diet serves as good protein source, which was also observed by D'Abramo *et al.* (1981) in lobsters, yet it needs some modification so as to enhance the poor survival

rate recorded. The survival rate recorded in other treatments fed with different protein sources were comparatively higher, since the ingredients were not purified before formulating the diet. It is also probable that growth promoting substances present in natural food ingredients could have influenced higher survival rate. For this reason dietary studies in prawns and other crustaceans are uncomparable because of numerous factors which are overlooked by prawn nutritionists of which the type and form of protein source used in the diet has a dominant role (Zein-Eldin and Meyers, 1973; New, 1976a).

Poor growth rates in prawns fed with purified diets were also recorded by number of researchers due to inadequate proportion of dietary nutrients supplementation such as minerals and vitamins. However, minerals are abundant in sea water (Shewbart et al., 1973) excepting for phosphorus, which needs to be supplemented because prawns require higher quantities (New, 1976a). Yet, in the present study minerals have been added in adequate levels to avoid dietary mineral deficiency. On the other hand, vitamins supplementation in the purified diet is essential as the vitamins can only be derived by the animals from the diet. However, vitamins supplementation has to be done cautiously in compounded feeds as the vitamins already present in ingredients may add to the supplemented, leading to hypervitaminosis (New, 1976a).

So the present study suggests that multiprotein diets are fruitful for the overall growth of juvenile prawns and that casein based purified diet can serve ably as a reference protein source diet for various basic nutritional studies in prawns.

CHAPTER-III

EFFECTS OF DELETION OF WATER SOLUBLE VITAMINS FROM THE DIETS

INTRODUCTION

The importance of vitamins as essential constituents in the diets of animals came to light in the early part of this century and during the past five decades active and rapid progress in vitamin research was made, almost in all the commercially important species. However, vitamin research is still in its infancy in aquatic species, especially in crustaceans.

Studies on the essentiality and requirements of vitamins suffer from different maladies, unlike other dietary nutrient requirement studies. In vitamin studies, requirements in animals cannot be established on the basis of vitamin expenditure or vitamin balances, because the end products of vitamin metabolism include not only the vitamins themselves and their known derivatives but also quite probably unknown metabolites (Mitchell, 1964). Again, the rate of depletion of the body stores of a vitamin is not a reliable basis for computing the daily requirements (Baumann et al., 1934; Frey and Jansen, 1947). Some of these practical difficulties led to the slow progress in vitamin research. Till date, all the studies are based on the prophylactic or curative procedures. The minimum dosage recommended is based on the fact, so as to prevent or cure some incipient symptoms of deficiency of the specific vitamin in question.

Vitamin requirements of animal species is also much dependant on their 'Physiological State' besides the influence of other dietary nutritional factors which may be synergistic or antagonistic (Frasier and Friedemann, 1946). Age, size, sex, species, food intake and calorie intake were found to influence the vitamin requirements of animal groups (Beaton et al. 1952).

Studies have shown that most of the B vitamins - thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, biotin, cyanocobalamine and, folic acid, are essential nutritional factors and are required in microquantities in the diets. The other nutritionally essential water soluble vitamins are choline, inositol and ascorbic acid, which are required in appreciably high dosages compared to the former group.

While contributions to vitamin requirement research from mammals and poultry are numerous (Mitchell, 1964), contributions from aquatic species are relatively less and mostly came from studies with finfish (Cowey and Sargent, 1972, Halver, 1972, 1978, 1982; Lovell and Li, 1978, Mahajan and Agrawal, 1979, 1980a; Millikin, 1982). In recent years, active research has been in progress on the vitamin requirements of crustaceans, because of their growing commercial importance (Deshimaru and Kuroki, 1976b, 1979; Guary et al., 1976; Kanazawa et al., 1976; Deshimaru and Yone, 1978; Hunter et al., 1979, D'Abramo and Baum, 1981, D'Abramo et al., 1982; Heinen, 1984). However, the major contributions

to vitamin requirements of prawns came from studies with ascorbic acid and vitamin A, and only meagre information exist on the requirements of other vitamins (New, 1976). Recent studies have shown conflicting results as to the requirement of vitamins in various crustacean species. While most penaeids were found to require vitamin C in their diets (Kitabayashi et al., 1971 b; Guary et al., 1976; Kanazawa et al., 1976; Lightner et al., 1977, 1979; Magarelli et al., 1979; Heinen, 1984), Artemia, Moina, and Homarus americanus have been reported to have no requirement for vitamin C(Conklin and Provasoli 1977; Heinen, 1984). Most of the B Vitamins have also been reported to be essential in the diets of most crustaceans and the quantiative requirements significantly vary among the various species studied.

Vitamin requirement studies are difficult in crustaceans because of the influence of a number of factors, of which molting in crustaceans, and leaching of vitamins from diets into the culture medium are very important. Besides these, contributions from the microbial flora present in the gut may also complicate the vitamin requirement studies. It has also been observed that vitamins and their precursors, since already present in the raw materials, 'Blanket Applications' of vitamin premixes in multi-ingredient shrimp diets may result in some excesses (New, 1976). Conversely, as the vitamin requirements of shrimps remain unknown, formulated diets may still be deficient in other vitamins even after supplementation. For instance Oregon salmon vitamin mixture

when administered to the prawn, Palaemon serratus (Forster and Beard, 1973) in a multi-ingredient diet, no advantage was observed. Thus, prior knowledge of the vitamin requirements of prawns will be advantageous to economise the formulation and preparation of diets and also to avoid wasteful expenses of some vitamins, which may be even toxic or antagonistic to shrimps, when supplied in excess.

McLaren et al.(1947b) developed a vitamin test diet containing crystalline vitamins, casein, dextrin and oils with crab meal or dried liver as the source for the antianemic factor. Thus, these pioneer fish nutritionists paved the way for vitamin requirement studies in aquatic species and reported the qualitative and quantitative requirement of thiamine, riboflavin, pyridoxine, pantothenic acid, inositol, biotin, folic acid, choline and niacin for rainbow trout. These studies were based on the fish's growth responses and food conversion. Dietary vitamin deficiency symptoms associated with 11 water soluble vitamins were identified in a short time in many finfish species using vitamin free casein in purified diets (Halver, 1957 ; Coates and Halver, 1958; Kitamura et al., 1967; Hashimoto et al., 1970).

However, only few studies have been carried out on the effect of deletion of water soluble vitamins from the diet of crustaceans. Heinen (1984) reported the symptoms associated

with the deficiency of some of the water soluble vitamins in juveniles of the prawn, M. rosenbergii. He reported that supplementation of vitamins such as riboflavin, p-aminobenzoic acid (PABA), cyanocobalmin and pantothenic acid in diets resulted in reduced growth rate, compared to fast growth rate in prawns, fed on diets deficient in these vitamins. This reduced growth rate in vitamin supplemented diet fed prawns was attributed to the detrimentally high concentration of vitamin used. Heinen (1984) also reported few deficiency symptoms in prawns associated with the omission of water soluble vitamins from diets.

Thus, the foregoing short review indicates the paucity of information on the vitamin requirement of crustaceans. The existing few studies mainly relate to dietary deficiency symptoms and in few cases, quantitative requirements of specific vitamins have been worked out based on survival and growth. In most of the studies existing, the influence of vitamins on food conversion, protein efficiency ratio and body composition has not been worked out. Once the essentiality and requirement of the vitamins is understood, it should be possible to formulate cost-effective diets which would promote growth rate significantly.

Moreover, there is no published information on the essentiality or requirement ofvvarious water soluble vitamins for Penaeus indicus. Therefore, the present investigation in early juveniles of P. indicus was taken up to understand the

effect of water soluble vitamins on the survival, growth and to study dietary deficiency syndromes associated with the deletion of the vitamins from the diets.

MATERIAL AND METHODS

An experimental study was conducted for 45 days in triplicate aquaria using isonitrogenous and isocaloric purified diets with vitamin free casein as protein source. The water-soluble vitamins deleted from the diets were ascorbic acid, riboflavin, choline, thiamine, pyridoxine, niacin, pantothenic acid and inositol.

Post-larval prawns were obtained from Narakkal Prawn Culture Laboratory of the Central Marine Fisheries Research Institute, for experimental study. The prawns were reared under laboratory conditions for a period of 15-20 days till the appropriate size for experimental study was obtained. All conditions for rearing before and during experimental study were similar to those presented in earlier experiments and described in Chapter -I. Table 11 shows environmental conditions maintained and the initial length and weight of the prawns used for the study.

Dietary formulation for vitamin deficiency studies were based on earlier work in fishes by Halver et al. (1957). Supplementation of various vitamins in the prawn's diet was also made based on earlier works in various crustacean species

TABLE 11: ENVIRONMENTAL PARAMETERS AND STOCKING SIZE OF JUVENILE PRAWNS

Parameter	Mean values
Temperature (°C)	27.7 ± 1.293
Salinity (ppt)	21.1 ± 1.896
pH	7.54 ± 0.310
Ammonia concentration in the water (NH_4-N mg/l/d)	0.0959 ± 0.0181
Initial length (mm)	23.436 ± 1.2814
Initial weight (mg)	75.9 ± 0.0025

(Kanazawa et al., 1970, 1976; Cowey and Forster, 1971; Adelung and Ponat, 1977; Aquacop, 1978; Conklin et. al., 1978; Bages and Sloane, 1981).

Based on the protein requirement of the species found out in the present study (Chapter-I), isonitrogenous diets (37.5% protein) were used for the experimental study. Vitamin-free casein obtained from ICN biochemicals, USA, was used as protein source. Most of the other ingredients were same as that used for the formulation of protein requirement study at 37.5% level (Chapter-1); except for lecithin (phospholipid) and vitamin mixture (Table 8). Lacithin was added at a level of 3% in the diet as it has been reported to improve the survival rate in crustaceans (Conklin, 1980; D'Abramo et al., 1981; Kanazawa, 1983; Heinen, 1984). However, the total lipid content was maintained at 9% by adjusting with fish oil and corn oil.

Diets deficient in each of the selected vitamins namely, ascorbic acid, choline, thiamine, pyridoxine, niacin, pantothenic acid, riboflavin and inositol were prepared by replacing with equal quantity of α-cellulose, an inert material (Halver, 1972; Guary et al., 1976; Deshimaru and Kuroki, 1979; Heinen, 1984). Additionally two diets were prepared in which one had all the essential vitamins (control diet with fat and water soluble) and the other had no vitamin supplementation. Table 12 shows the composition of diets and various experimental vitamin mixtures used for the study.

TABLE 12: VITAMIN COMPOSITION OF EXPERIMENTAL DIETS.

Total 3.3295 g vitamin/100 g dry diet

Vitamin	Amount of vitamin g/100 g dry diet								
	Ascorbic Acid	Choline	Thiamine	Pantothenic acid	Pyridoxine	Niacin	Riboflavin	Inositol	Other Vitamins
All vitamins	2.00	0.60	0.01	0.30	0.008	0.032	0.008	0.30	0.0715
No vitamins	-	-	-	-	-	-	-	-	-
Ascorbic Acid	D	0.60	0.01	0.30	0.008	0.032	0.008	0.30	1.3295
Choline	2.00	D	0.01	0.30	0.008	0.032	0.008	0.30	2.7295
Thiamine	2.00	0.60	D	0.30	0.008	0.032	0.008	0.30	3.3195
Pantothenic acid	2.00	0.60	0.01	D	0.008	0.032	0.008	0.30	3.0295
Pyridoxine	2.00	0.60	0.01	0.30	D	0.032	0.008	0.30	3.2975
Niacin	2.00	0.60	0.01	0.30	0.008	D	0.008	0.30	3.2975
Riboflavin	2.00	0.60	0.01	0.30	0.008	0.032	D	0.30	3.3215
Inositol	2.00	0.60	0.01	0.30	0.008	0.032	0.008	D	3.0295

D - deleted

TABLE 12: COMPOSITION OF FEEDS
Contd.

Ingredient	g/100 g
Casein (Vitamin-free)	39.0
Gelatin	1.0
Egg-albumin	1.0
Glucosamine-HCl	1.0
Sucrose	6.28
Glucose	4.20
Starch	12.6
Codliver-Oil	6.77
Corn oil	3.38
Lecithin	3.38
Cholesterol	00.50
Sodium succinate	0.30
Sodium citrate	0.30
Mineral mixture*	7.41
Vitamin Mixture**	3.35
Agar-agar	2.0
Additives+	4.5
Cellulose	4.0
TOTAL	100.97

* same as in Chapter 1 Table 3

\+ same as in Chapter 2 Table 7

The experimental diets were prepared following similar, procedures as for the dietary protein requirement study, (Chapter-I). However, lecithin was mixed thoroughly with the dry powdered ingredients before mixing with the preheated geletin-cellulose-oil mixture.

The effects of deletion of various vitamins from the diets were studied based on the parameters similar to that considered for protein requirement study. Deficiency symptoms observed during the experimental study were also recorded. Data obtained from various parameters were statistically analysed as described in Chapter 1.

R E S U L T S A N D O B S E R V A T I O N S

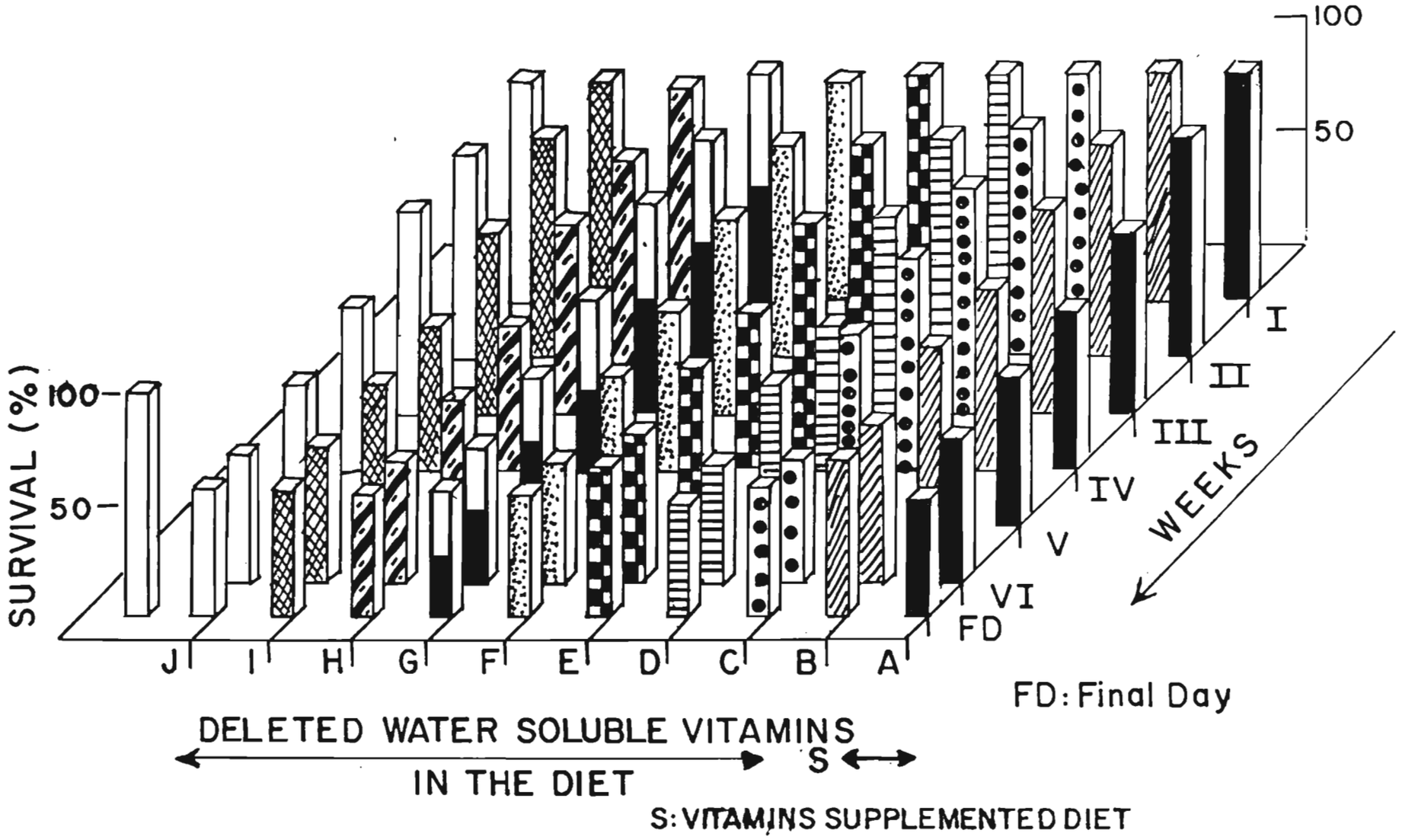
The results of the experiment conducted to ascertain the essentiality of water-soluble vitamins are presented here.

Survival:

Survival rate (Fig. 19) was significantly ($P < 0.05$) influenced by the supplementation or deletion (either individually or complete) of vitamins from the diet. Prawn groups fed on the control diet (vitamin supplemented diet) showed significantly ($P < 0.05$) higher survival compared to all the vitamin deficient diets. However, no significant ($P > 0.05$) differences in survival rates were observed between prawn groups fed on the diet with no vitamin supplementation and those

Fig. 19. Weekly percent survival of prawns fed diets with and without water-soluble vitamins. A-no vitamins, B-all vitamins, C-ascorbic acid deleted, D-thiamine deleted, E-choline deleted, F-pantothenic acid deleted, G-pyridoxine deleted, H-niacin deleted, I-riboflavin deleted, J-inositol deleted.

FIG. 19


100
50
SURVIVAL (%)
100
50
J
I
H
G
F
E
D
C
B
A
FD
VI
V
IV
III
II
I
WEEKS
FD: Final Day
DELETED WATER SOLUBLE VITAMINS
IN THE DIET
S
S: VITAMINS SUPPLEMENTED DIET

fed on diets in which individual vitamins were deleted. The highest survival rate (70%) was obtained by feeding the control diet, which had all the essential vitamins and the lowest survival (50%) was recorded in prawn groups fed with the thiamine deficient diet, after 45 days of experimentation. The survival rates obtained by feeding diets deficient in all the water soluble vitamins, pantothenic acid and niacin were not significantly different from each other (53.3%). There were also no significant differences among the survival rates of prawns fed on diets deficient in ascorbic acid, pyridoxine, riboflavin and inositol. However, prawns fed with the choline deficient diet showed relatively higher survival rate (66.7%) than most other treatment groups.

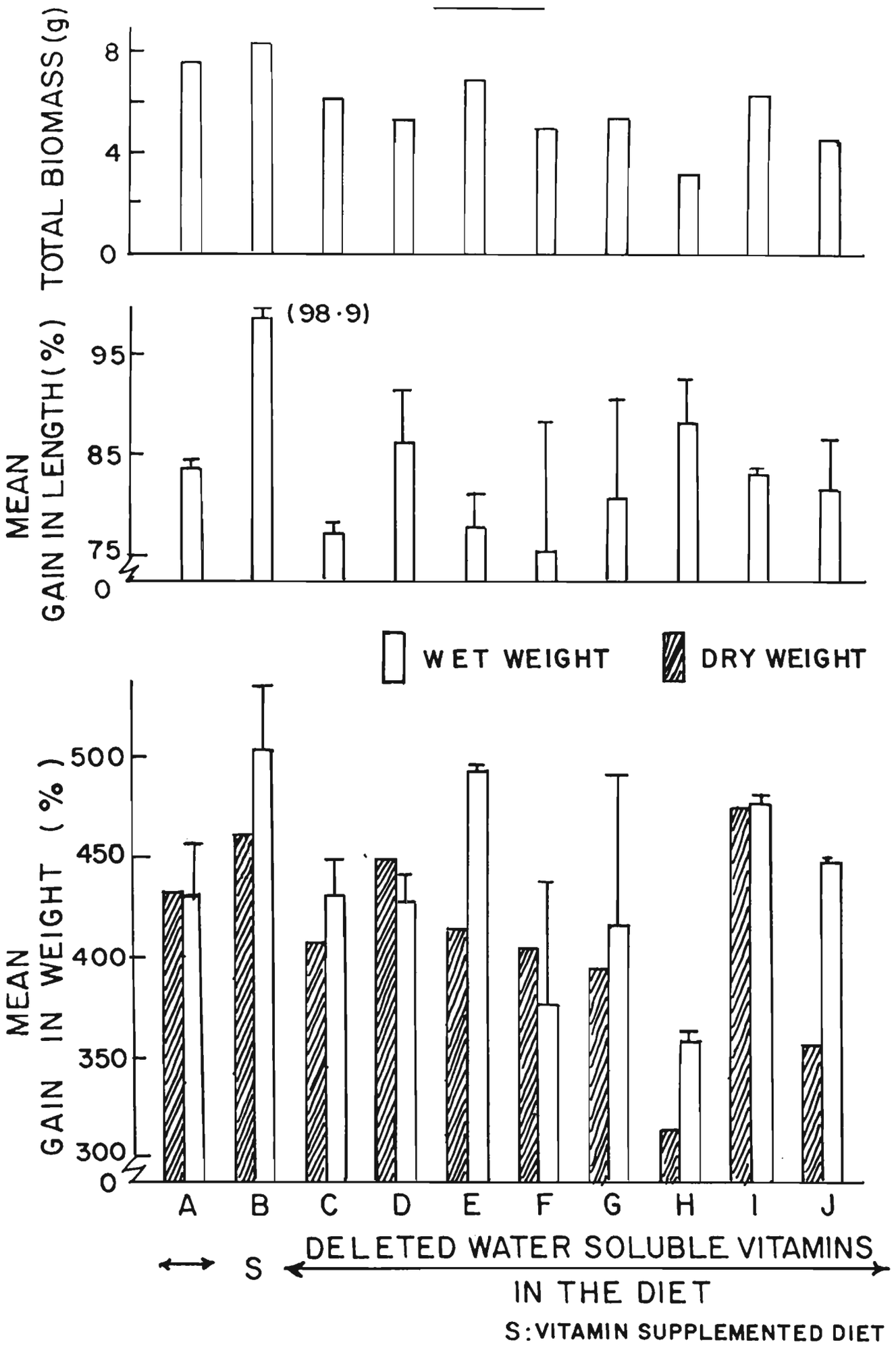
Data on weekly survival rates (Fig. 19) showed relatively higher mortality rates from third week onwards in prawns groups fed diets deficient in all vitamins, niacin and riboflavin. However, prawn groups fed on diets deficient in thiamine, pantothenic acid, pyridoxine and inositol, showed low survival rates only from the fourth week onwards. The mortality in other treatments groups of prawns was however, gradual.

Growth:

Analysis of the data obtained from the experiment showed that the diets (control as well as test diets) had significant effect ($P < 0.05$) on the mean percent gain in length

Fig. 20. Percent gain in length and weight, and total biomass (g) of prawns fed diets with and without water soluble vitamins. A-no vitamins, B-all vitamins, C-ascorbic acid deleted, D-thiamine deleted, E-choline deleted, F-pantothenic acid deleted, G-pyridoxine deleted, H-niacin deleted, I-riboflavin deleted, J-inositol deleted.

FIG. 20


TOTAL BIOMASS (g)
8
4
0
MEAN GAIN IN LENGTH (%)
(98·9)
95
85
75
0
WET WEIGHT
DRY WEIGHT
MEAN GAIN IN WEIGHT (%)
500
450
400
350
300
0
A
B
C
D
E
F
G
H
I
J
S
DELETED WATER SOLUBLE VITAMINS IN THE DIET
S: VITAMIN SUPPLEMENTED DIET

(Fig. 20). The control diet gave significantly higher percent gain in length (98.9%) than all other diets. However, there were no significant differences in the mean percent gains in length among prawns fed on diets deficient in all the essential vitamins (84.1%), thiamine (86%), pyridoxine (80.7%), niacin (88.2%), riboflavin (83%) and inositol (81.9%) and these results were significantly higher than that obtained for prawns fed with pantothenic acid, ascorbic acid and choline deficient diets (75-78%).

The mean percent gain in wet weight of prawns (Fig. 20) obtained from various treatments showed some deviation from that of percent gain in length. However, the deletion or supplementation of vitamin also had significant ($P < 0.05$) influence on the mean percent gain in wet weight of prawns. The prawns fed with the control diet, containing all the essential vitamins, recorded significantly ($P < 0.05$) higher percent gain in wet weight than prawns from all other groups, fed with vitamin deficient diets. While the niacin deficient diet gave better mean percent gain in length of prawns, the pantothenic acid deficient diet gave relatively better mean percent wet weight gain. Among other treatment groups relatively, higher mean percent gain in wet weight was obtained in prawns fed with diets deficient in choline (492.5%), riboflavin (476.5%) and inositol (447.8%). There were also no significant differences in the mean percent wet weight gain of prawns among diets deficient in all the essential

vitamins (433.3%), ascorbic acid (430.3%), thiamine (428.3%) and pyridoxine (416.8%). Although prawns fed with choline and ascorbic acid deficient diets had almost same mean percent gain in length, there were significant differences between them in the wet weight gains.

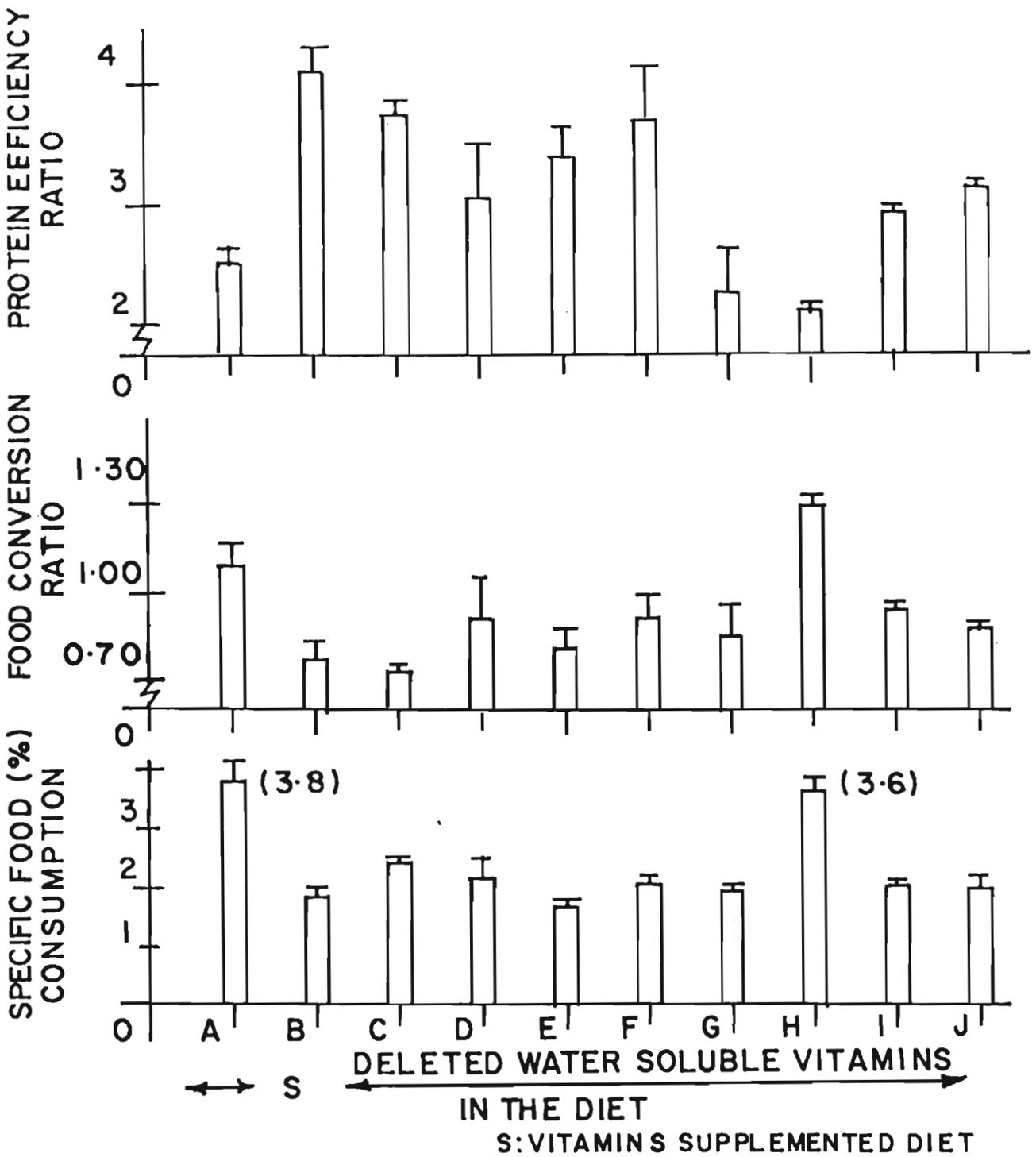
The mean percent gain in dry weight of prawns (Fig. 20) was also observed to be significantly ($P < 0.05$) influenced by the diets. While prawns fed with the control diet and riboflavin deficient diet had significantly higher dry weight gains, those fed on diets deficient in niacin and inositol had significantly ($P < 0.05$) lower mean percent gain. The highest percent gain in dry weight was observed in prawns fed on the riboflavin deficient diet (475.6%) which was followed by those fed on the control diet (461.4%). The lowest mean percent dry weight gain was observed in prawns fed with the niacin deficient diet (313.9%). However, no significant differences in percent gain in dry weight of prawns was observed between treatment groups fed on diets deficient in all the vitamins (446.9%), thiamine (448.9%) and choline (414.7%). There were also considerable differences between the percent gains in wet weight and dry weight of prawns fed on diets deficient in riboflavin, inositol, choline and thiamine, indicating the significant influence of these diets on the dry matter content of prawns.

Specific Food Consumption (SFC):

Specific food consumption in prawns (Fig. 21) was significantly ($P < 0.05$) influenced by the diets. Prawns fed

Fig. 21. SFC, FCR and PER for diets with and without watersoluble vitamins. A-no vitamins, B-all vitamins, C-ascorbic acid deleted, D-thiamine deleted, E-choline deleted, F-pantothenic acid deleted, G-pyridoxine deleted, H-niacin deleted, I-riboflavin deleted, J-inositol deleted.

FIG. 21


PROTEIN EEFICIENCY RATIO
4
3
2
0
FOOD CONVERSION RATIO
1·30
1·00
0·70
0
SPECIFIC FOOD (%) CONSUMPTION
(3·8)
(3·6)
3
2
1
0
A
B
C
D
E
F
G
H
I
J
S
DELETED WATER SOLUBLE VITAMINS
IN THE DIET
S: VITAMINS SUPPLEMENTED DIET

diets deficient in all the vitamins, ascorbic acid and niacin showed significant differences ($P < 0.05$) in SFC with that of other dietary treatment groups. The highest SFC was observed in prawn groups fed on the diet deficient in all the vitamins (3.84%), which was closely followed by the prawn groups fed on the niacin deficient diet (3.61%). On the other hand, lowest SFC was recorded in prawn groups fed on the choline deficient diet (1.64%), which was followed by prawns fed with the control diet containing all the essential vitamins (1.86%).

Food Conversion Ratio (FCR):

Food conversion ratio recorded from various treatments are shown in Fig. 21. Analysis of variance of the data gave a 'F' value which was significant ($P < 0.05$) indicating that the diets fed to the prawns significantly affect FCR. Prawn groups fed on the niacin deficient diet gave the highest FCR (1.29), which was followed by those fed on the diet deficient in all the vitamins (1.09). The FCRs recorded from the above two groups were significantly ($P < 0.05$) higher than that recorded in other groups. The FCRs recorded for prawn groups fed on the ascorbic acid deficient diet (0.72) and the diet containing all the vitamins (0.77) were significantly ($P < 0.05$) lower than that of other groups. However, no significant treatment differences could be observed in the FCRs between prawn groups fed on diets deficient in thiamine (0.91), choline (0.81), pantothenic acid (0.92), pyridoxine (0.85), riboflavin (0.93) and inositol (0.87).

Protein Efficiency Ratio (PER):

Protein efficiency ratios (Fig. 21) were significantly ($P < 0.05$) influenced by the diets. In almost all treatment groups, the PER values were observed to show an inverse trend with that of the FCR values. Prawns fed on the diet containing all the vitamins recorded the highest PER value (4.12) which was followed by prawns fed on the ascorbic acid (3.78) and pantothenic acid (3.72) deficient diets; and these PER values were significantly ($P < 0.05$) higher than that recorded from other treatment groups. Similarly, prawns fed on the niacin deficient diet (2.13) recorded the lowest PER. However, there were no significant differences in the PER recorded among prawn groups fed on diets deficient in thiamine (3.09), choline (3.13), riboflavin (2.97) and inositol (3.19).

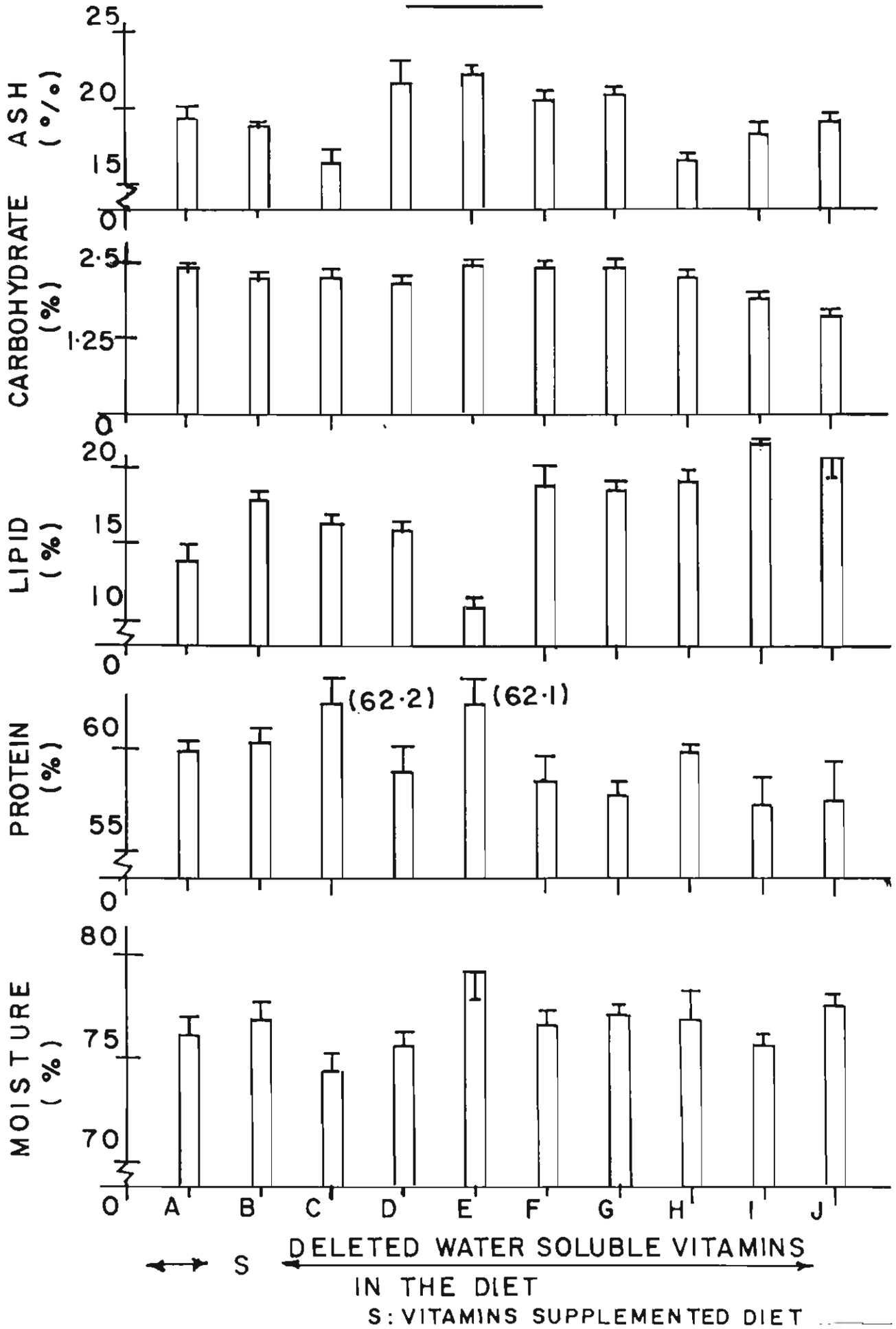
Biochemical Composition:

The data on moisture, ash, protein, lipid and carbohydrate contents of prawns from various treatments are shown in Fig. 22. Analysis of variance of the data showed that the diets had significant ($P < 0.05$) influence on the moisture and carbohydrate contents and highly significant ($P < 0.01$) effect on the protein, lipid and ash contents.

The prawns fed on the choline deficient diet had the highest moisture content (79.2%) and showed significant ($P < 0.05$) differences with most other treatments. The lowest moisture

Fig. 22. Biochemical composition of prawns fed diets with or without water-soluble vitamins
A-no vitamins, B-all vitamins, C-ascorbic acid deleted, D-thiamine deleted, E-choline deleted, F-pantothenic acid deleted, G-pyridoxine deleted, H-niacin deleted, I-riboflavin deleted, J-inositol deleted.

FIG. 22


ASH (%)
25
20
15
0
CARBOHYDRATE (%)
2·5
1·25
0
LIPID (%)
20
15
10
0
PROTEIN (%)
(62·2)
(62·1)
60
55
0
MOISTURE (%)
80
75
70
0
A
B
C
D
E
F
G
H
I
J
S
DELETED WATER SOLUBLE VITAMINS
IN THE DIET
S: VITAMINS SUPPLEMENTED DIET

content was recorded in prawns fed with the ascorbic acid deficient diet (74.4%). Among the treatment groups, prawns fed on diets deficient in thiamine (21.5%) and choline (22.3%) had significantly ($P < 0.05$) higher ash contents. Similarly, the ash contents of prawn fed on diets deficient in ascorbic acid (16.3%) and niacin (16.5%) were significantly lower than all other groups of prawns.

The protein content in prawns fed on diets deficient in ascorbic acid (62%) and choline (62%) were significantly ($P < 0.05$) higher than that of all other groups. However, there were no significant differences in the protein content among groups of prawns fed diets deficient in pyridoxine, riboflavin, inositol, thiamine and pantothenic acid. Relatively higher protein content was recorded in prawns fed on diets deficient in all the essential vitamins (59%), niacin (59%) and the control diet(60%).

The total lipid content of prawns was also significantly ($P < 0.01$) influenced by the dietary treatments. The lipid contents of prawns fed on diets deficient in riboflavin which had the highest total lipid content (22.2%) and inositol(20.4%) were significantly ($P < 0.05$) higher than that of other groups. The lowest lipid content was recorded in prawns fed with the choline deficient diet (10.1%), which was followed by those fed on diet deficient in all the vitamins (13.7%). In most of the other treatment groups of prawns, the lipid content varied insignificantly.

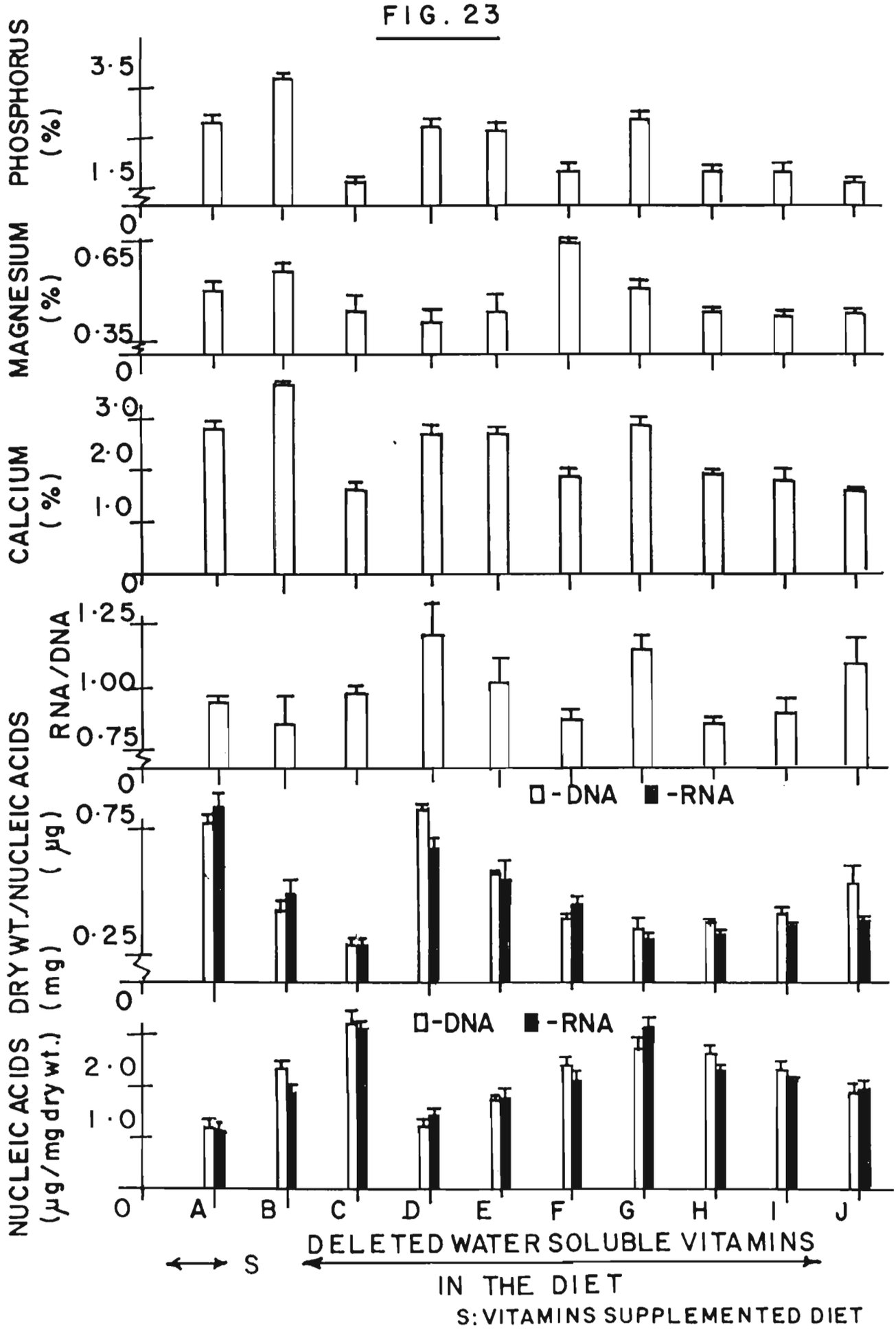
The carbohydrate content of prawns (Fig. 22), from various dietary treatments ranged from 1.61 to 2.41, and prawns fed diets deficient in choline (2.41%), pantothenic acid (2.39%), pyridoxine (2.39%) and control diet (2.35%) had significantly higher carbohydrate contents than the other groups. The lowest carbohydrate content was recorded in prawns fed on inositol deficient diet (1.61%), followed by those fed on the diet deficient in riboflavin (1.19%), and these values were significantly lower than that obtained for all other groups.

The RNA content and dry weight/RNA ratio of prawns were also significantly ($P < 0.01$) influenced by the experimental diets (Fig. 23). The prawns fed with diets deficient in all the vitamins, ascorbic acid and pyridoxine showed significant ($P < 0.05$) differences in the RNA content with all other treatment groups. The highest RNA content was recorded in prawns fed on diets deficient in ascorbic acid (3.20 µg/mg) and pyridoxine (3.21 µg/mg) and the lowest in prawns fed on the diet deficient in all the vitamins (1.19 µg/mg). There were no significant differences between RNA contents of prawns fed diets deficient in pantothenic acid (2.17 µg/mg), niacin (2.33 µg/mg), riboflavin (2.12 µg/mg) and inositol (2.02 µg/mg).

The significant ($P < 0.05$) influence of the diets was also evident from the data on dry weight/total RNA ratio (Fig. 23). While the prawns fed on the diet deficient in all the vitamins had significantly higher dry weight/RNA ratio (0.84), those

Fig. 23. Biochemical composition of prawns fed diets with or without water-soluble vitamins
A-no vitamins, B-all vitamins, C-ascorbic acid deleted, D-thiamine deleted, E-choline deleted, F-pantothenic acid deleted, G-pyridoxine deleted, H-niacin deleted, I-riboflavin deleted, J-inositol deleted.

FIG. 23


PHOSPHORUS (%)
3·5
1·5
0
MAGNESIUM (%)
0·65
0·35
0
CALCIUM (%)
3·0
2·0
1·0
0
RNA/DNA
1·25
1·00
0·75
0
□-DNA ■-RNA
0·75
0·25
0
□-DNA ■-RNA
2·0
1·0
0
A
B
C
D
E
F
G
H
I
J
S
DELETED WATER SOLUBLE VITAMINS
IN THE DIET
S: VITAMINS SUPPLEMENTED DIET
NUCLEIC ACIDS (μg/mg dry wt.)
DRY WT./NUCLEIC ACIDS (mg) (μg)

fed on diets deficient in ascorbic acid and pyridoxine had significantly lower ratio (0.32), compared to prawns from other treatments. However, there were no significant differences between the dry weight/total RNA ratios recorded in the various groups of prawns fed on diets deficient in choline (0.56), pantothenic acid (0.46), niacin (0.43), riboflavin (0.47), inositol (0.49) and the control diet (0.51).

The DNA contents of prawns showed similar trend (Fig. 23) as that of RNA content and was significantly ($P < 0.05$) influenced by the diets. There were also significant ($P < 0.05$) differences between the DNA content of prawns fed on the different experimental diets. The highest DNA content was recorded in prawns fed on the choline deficient diet (3.25 µg/mg); whereas the lowest DNA content was recorded in prawns fed on the thiamine free diet (1.23 µg/mg), closely followed by the prawn groups fed on the diet deficient in all vitamins (1.28 µg/mg). However, there were no significant differences between the DNA contents of prawns fed on diets deficient in pantothenic acid (2.47 µg/mg), riboflavin (2.35 µg/mg) or control diet containing all the vitamins (2.3 µg/mg).

The dry weight/total DNA ratio obtained from the different treatment groups of prawns (Fig. 23) showed that the experimental diets significantly ($P < 0.05$) influence the dry weight/total DNA ratio in prawns. A comparison of the data on dry weight/total RNA ratio and dry weight/total DNA ratio for various treatment groups of prawns would show considerable differences in the trends in values between these two parameters. The highest dry weight/

total DNA ratio was observed in prawn groups fed on the thiamine deficient diet (0.83), followed by prawn groups fed on the diet deficient in all the vitamins (0.70). The lowest dry weight/ total DNA ratio was observed in prawns fed on the ascorbic acid deficient diet (0.31). There were no significant differences between the dry weight/DNA ratios of prawn groups fed on diets either deficient in pantothenic acid (0.41), riboflavin (0.43), choline (0.57), inositol (0.54) or the control diet (0.44).

The RNA/DNA ratios recorded for different groups of prawns (Fig. 23) also showed significant ($P < 0.01$) effect by the experimental diets. However, only prawns fed diets deficient in thiamine (1.22), pyridoxine (1.16) and inositol (1.09) had significantly ($P < 0.05$) higher RNA/DNA ratios.

The calcium, magnesium and phosphorus contents of the experimental prawns recorded from the dietary treatments are shown in Fig. 23. Analysis of variance of the data showed the highly significant ($P < 0.01$) effect of diets on the calcium content of prawns. Prawns fed on diets deficient in ascorbic acid, pantothenic acid, niacin, riboflavin and inositol had significantly ($P < 0.05$) lower calcium compared to prawns fed on other diets. The highest calcium content was recorded in prawns fed on the control diet with all vitamins (3.71%) and the lowest in prawns fed on the ascorbic acid deficient diet (1.63%).

Magnesium content of prawns (Fig. 23) was not significantly affected by the diets fed to them. However, prawns fed on diet

deficient in pantothenic acid had slightly higher magnesium content (0.65%) than those fed on the control diet (0.57%). The magnesium contents recorded in prawns from other treatment groups did not differ markedly and ranged from 0.42% to 0.52%.

Phosphorus content in prawns (Fig. 23) was also significantly ($P < 0.01$) influenced by the experimental diets fed to them. The diets deficient in pyridoxine (1.84) and choline (1.77) produced significantly ($P < 0.05$) higher phosphorus contents in prawns than other diets. Diets deficient in pantothenic acid (0.70%), riboflavin (0.74%) inositol (0.88%) and all the essential vitamins (0.82%) produced significantly ($P < 0.05$) lower phosphorus content in prawns.

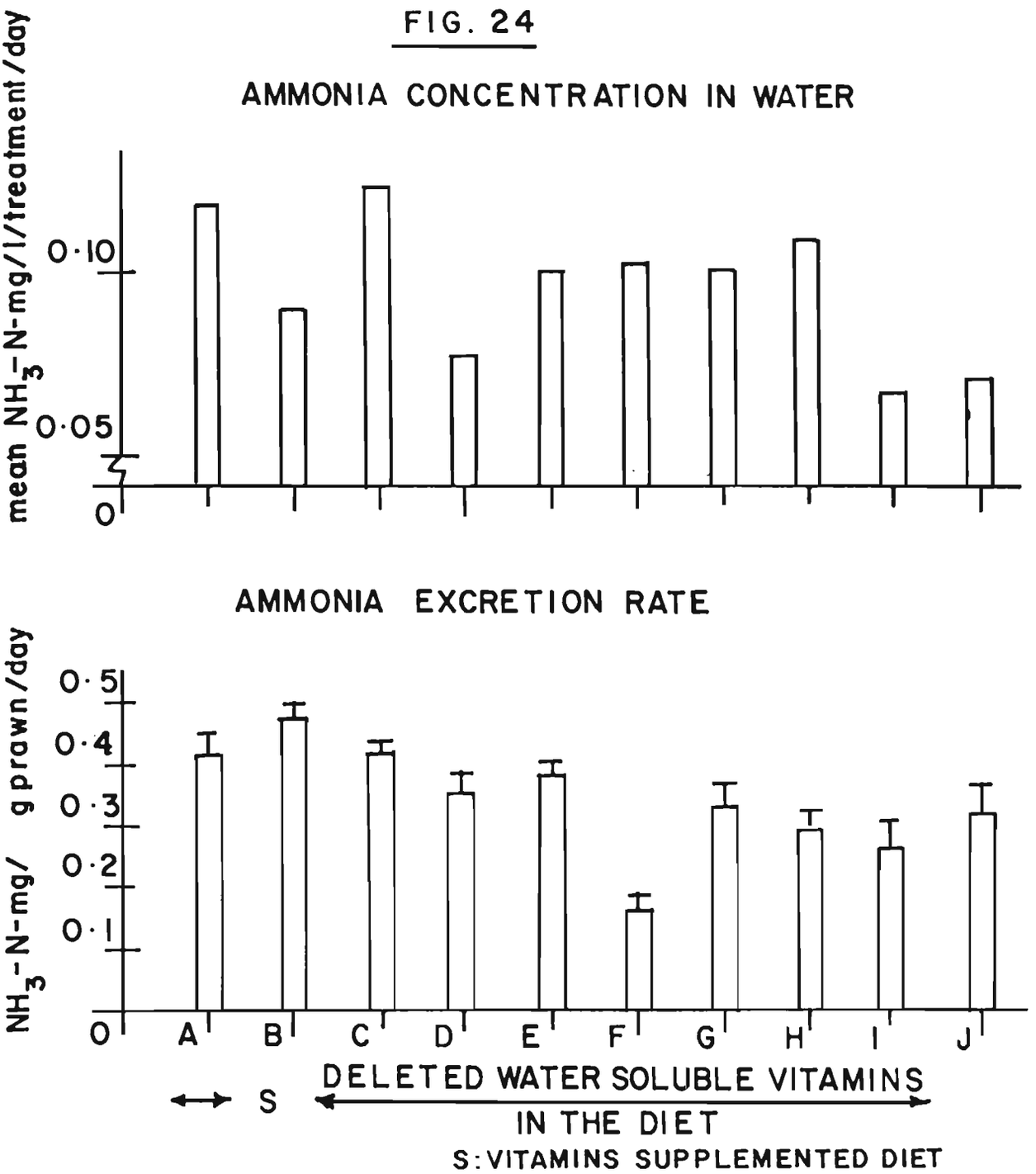
Ammonia Excretion:

With a view to studying the influence of the experimental diets on ammonia excretion by the prawns, ammonia concentration in the water of the experimental tanks was determined, twice weekly, during the 45 days of experimentation. The data were analysed and the mean ammonia concentration per day per treatment was recorded (Fig. 24).

The mean ammonia concentration in water Fig. 24 showed that the experimental diets significantly influence the ammonia excretion by the prawns, and the prawn groups fed on diets deficient in ascorbic acid, niacin, and inositol showed maximum significant ($P < 0.05$) differences in the ammonia excretion with that of other groups.

Fig. 24. Ammonia concentration in seawater and ammonia excretion rate in prawns fed diets with or without watersoluble vitamins. A-no vitamins, B-all vitamins, C-ascorbic acid deleted, D-thiamine deleted, E-choline deleted, F-pantothenic acid deleted, G-pyridoxine deleted, H-niacin deleted, I-riboflavin deleted, J-inositol deleted.

FIG. 24


AMMONIA CONCENTRATION IN WATER
mean NH_3-N-mg/l/treatment/day
0·10
0·05
0
AMMONIA EXCRETION RATE
NH_3-N-mg/ g prawn/day
0·5
0·4
0·3
0·2
0·1
0
A
B
C
D
E
F
G
H
I
J
S
DELETED WATER SOLUBLE VITAMINS
IN THE DIET
S: VITAMINS SUPPLEMENTED DIET

Ammonia Excretion in Prawns Subjected to the Experimental Diets:

Ammonia excretion rates in intermolt stage prawns, from each experimental treatment, were monitored for a period of 24 hrs, after the 45 days of experimentation. The ammonia excretion rates expressed as mg NH_4-N/g prawn/day recorded from different treatments are shown in Fig. 24. Analysis of variance of the data showed the highly significant ($P < 0.01$) effect of experimental diets on ammonia excretion rates. Least significance difference test showed that prawn groups fed on diets deficient in pantothenic acid excrete significantly less ammonia (0.17 mg/g/day) compared to all other groups. Similarly, the ammonia excretion rates of prawn groups fed on the control diet (0.48 mg/g/day), and those fed on diets deficient in all the essential vitamins (0.42 mg/g/day) and ascorbic acid (0.41 mg/g/day) were significantly higher than all other experimental groups of prawns. There were no significant differences in ammonia excretion rates among prawn groups fed on diets deficient in thiamine (0.35 mg/g/day), pyridoxine (0.33 mg/g/day), niacin (0.29 mg/g/day), inositol (0.32 mg/g/day) and riboflavin (0.26 mg/g/day). However, the ammonia excretion rate was significantly ($P < 0.05$) higher in prawn groups fed on the choline deficient diet (0.39 mg/g/day) compared to the above groups.

O B S E R V A T I O N S

Prawn groups fed on the vitamin deficient diet for 45 days were observed to show some differences in molting activity, response to light and external morphology.

Molting:

There were differences in the number of exuviae collected and post-molt deaths encountered from the various treatment groups during the experimental study (Table 13). Relatively more number of exuviae (25 Nos) were collected from the treatments, where prawns were fed with the control diet, compared to all other groups. The number of exuviae collected from treatments with diets deficient in choline (20 Nos), pantothenic acid (19 Nos) and pyridoxine (18 Nos) were also relatively higher than that recorded in most of the other treatment groups. The exuviae recovered from most treatments were almost complete, though in some cases they were half-eaten by the cohabiting individuals.

Prawn groups fed on diets deficient in all the vitamins, niacin, inositol and riboflavin showed relatively less number of post-molt deaths compared to those fed with diets deficient in ascorbic acid and thiamine. However, prawn groups fed on diets deficient in thiamine pantothenic acid and pyridoxine showed maximum post-molt deaths compared to all other treatment groups.

TABLE 13: OBSERVATIONS IN PRAWNS FED WITH DIFFERENT EXPERIMENTAL DIETS

Dietary treatments	Mean nos. of molts recovered	Mean nos of post-molt deaths	Texture of the body
Diets deficient in all vitamins	11	3	SO
Control diet with all vitamins	25	3	H
Ascorbic acid deficient	13	4	SO
Choline deficient	20	3	H
Thiamine deficient	12	6	H
Pantothenic acid deficient	19	5	SO
Pyridoxine deficient	18	4	SO
Niacin deficient	11	3	H
Riboflavin deficient	10	3	H
Inositol deficient	10	3	H

H-hard, So-soft.

Food Intake:

Food intake by prawns in various treatments did not differ markedly from each other during the first 15 days of experimental study. However, variations in food intake between treatment groups became apparent from the third week onwards. Prawns fed on diets deficient in all the vitamins, choline, niacin and inositol ingested relatively less food with increase in experimental duration. The prawns were observed to move away from the food, in the above treatments probably due to lack of appetite or aversion towards the food. Prawns also did not quickly respond to the feed on introduction into the tank. Similar type of symptoms were observed after four weeks of experiment in prawns fed on diets deficient in ascorbic acid and pantothenic acid. In all other treatment groups normal food intake was observed.

Behaviour Towards Light:

When a sudden flash of light from a table lamp (1625×10^2 lux) was directed into the experimental tanks, variations in the response of prawns were observed between different treatments. During the first two weeks, there were no observable differences in the response of the prawns from various treatments, however, prawns started showing variation in response to light from the third week onwards. The prawns fed on diets deficient in ascorbic acid, pyridoxine, riboflavin and choline showed aggresive and irregular movements, till the light source was removed. Comparatively, passive response was observed in the case of prawns fed on diets deficient in all vitamins, thiamine,

pantothenic acid, niacin and inositol. No significant responses could be initiated even on disturbing the water column or on hitting the side of the experimental tanks. But, the prawns fed on the control diet containing all vitamins, showed normal light evading responses, which lasted for few seconds.

External Morphology:

Differences were also observed between treatments in the distribution and density of certain brownish-black spots on the abdominal region and rostrum of prawns. Prawns fed with the diet deficient in all the vitamins and those fed on diets deficient in ascorbic acid, choline, thiamine, pyridoxine, riboflavin and inositol had spots on the abdomen, rostrum and in certain cases on gills as shown in Table 13.

D I S C U S S I O N

Vitamins as non-energy micronutrients have prominent role in metabolism and significantly influence the growth and survival of prawns. From the present study, it is evident that omission of all vitamins (fat and water-soluble) or individual water-soluble vitamins such as ascorbic acid, thiamine, pantothenic acid, pyridoxine, niacin, riboflavin or inositol from the diet of prawns results in low survival rates. However, deficiency of choline in the diet does not affect the survival significantly.

Amongst treatment, diets deficient in all vitamins, thiamine and niacin resulted in relatively poor survival. This indicates that thiamine and niacin constitute very important indispensable vitamins for prawn diets. Comparatively, the higher survival rates recorded in other treatments fed on diets deficient in any of the other water-soluble vitamins, suggest that may be the body stores of these vitamins are slowly exhausted (Mitchell, 1964; Halver, 1972).

On the other hand, the low mortality rates occurred in prawns fed on the diet deficient in choline, indicates that possibly deletion of choline from diets may not have significant effect when lecithin is included (vide Chapter V). Lecithin, which contains the nitrogenous base choline, has been reported to improve survival in crustaceans (Conklin et al., 1980; D'Abramo and Baum, 1981; Kanazawa, 1983) and probably, crustaceans are able to subsist on choline derived from this phospholipid (phosphatidyl choline), resulting in higher survival (Conklin et al., 1980; D'Abramo and Baum, 1981). On the other hand, the highest survival in prawns fed on the control diet indicates that all the essential vitamins are required to maintain the metabolic balance in prawns, without causing any stress. In addition to the influence of the other vitamins, lecithin, choline and inositol in the diets must have contributed for the higher survival rate in the control, as they have been shown to improve survival in P. japonicus (Kanazawa, 1983), Homarus americanus (Conklin et al., 1980; D'Abramo et al., 1981) and

Moina (D'Abramo and Baum, 1981). Thus, dietary supplementation of vitamins form an essential aspect in purified diets, when fed to the prawns (New, 1976a; Ponat and Adelung, 1980).

The present findings in P. indicus do not support the observations of many of the earlier workers who found that the deletion of thiamine (Deshimaru and Kuroki, 1979; Heinen, 1984) choline (Deshimaru and Kuroki, 1979) pyridoxine, niacin and riboflavin (Heinen, 1984) from diets do not significantly affect the survival rates in P. japonicus or Macrobrachium rosenbergii. However, the present study shows that deletion of any of these vitamins from the diet results in lower survival rate than the control diet containing all the essential vitamins. The present findings agrees with the observations of Guary et al.(1976) and Kanazawa et al.(1976)in P. japonicus; D'Abramo and Baum(1981) in Moina; D'Abramo et al.(1981)in H. americanus and Ponat and Adelung (1980)in Cancer pagurus where in the deficiency of these vitamins have been shown to cause high mortality.

The growth of prawns fed on the control diet with all the vitamins was significantly higher than prawns from most other treatments. Although, gain in wet weight of prawns fed with the control diet did not differ significantly from that of prawns fed with choline or riboflavin deficient diets, on dry weight basis, prawns fed with the choline and riboflavin deficient diets showed improved growth compared to those fed on the control diet. Probably, the dietary concentration of riboflavin in the control diet may be in excess and thereby acted

detrimental to the prawns, as reported for juvenile M. rosenbergii also (Heinen, 1984).

It was also observed that growth of prawns fed with thiamine and niacin deleted diets were significantly affected, suggesting that these prawns are unable to grow normally on deletion of the vitamins from the diet. On the other hand, prawns fed with ascorbic acid, pantothenic acid and pyridoxine deficient diets showed insignificant variations between them in dry weight but showed variation in percent gain in length and wet weight, indicating that probably the prawns are able to subsists on body reserves unlike thiamine and niacin deficient diet fed prawns.

Prawns fed with inositol deficient diet showed higher growth on percent gain in length and wet weight,but poor growth on dry weight basis, compared to most other treatment groups fed prawns. The variation in wet weight and dry weight observed in prawns, is primarily due to the difference in moisture content which reflects on the deposition of organic and inorganic nutrients in tissues. The significantly higher growth recorded in prawns fed on diets deficient in all vitamins cannot be considered as proportionately equivalent to the growth obtained in prawns from other treatments, as the number of surviving animals was extremely low due to cannibalism, molt eating behaviour and devouring of dead prawns, which could have been the reason for higher growth recorded from the surviving prawns.

The study also shows that the prawns show a requirement for all the vitamins, though choline and riboflavin appears to be partially dispensable. Of course, long periods of deletion of these vitamins may not sustain the positive growth and may result in dietary deficiency symptoms. Amongst the vitamins -thiamine, niacin and pantothenic acid seems to be the most important ones as growth and survival studies indicate. However, the present findings differ markedly from that of Heinen (1984) in the survival rates, especially for thiamine, niacin and pantothenic acid deficient diets; who reported higher survival rate in juvenile M. rosenbergii. These variations may be due to the experimental procedures adopted in either case or may be due to the differences in the requirement of vitamins by penaeid and non-penaeid prawns.

Observations on specific food consumption (SFC), food conversion ratio (FCR), protein efficiency ratio (PER), carcass composition, ammonia excretion rates and some of the observations made during the experimental study, further reveal the influence of the vitamins on the prawns.

Prawns in various treatments seem to show distinct variations in food intake and conversion for body growth. In the case of prawns fed on diets deficient in all vitamins and niacin, poor food consumption, poor food conversion and protein conversion were evident. On the other hand, in prawns fed with the thiamine deficient diet, though the survival was low, the values obtained for SFC, FCR and PER were not significantly different from those fed with other vitamin deficient diets.

However, the growth in thiamine deficient diet fed prawns was same as the other vitamin deleted diet fed prawns, which signifies that thiamine does not significantly affect the food intake or conversion as that of prawns fed with niacin deficient diet.

The prawns fed with ascorbic acid, pantothenic acid, pyridoxine and inositol deficient diets showed insignificant variation in the SFC and FCR values but showed significant variation in PER. The PER values obtained for ascorbic acid and pantothenic acid deficient diets were higher than that of inositol and pyridoxine deficient diets. This suggests, that possibly, ascorbic acid and pantothenic acid may not have direct influence on the protein utilization compared to pyridoxine or inositol. The SFC, FCR and PER values obtained for ascorbic acid and pantothenic acid deficient diets did not vary significantly from that of prawns fed with the control diet, even though they significantly varied in growth. So the influence of these vitamins may not be on the food consumption and utilization but may be on other metabolic processes that influence growth and survival.

Further, the effects of deletion of various vitamins from diets can be substantiated from the body chemical composition and ammonia excretion rates of prawns. The moisture content in prawns does not seem to be much influenced by the deletion of vitamins from diets, excepting for those prawns fed on diets deficient in ascorbic acid, thiamine, choline and

riboflavin. The higher moisture content in the case of prawns fed on the choline deficient diet could be as a result of lowest lipid content. On the other hand, ascorbic acid deficient diet fed prawns did not differ significantly in the different chemical constituents with that of other treatment group fed prawns, even though, the moisture content recorded was slightly less than the others.

Low lipid levels and higher moisture content in prawns fed on the choline deficient diet, suggests that under this vitamin deficiency prawns were actively utilizing lipids as a source of metabolizable energy, sparing protein for growth. On the other hand, addition of choline in the diet (control diet) resulted in comparatively higher lipid content than the choline deficient diet which may be due to the synergistic effect of the other vitamins present in the diet. However, the high ash content in prawns fed on the choline deficient diet, suggests that possibly some changes might have resulted in the composition of biomembrane phospholipids, as they form an integral part (Clude, 1949). The high ash content in the choline deficient diet fed prawns is also reflected in the high phosphorus and calcium values, which suggests that the dietary uptake of these inorganic constituents may be influenced by choline deficiency.

In most of the treatment groups, excepting in the choline deficient and to some extent in ascorbic acid deficient treatments the protein content of prawns seems to be unaffected by the test diets. However, higher protein content was recorded in prawns fed with choline deficient and ascorbic acid deficient

diets. On the other hand, lipid and ash were observed to show significant differences between treatment groups. The lipid content was relatively high especially in prawns fed with niacin, riboflavin and inositol deficient diets. In these treatment groups of prawns, the ash and protein contents were relatively less which suggests that may be lipid were not influenced by the deletion of these vitamins from the diet and were not catabolised efficiently for metabolizable energy purpose. The poor protein deposition and high lipid deposition, however, are reflected in the poor growth and survival in the case of prawn groups fed on niacin or inositol deficient diets.

However, prawns fed on riboflavin deficient diet showed high growth rate, even when low protein and high lipid contents were recorded, compared to the prawns fed on riboflavin supplemented control diet. Presumably, growth may not be enhanced by riboflavin deficiency, but it is suspected that the concentration of riboflavin used in the control diet may be too high to induce reduced growth as reported in M. rosenbergii (Heinen, 1984).

Pantothenic acid and pyridoxine deficient diet fed prawns showed characteristically high lipid and ash, but lower protein contents. The protein content was higher but lipid and ash contents were lower in prawns fed on the control diet compared to the former two treatment groups. These results indicate that deletion of vitamins from diets results in increased rate of catabolization of proteins than lipids and thus poor growth and survival results, compared to prawns fed on the control diet with all the vitamins.

From the data on growth, survival and chemical composition of prawns, it appears that when all vitamins or individual vitamins are deleted from diets, they affect the metabolism resulting in imbalances in growth and chemical composition. Amongst the energy rich nutrients, the metabolism of proteins and lipids seems to be affected most, leading to faster catabolization of proteins and storage of lipids.

The nucleic acids content in prawns fed on the diet deficient in all vitamins was lower than those fed with other diets. This indicates that probably vitamins deletion from diets results in inhibition of nucleic acid synthesis. Ascorbic acid and pantothenic acid are important vitamins and deficiency of these are reported to inhibit RNA synthesis (Mitchell, 1964; Levin, 1976). The low content of nucleic acids accompanied by high ammonia excretion rates in prawns fed on the all vitamin deficient diet, clearly shows that deficiency of vitamins will significantly affect protein synthesis, growth and survival.

The riboflavin deficient diet fed prawns had slightly higher nucleic acid concentration than the prawns fed with the control diet. However, they did not show any significant variation in growth indicating that proteins were less catabolized and used for growth under riboflavin deficiency. However, Heinen (1984) observes that in M. rosenbergii, riboflavin deficient diet fed prawns show higher growth than those fed on

a diet containing riboflavin. The relatively poor growth in prawns fed with riboflavin may be due to hypervitaminosis and the same explanation may also hold true for the present study.

On the other hand, prawns fed on diets deficient in pantothenic acid, niacin, riboflavin and inositol did not show any significant variation in nucleic acid contents, but showed variation in the ammonia excretion rates. Even though, the nucleic acids contents was almost same, the ammonia excretion rates in pantothenic acid deficient diet fed prawns was the lowest among the treatment groups, indicating that possibly protein catabolism is greatly reduced under pantothenic acid defifiency, probably due to low concentration of coenzyme A (Mitchell, 1964), which is an essential coenzyme during metabolic reactions and of which pantothenic acid forms an integral part. But on the other hand, in the case of niacin deficient diet fed prawns, high ammonia excretion with poor protein deposition, growth and survival, indicate that proteins were utilized more efficiently for energy purpose than for growth.

However, the concentration of nucleic acids was highest in prawns fed with ascorbic acid and pyridoxine deficient diets amongst all the treatments, indicating that proteins are synthesized efficiently even in the absence of these vitamins. In the case of prawns fed on ascorbic acid deficient diet, the ammonia excretion rates were as high as in prawns fed on the diet deficient in all vitamins. Also, the protein content of these prawns was also high, compared to all other treatments

excepting choline fed prawns. However, the growth and survival was not efficient as that of prawns fed with all vitamins or choline or riboflavin deficient diets. This suggests that though proteins are synthesized, (probably a certain amount is catabolized, as evidenced by the high ammonia excretion rates, and hence poor growth results. The high ammonia excretion rates may also be as a result of dietary stress on physiological processes occurring in these prawns.

On the other hand, the nucleic acids content indicated efficient synthesis of proteins by the prawns fed on the pyridoxine deficient diet, but the ammonia excretion rate indicate, increased catabolism of proteins, resulting in lower protein deposition and poor growth, unlike in ascorbic acid deficient diet fed prawns where protein deposition was higher, eventhough growth was almost same as that recorded in pyridoxine deficient diet fed prawn

The various experimental diets also influenced the general activity, molting and feed intake in prawns. In most cases of vitamin deficiency, excepting choline, the post-molt deaths were primarily due to the treatment effects, as these prawns, prior to death, tend to show hypo or hyper activity, anorexia and aversion towards feed. This was more pronounced in treatment groups fed on diets deficient in all the vitamins, thiamine and niacin, wherein hyper-activity of prawns was evident, accompanied by reduced food intake from the third week onwards. In other treatments, prawns s owed such symptoms

from the subsequent weeks only. So these observations indicate that prawns require vitamins for general maintenance and growth in concentrations optimal for each stage of prawns. However, excessive amounts of vitamins, like riboflavin, may have detrimental effect on the growth and survival of prawns as evidenced from the present study and that on M. rosenbergii (Heinen, 1984).

Thus, deletion of vitamins from the diet of prawns causes poor food intake, aversion towards feed, hypo activity followed by poor molting, resulting in poor growth and high mortality rates. Some of the deficiency symptoms observed from the various treatments are summarised in Table 36. In the subsequent chapters (IV to IX), the results of the experiments to determine the dietary requirement of some of the most important water soluble vitamins, using graded concentration of the selected vitamins have been presented and the possible physiological changes exhibited by the prawns when fed below or above their requirements are discussed.

C O N C L U S I O N

The present findings clearly show that juvenile P. indicus have dietary requirement for most of the water soluble vitamins. The prawns grow more efficiently when the diets are supplemented with vitamins compared to when they are either omitted individually or collectively.

Amongst the water soluble vitamins prolonged deficiency of ascorbic acid, thiamine, niacin, pantothenic acid, pyridoxine and inositol results in poor growth and survival.

Characteristically, deficiency of choline and riboflavin does not seem to have any significant influence compared to the deficiency of other vitamins. In the case of choline, it appears that lecithin, a phospholipid containing choline, included in the diet, may supply choline when not available from the diet (D'Abramo and Baum, 1981; D'Abramo et al., 1981; Kanazawa, 1983). So the choline omission may be off-setted by supplementing lecithin in the diet.

Deficiency of riboflavin in the diet of prawns did not affect growth, but improved growth, compared to prawns fed with riboflavin in the control diet. The relatively poor growth obtained with the control having riboflavin may be due to hypervitaminosis as a result of using high concentration of riboflavin in the control diet. However, it does not mean that complete deletion of riboflavin will be useful, since the survival was markedly lower than the control diet containing riboflavin fed prawns. So probably, lower concentrations of riboflavin supplementation is necessary in diets for efficient growth and survival of juvenile prawns but at higher concentrations, the prawns may not have the ability to get rid of excess vitamin (Heinen, 1984). Thus, the present study clearly shows that all the tested vitamins are essential in the diet of prawns.

CHAPTER-IV

ASCORBIC ACID REQUIREMENT

INTRODUCTION

Vitamin C or ascorbic acid was reported as antiscurvy agent, administered to sailors in the form of citrus fruit (Lind, 1753) (cited, Woodruff, 1964). Szent-Györgi (1928) (cited, Woodruff, 1964) isolated this vitamin and named it 'hexauronic acid' based on its molecular structure. For the first time Reichstein et al. (1933) synthesized and in the same year Szent-Györgi and Howorth (1933) renamed hexauronic acid as ascorbic acid.

It is now well documented that this vitamin participates actively in the metabolism of all species. In all the chlorophyll containing plants active, efficient, biosynthesis of ascorbic acid has been reported (Roy and Guha 1958; Chatterjee, 1973). Comparatively, in the animal kingdom, the biosynthesis of ascorbic acid has been reported to be discontinuous in the evolutionary scale and so most species have to solely depend on dietary sources or derive from the microorganisms thriving (along with them in the environment. Thus among animals, biosynthesis of ascorbic acid is restricted to amphibians, reptiles, lower order birds, most mammals and in certain cases of higher order birds (Passeriformes) (Chatterjee et al., 1961). Certain vertebrates such as flying mammals, many primates, including man, and fishes are unable to biosynthesize ascorbic acid (Roy and Guha, 1958; Gupta et al., 1972).

Likewise, certain invertebrates (arthropods) are also unable to biosynthesise ascorbic acid (Gupta et al., 1972; Magarelli and Colvin, 1978; Magarelli et al., 1979). This inability in these specific groups of animals is attributed to the genetic failure of enzyme synthesis or lack of expression of the same (Levin, 1976).

Ascorbic acid participates in the metabolism of living organisms, whether biosynthesized by the organism or derived exogenously from the diet (Levin, 1976). An excellent review by Knox and Goswami (1961) highlights its specific roles in the intermediary metabolism. One of the major functions assigned to ascorbate was as protector of enzymes and hormones from oxidation and inhibition (Woodruff, 1964). Ascorbic acid has been reported to be involved in the functioning of neural stimuli transmitter (Bordie and Costa, 1962) and in RNA synthesis (Price, 1966). Recent studies have shown that this vitamin has 'mild detergent' action responsible for the dissolution of fat-cholesterol and cholesterol-phospholipid-Ca complexes (Levin, 1976).

Ascorbic acid and -SH radicals are also important endogenous radioprotectors (Bacq and Goutier, 1967; Pennington and Maloan, 1968) and growth regulators (Chinoy, 1969; Chinoy et al., 1971b). In crustaceans, vitamin C has also been reported to influence alkaline phosphatase activity during the synthesis of chitin and sclerotization of the epicuticle (Conklin, 1983). Ascorbic acid takes part as a reactant in a

number of defined enzyme systems such as in the hydroxylation of epinephrine and tryptophan, and in the oxidation of tyrosine (Levin, 1976).

Many workers have reported vitamin C having synergistic roles with other vitamins. Deletion of vitamin A (Kutsky, 1973) and vitamin E (Mitchell, 1964) from diets of animals, causes reduction in vitamin C levels in the plasma. Vitamin C on the other hand protects these vitamins from being oxidized (Terroine, 1953). Similarly, deficiency of folic acid and pantothenic acid and other B vitamins can be off-set by vitamin C supplementation in most cases (Terroine, 1953), and thus, the property of ascorbic acid in large dosages to act as a vicarious agent has been correctly coined by Fridericia (1926).

One of the important functions of ascorbic acid is in the collagen synthesis (Robertson and Schwartz, 1953; Stone and Meister, 1957), which is an important aspect in muscle development of all animals including arthropods (Smith and Wigglesworth, 1959; Harper et al. 1967). Stone and Meister (1957) demonstrated the necessity of vitamin C for the hydroxylation of proline and lysine to form hydroxyproline an unusual amino acid that exclusively occurs in collagen.

Deficiency of these ascorbic acid molecules in the diets of animals results in metabolic disorders leading to diseases. Deficiency symptoms could be clearly delineated on deletion of the vitamin (Briggs, 1960; Gupta et al., 1972; Reddy and Chippendale, 1972) from diet. The major symptom observed is

the malformation of collagen tissue culminating in the melanization of hemocytic lesions leading to death (Lightner et al., 1979; Lightner, 1983). In crustaceans, similar symptoms in collagen tissue, designated as "Black Death Disease" have been demonstrated in the prawns - Penaeus californiensis and P. stylirostris (Lightner, 1977; Lightner et al., 1977). Earlier, the same symptoms were reported as blackened lesions by Guary et al. (1976) and Deshimaru and Kuroki (1976b) in the Kuruma prawn, P. japonicus and by Magarelli and Colvin (1978) in P. stylirostris. The occurrence of black death disease symptoms in crustaceans, especially in shrimps is noted within 19-42 days, when ascorbic acid deficient diets were administered (Magarelli et al, 1979) and on supplementation with the vitamin, the shrimps return to normal condition (Lightner et al., 1979).

The studies of Beerstcher et al. (1954b) in Oniscus ascellus; Provasoli and D'Agostino (1969) and Provasoli and Pinter (1980) in Artemia salina; Conklin and Provasoli (1977) in Moina macrocopa; Castell et al. (1983) in H. americanus; Kitabayashi et al. (1971b), Deshimaru and Kuroki (1976b), Guary et al., (1976), Kanazawa (1983) and Lightner (1983) in Penaeus japonicus; Lightner (1983) in Penaeus azetecus; Magarelli et al. (1979) and Lightner (1983) in P. stylirostris and P. californiensis, and Heinen (1984) in Macrobrachium rosenbergii have highlighted the essentiality of vitamin C in the diet of crustaceans. However, requirements in each species varies with age, physiological state, molting cycle and abiotic

stresses, which influence the metabolic role of vitamin C in the tissues.

In P. japonicus, it was demonstrated that 0.1% or less ascorbic acid in the diet produces high mortality rate and gill disease at a temperature of 28-30°C; and these prawns developed a greyish white color on the margin of carapace, lower part of the abdomen, and tips of walking legs (Lightner, 1977). Lightner et al., (1979) reported that adequate levels of ascorbic acid was about 0.6% of the dry diet for P. californiensis and P. stylirostris, and Guary et al. (1976) found 1.0% for P. japonicus. Interestingly, Deshimaru and Kuroki (1976) studied the requirements of ascorbic acid, within the range of 0 to 1.0% in the diet and found the best growth in ascorbic acid-free diet. The above authors also reported that the feed intake decreased with increasing ascorbic acid concentration in the food. Aside from the above works, studies on the effect of ascorbic acid on the survival, molting frequency, growth, conversion efficiency and concentration of ascorbic acid in tissues of penaeid prawns were restricted to those of Guary et al. (1976) in P. japonicus and Magarelli et al. (1978) in P. californiensis and P. stylirostris. However, most authors are of the opinion that ascorbic acid forms an essential ingredient in the diet of crustaceans (New 1976a).

Despite the importance of ascorbic acid in prawns, there has not been any attempt to study and establish the essentiality

and requirements of ascorbic acid for Penaeus indicus, and therefore, the present investigation was carried out to determine the dietary ascorbic acid requirement of juveniles of P. indicus.

MATERIAL AND METHODS

Ascorbic acid requirement of juvenile prawns was studied using isonitrogenous diets containing graded levels of ascorbic acid as shown in Table 15. α-Cellulose was used to adjust the total concentration of vitamin mixture in all the diets. The experimental set up, rearing of animals and monitoring and maintenance of environmental conditions during the experimental study were similar to that described in earlier chapters. Table 14 gives the mean environmental conditions maintained and the initial length and weight of the animals used for the experimental study. Purified diet containing graded levels of ascorbic acid were formulated and prepared using vitamin-free casein as the protein source employing similar procedures adopted in Chapter 1 and 3. Similar parameters as reported in protein requirement studies (Chapter 1) were considered for the present study. Methods of determination of various parameters and statistical analysis of the data were carried out following similar procedures as given in Chapter 1.

Histological Studies:

Histological studies were done on the juvenile prawns fed on various diets containing different levels of ascorbic

TABLE 14: ENVIRONMENTAL PARAMETERS AND STOCKING SIZE OF JUVENILE PRAWNS.

Parameter	Mean values
Temperature (°C)	26.60 ± 1.071
Salinity (‰)	21.10 ± 1.627
pH	8.01 ± 0.083
Ammonia concentration in the water (NH_4-N mg/l/d)	0.0344 ± 0.006
Initial length (mm)	16.32 ± 0.056
Initial weight (mg)	34.3 ± 0.0036

TABLE 15: DIETARY COMPOSITION OF EXPERIMENTAL DIETS WITH GRADED LEVELS OF α-ASCORBIC ACID.

Ingredient	9/100 g						
α-Ascorbic acid	0.00	0.40	0.80	1.20	1.60	2.00	2.40
α-Cellulose	2.40	2.00	1.60	1.20	0.80	0.40	0.0

acid. At the end of the experiment, few prawns from each treatment were dissected and their hepatopancreas and abdominal muscle were isolated. These tissues were fixed in 10% neutral buffered formalin for 48 hrs, thoroughly washed with distilled water and then transferred to 10% alcohol. Three changes were given each of 2 hrs duration before transferring to 80% alcohol. After 30 mins, transferred the tissues to 90% and then to absolute alcohol, after 30 mins interval. At absolute alcohol two changes were given before the tissues were transferred to xylene and alcohol mixture (1:1) for 45 mins. Subsequently, transferred the tissue to pure xylene and paraffin mixture for another 30 mins. Molten wax (56-58°C) was poured into small aluminium boats and quickly transferred the tissues into the solidifying wax. The embedded tissue in wax was then trimmed to small blocks and mounted on wax holder of the microtome.

Thin sections (5-7 microns) of various tissues were cut with a manually operated microtome. The wax ribbons were then mounted on glycerine applied clean slides. The slides were then warmed over warm water to spread the wax ribbon uniformly. The sections were then hydrated initially with xylene followed by alcohol grades (100%, 90%, 50%) and finally with distilled water, five mins in each grade. After hydration, the sections were stained with hematoxylin(Delafield's hematoxylin) for 45 mins. The

stained slides were then dehydrated with upseries of alcohol grades (50% to 70%) and then counter stained with eosin for 10 mins. The tissues were then transferred to 90% alcohol and finally to absolute alcohol with two changes for 5 mins each. The slides were then cleared in xylene and permanent slides were made using DPX mountant. The slides were observed under compound microscope and recorded the structural changes in the tissues.

RESULTS AND OBSERVATIONS

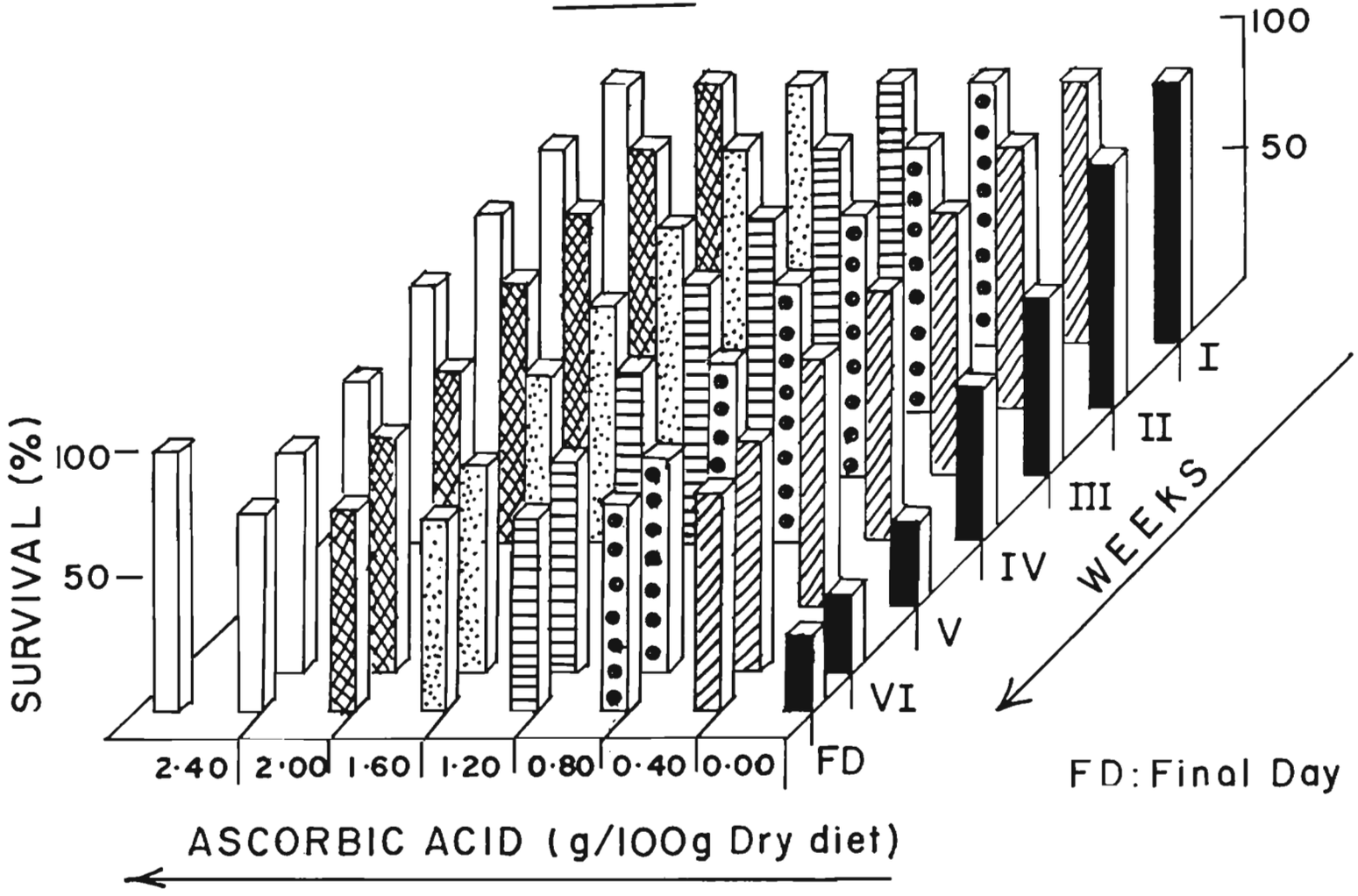
From the results of the experiment presented in Chapter 3 the essentiality of ascorbic acid in the diet of juvenile Penaeus indicus was established. Subsequently, with a view to determining the near optimal requirement for ascorbic acid by juvenile P. indicus, an experiment was carried out using purified diets containing different concentrations of $\mathcal{L}$-ascorbic acid (0.09- 2.4 g/100 g dry diet) in the diet and the results are presented here.

Survival:

Figure 25 clearly shows that concentration of ascorbic acid in the diet markedly affects the survival rate in juvenile prawns and the effect was found to be highly significant ($P<0.05$). However, there was no consistent trend in the percent survival of prawns with increasing concentrations of ascorbic acid in the diets. As observed in the previous study prawn groups fed with the ascorbic acid deficient diet produced significantly ($P<0.05$) lower survival rates compared to other treatment groups. However,

Fig. 25. Weekly percent survival of prawns fed diets with different levels of ascorbic acid.

FIG. 25


SURVIVAL (%)
100
50
100
50
I
II
III
IV
V
VI
WEEKS
2·40
2·00
1·60
1·20
0·80
0·40
0·00
FD
FD: Final Day
ASCORBIC ACID (g/100g Dry diet)

no significant differences in survival rates were observed between prawn groups fed diets containing different concentrations of ascorbic acid. The highest survival rate (92.5%) was recorded in prawn groups fed on diets containing 2g ascorbic acid and lowest with ascorbic acid deficient diet (30%). However, the highest survival rate recorded in treatment with 2 g ascorbic acid was not significantly different from that recorded in treatment containing 0.4 g of ascorbic acid (87.5%). The prawn groups fed diets containing 0.8 g, 1.2 g, 1.6 g and 2.4 g resulted in survival rates ranging from 72.5% to 80% and are not significantly different from each other.

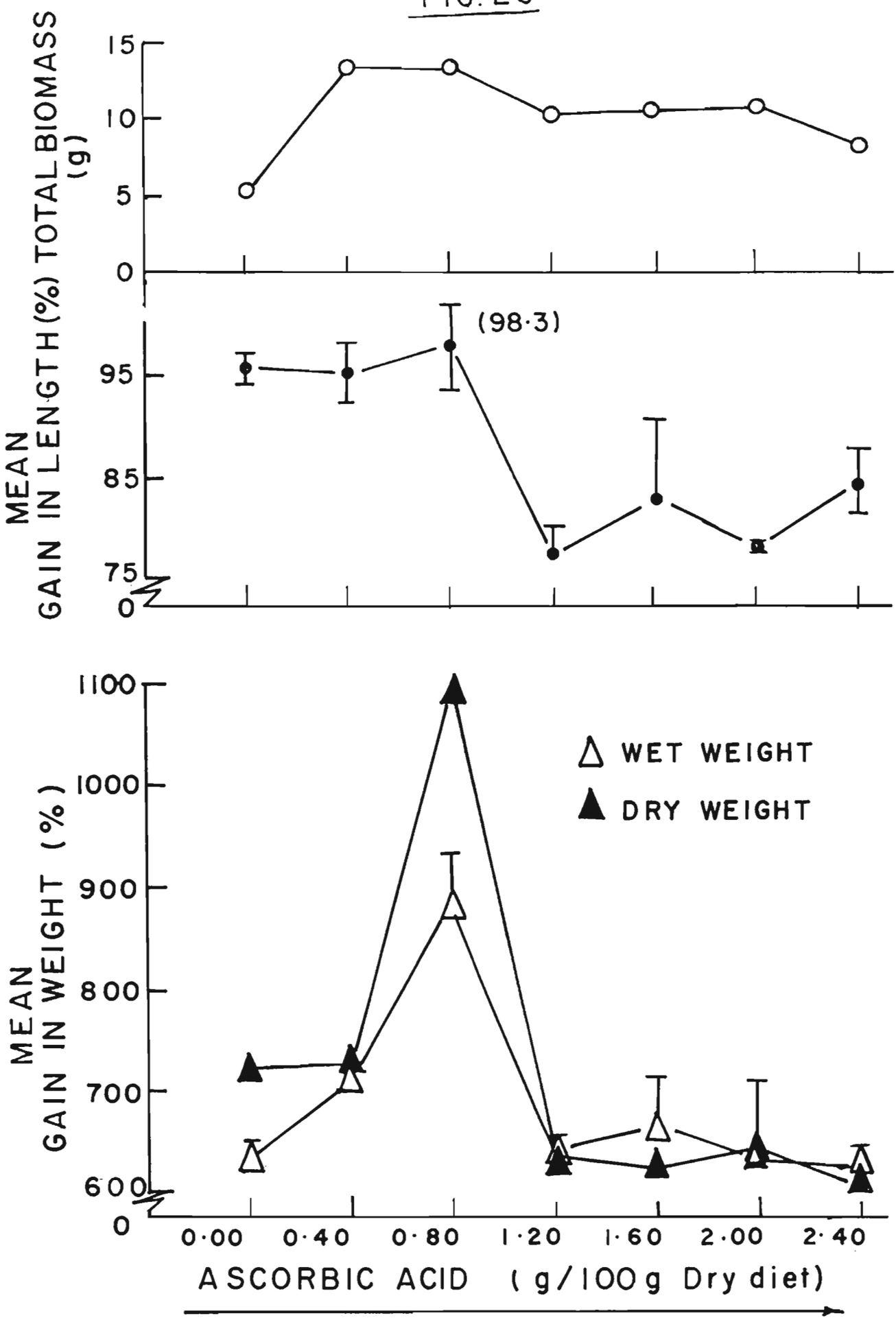
Growth:

The mean percentage gain in length, wet weight and dry-weight recorded from various treatments of increasing ascorbic acid concentrations are shown in Fig. 26. Analysis of variance of the data showed that the diets containing different concentrations of ascorbic acid significantly ($P < 0.01$) influence the mean percent gain in length, wet weight and dry weight of prawns. The highest mean percent gain in length (98.3%), wet weight (880%) and dry weight (1093.8%) were observed in prawn groups fed on the diet containing a concentration of 0.8 g ascorbic acid in the diet.

Prawns fed on the ascorbic acid deficient diet had relatively higher mean percent gain in length (95.8%), wet weight (633.4%) and dry weight (722.2%). However, the growth

Fig. 26. Percent gain in length and weight, and total biomass (g) of prawns fed diets with different levels of ascorbic acid.

FIG. 26


MEAN GAIN IN LENGTH (%) TOTAL BIOMASS (g)
15
10
5
0
95
85
75
0
(98·3)
MEAN GAIN IN WEIGHT (%)
1100
1000
900
800
700
600
0
WET WEIGHT
DRY WEIGHT
0·00
0·40
0·80
1·20
1·60
2·00
2·40
ASCORBIC ACID (g/100 g Dry diet)

observed in prawns of this group was mainly due to cannibalism and devouring of the dead prawns by surviving ones. Though, there was slight differences between the mean percent gains in length of prawns fed on diets containing ascorbic acid beyond 0.8 g, no specific trend could be observed. However, the percent gain in wet weight and dry weight followed a similar pattern without any significant differences between prawn groups fed on diets with ascorbic acid concentrations beyond 0.8 g. The maximum significant differences were observed between the prawn group fed on the diet with 0.8 g ascorbic acid and all other treatment groups.

Specific Food Consumption (SFC):

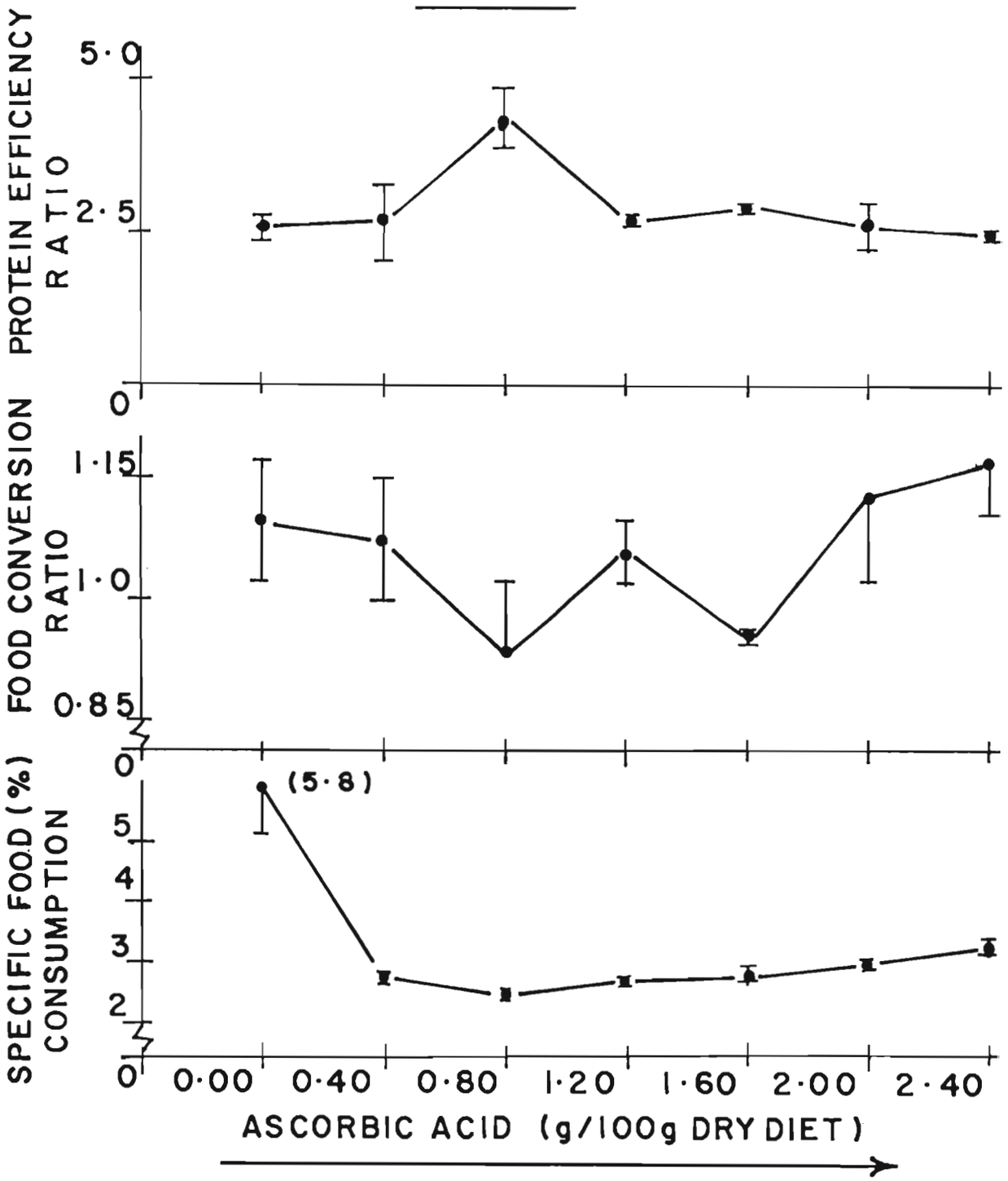
Specific food consumption (Fig. 27) showed distinct variation between different groups of prawns fed on the experimental diets and the influence of ascorbic acid diets on the SFC was highly significant ($P < 0.01$). The highest SFC was recorded in the treatment without ascorbic acid in the diet (5.81%) and the lowest in prawns fed with 0.8 g ascorbic acid (2.42%) in the diet. In all other treatment groups, the SFC ranged between 2.76 and 3.28% and the differences observed between these groups were not significant.

Food Conversion Ratio (FCR):

Food conversion ratios obtained for this experiment are shown in Fig. 27. The FCRs were significantly ($P < 0.05$)

Fig. 27. SFC, FCR and PER for diets with different levels of ascorbic acid.

FIG. 27


PROTEIN EFFICIENCY RATIO
5·0
2·5
0
FOOD CONVERSION RATIO
1·15
1·0
0·85
0
SPECIFIC FOOD (%) CONSUMPTION
(5·8)
5
4
3
2
0
0·00
0·40
0·80
1·20
1·60
2·00
2·40
ASCORBIC ACID (g/100g DRY DIET)

influenced by the diet. However, the FCRs recorded for prawn groups fed diets containing 0.8 g and 1.6 g of ascorbic acid showed significant differences with that of prawn groups fed with other dietary levels of ascorbic acid. The highest FCR (1.17) was recorded in prawns fed on the diet with 2.4 g of ascorbic acid and the lowest (0.93) was recorded in prawns fed on the diet with 0.8 g of ascorbic acid, closely followed by 1.6 g of ascorbic acid (0.95). In all other treatment groups, no significant variation in FCRs was recorded and the FCRs in these treatments, ranged between 1.06 and 1.13. The FCRs showed a declining trend upto 0.8 g of ascorbic acid in the diets and thereafter showed an increase with further increasing concentrati of the vitamin in the diet, except for the unexpected low FCR value recorded at 1.6 g treatment group.

Protein Efficiency Ratio (PER):

Protein efficiency ratio (Fig. 27) showed an increasing trend upto 0.8 g ascorbic acid and thereafter, it declined with further increase in ascorbic acid concentration in the diet. Analysis of variance of the data showed that the diets containing different concentrations of ascorbic acid significantly ($P < 0.05$) influenced the PER. Among treatment groups, prawns fed on diets with 0.8 g and 2.4 g ascorbic acid showed significant differences in the PER with that of other prawn groups. The maximum and the minimum PER values were recorded in prawns fed on diets with 0.8 g ascorbic acid (4.3) and 2.4 g ascorbic acid (2.45), respectively. In other treatment groups, the PER ranged between 2.54 and 2.9 and there were no significant differences between t

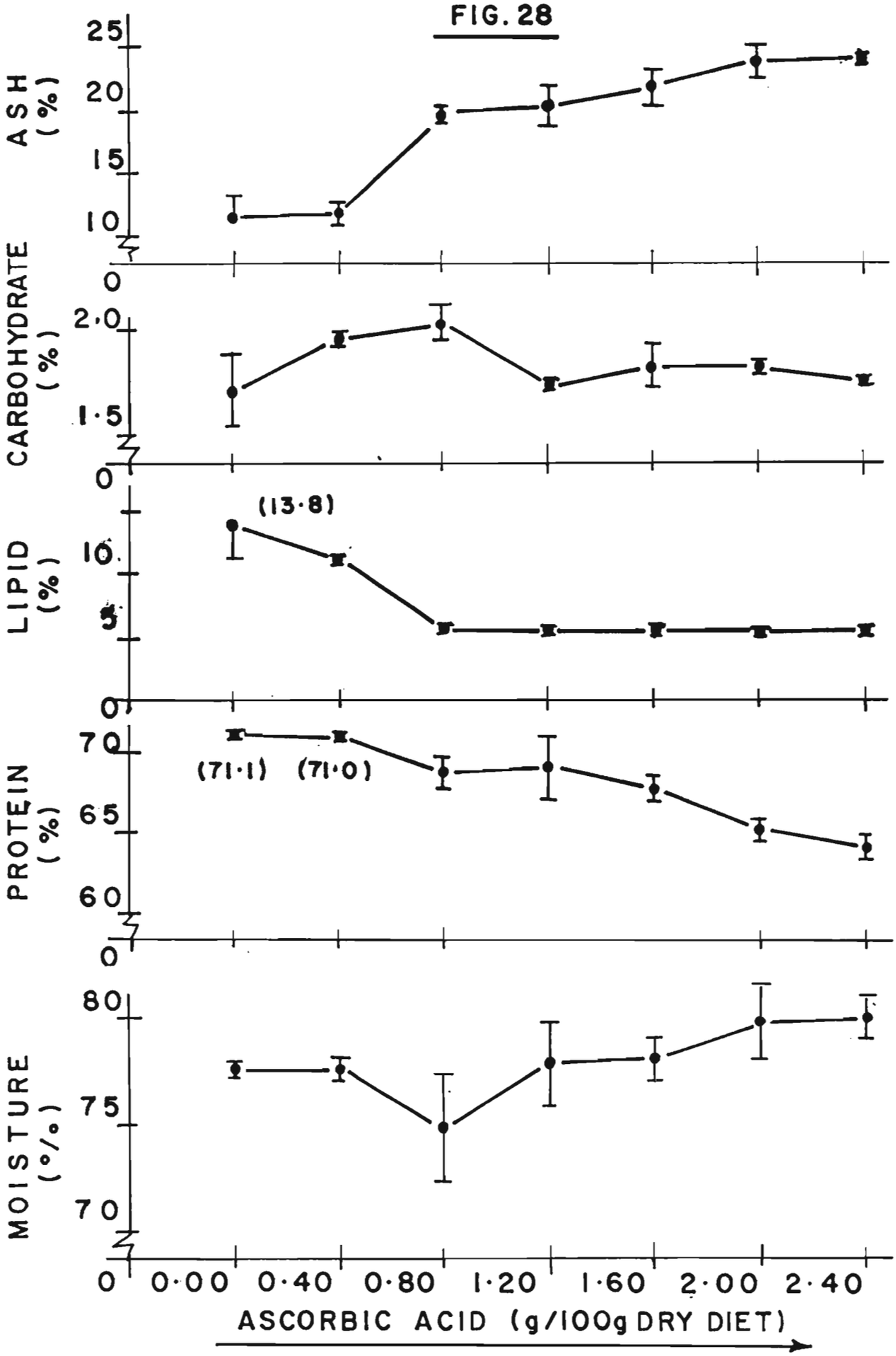
Biochemical Composition:

Data on moisture, ash, protein, lipid and carbohydrate contents of prawns from various treatments are shown in Fig.28. Statistical analysis of the data showed that the diets, containing various concentrations of ascorbic acid, significantly ($P < 0.01$) influence the moisture, ash, protein and lipid contents of prawns.

The highest moisture (80.1%) and ash (24.2% by dry weight) contents were observed (Fig. 28) in prawns fed on the diet with the maximum ascorbic acid concentration of 2.4 g. While the lowest moisture content was recorded in prawns fed on the diet containing 0.8 g ascorbic acid (74.9%), the lowest ash content was recorded in prawns fed on the ascorbic acid deficient diet (11.6%). The above results were significantly ($P < 0.05$) different from that observed for all other treatments, in which the moisture content ranged between 77.6% and 79.87% and ash content ranged between 19.7% and 23.9%. However, the ash content (11.9%) in prawns fed on the diet with 0.4g of ascorbic acid did not significantly vary from prawns fed without ascorbic acid in the diet. The moisture content in prawns decreased as the concentration of ascorbic acid in the diet increased upto 0.8 g and thereafter it increased with further increase in ascorbic acid concentration in the diet. On the other hand with increasing concentration of ascorbic acid in the diet of prawns, there was steady increase in the ash content of prawns (Fig.28).

Fig. 28. Biochemical composition of prawns fed diets with different levels of ascorbic acid.

FIG. 28


ASH (%)
25
20
15
10
0
CARBOHYDRATE (%)
2·0
1·5
0
LIPID (%)
(13·8)
10
5
0
PROTEIN (%)
70
65
60
0
(71·1)
(71·0)
MOISTURE (%)
80
75
70
0
0·00
0·40
0·80
1·20
1·60
2·00
2·40
ASCORBIC ACID (g/100g DRY DIET)

The protein content in prawns decreased with increase in concentration of ascorbic acid beyond 0.4 g (Fig. 28) and there was no significant difference between the protein content of prawns fed on the ascorbic acid deficient diet and those fed on diet with 0.4 g of ascorbic acid. However, significant ($P < 0.05$) differences were observed between the protein content of prawn groups fed on diets with higher concentration of ascorbic acid (2 g and above) and that of prawns from treatments with lower concentration of ascorbic acid. The highest protein content was observed in prawns fed on the ascorbic acid deficient diet, due to cannibalism and 0.4 g ascorbic acid (71%) in the diet but the lowest protein content was recorded in prawns fed on the diet with 2.4 g ascorbic acid (64%) in the diet. In prawns from all other treatment groups, the protein content ranged between 65 and 69%.

The lipid content in prawns showed a declining trend with increasing concentration of the vitamin in the diet upto 0.8 g and thereafter not much variations was observed in the lipid content (Fig. 28). However, among prawns from various treatment groups; those fed on the ascorbic acid deficient diet and those fed on the diet containing 0.4 g ascorbic acid had significantly ($P < 0.05$) higher lipid contents. No significant differences were observed between the lipid contents of prawns fed on diets containing different levels of ascorbic acid, beyond 0.4 g concentration in the diet. While the maximum lipid content was observed in prawns fed on the ascorbic acid deficient diet (14.8%),

the minimum (5.67%) was observed in prawns fed on the diet with 0.8 g and more ascorbic acid.

The carbohydrate content was significantly ($P < 0.05$) higher in prawns fed on diets with 0.8 g and 0.4 g ascorbic acid, compared to prawns fed on diets with higher concentrations of ascorbic acid. The carbohydrate content in prawns was found to increase with the ascorbic acid concentration in the diet upto 0.8 g and thereafter it showed a decline.

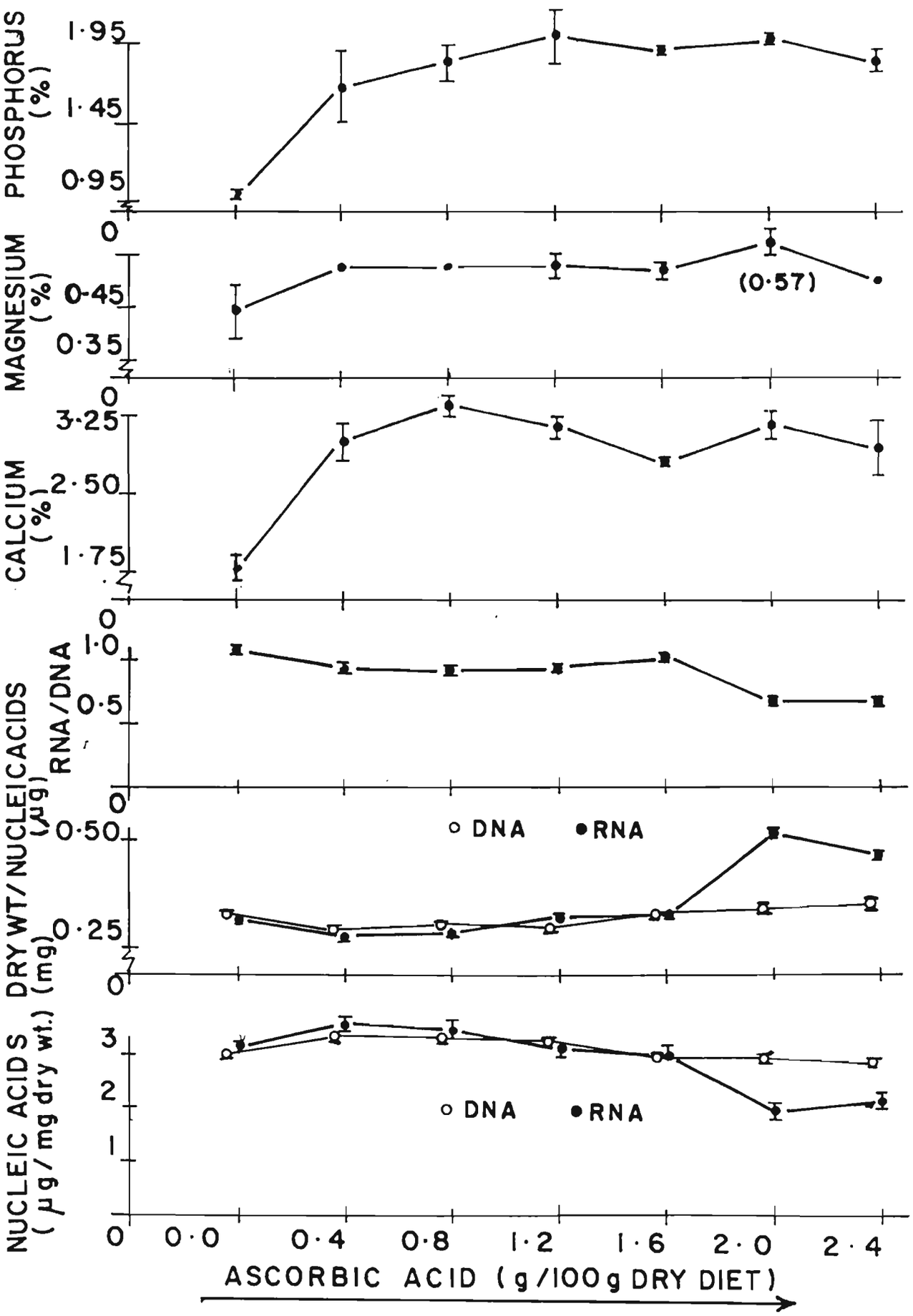
The RNA content of prawns was significantly ($P < 0.05$) influenced by the diets containing different levels of ascorbic acid fed to them. The prawns fed on diets with ascorbic acid levels of 2 g and 2.4 g had significantly ($P < 0.05$) lower RNA than those fed on diets containing lower concentrations of ascorbic acid. The highest RNA content (Fig. 29) was recorded with ascorbic acid concentration of 0.4 g (3.6 µg/mg) and the lowest with 2 g (1.9 µg/mg) of ascorbic acid. However, prawns fed on the diet with 2.4 g ascorbic acid (2.2 µg/mg) had slightly higher RNA than the lowest. In all other treatments RNA content in prawns ranged from 3.04 to 3.49 µg/mg, but there were no significant differences between treatments. The ratio between dry weight and total RNA was also significantly ($P < 0.05$) affected by the ascorbic acid concentration in the diets (Fig. 29). However, significant ($P < 0.05$) treatment difference was observed only between prawns fed on diets with ascorbic acid concentrations of 1.6 g and above, and these fed

on diets containing lower concentrations ($\leq$1.2 g) of ascorbic acid. There were also no significant differences in the dry weight/total RNA ratio between prawn groups fed on diets with less than 1.6 g ascorbic acid. While prawns fed on the diets with 2.4 g ascorbic acid showed the highest ratio (0.51), to those fed on the diets with 0.4 g ascorbic acid (0.3), showed the lowest ratio. An increasing trend in the ratio was observed with the increasing levels of ascorbic acid in prawn's diet.

DNA content in prawns did not show any significant ($P > 0.05$) variation in relation to the increasing levels of ascorbic acid in the diet. The highest DNA content (Fig. 29) was recorded in prawns fed on the diet containing 0.4 g of ascorbic acid (3.4 µg/mg) and the lowest in prawns fed on the diet with 2.4 g of ascorbic acid (2.8 µg/mg). In all other treatments, the DNA contents ranged between 2.9 and 3.3 µg/ mg. The prawns fed on the ascorbic acid deficient diet had relatively lower DNA content compared to prawns fed on the diet with 0.4 g ascorbic acid. A gradual decline in the DNA content was observed in prawn groups fed on diets beyond 0.4 g of ascorbic acid. The dry weight/DNA ratio, similarly, did not show any significant variation, in response to the ascorbic acid concentration in diets. However, there was a gradual increase in the ratio, with the increase in concentration of ascorbic acid in the diet of prawns (Fig. 29).

Fig. 29. Biochemical composition of prawns fed diets with different levels of ascorbic acid.

FIG. 29


PHOSPHORUS (%)
1·95
1·45
0·95
0
MAGNESIUM (%)
0·45
0·35
(0·57)
0
CALCIUM (%)
3·25
2·50
1·75
0
RNA/DNA
1·0
0·5
0
DRY WT/ NUCLEICACIDS (µg)
0·50
0·25
0
○ DNA ● RNA
NUCLEIC ACIDS (µg/mg dry wt.) (mg)
3
2
1
0
○ DNA ● RNA
0 0·0 0·4 0·8 1·2 1·6 2·0 2·4
ASCORBIC ACID (g/100g DRY DIET)

The RNA/DNA ratio showed a decreasing trend with increasing levels of ascorbic acid in the prawn's diet (Fig. 29) and it was significantly ($P < 0.05$) influenced by the experimental diets fed to the prawns. Significant ($P < 0.05$) differences were observed between treatment groups of prawns fed on diets containing higher concentrations of ascorbic acid (2 g and 2.4 g) and those fed on diets containing ascorbic acid concentrations less than 2 g. The highest RNA/DNA ratio was recorded in prawns fed on the diet with 0.4 g and 1.6 g of ascorbic acid (1.03) and the lowest ratio in prawns fed on the diet with 2.4 g of ascorbic acid (0.71). The prawns fed on diets with 2 g and more of ascorbic acid showed significantly ($P < 0.05$) lower RNA/DNA ratios than other treatment groups.

The diets containing various levels of ascorbic acid also had significant ($P < 0.05$) effect on the calcium content of prawns (Fig. 29). The prawns fed on the ascorbic acid deficient diet had significantly ($P < 0.05$) lower calcium than the prawns fed on all other diets containing graded levels of ascorbic acid. The highest calcium content was recorded (Fig. 29) in prawns fed on the diet containing 0.8 g of ascorbic acid (3.34%) and the lowest in prawns fed on the ascorbic acid deficient diet 1.76%). However, among prawn groups fed on diets containing different ascorbic acid concentrations, the lowest calcium content (2.93%) was recorded in prawns fed on the highest concentration level of ascorbic acid (2.4 g). The calcium content increased with the increase in dietary ascorbic acid level upto 0.8 g and there after showed a steady decline.

The magnesium content of prawns was not significantly influenced by the ascorbic acid concentration in diets. The highest magnesium content was observed (Fig. 29) in prawns fed on the diet with a concentration of 2 g of ascorbic acid (0.57%) and the lowest in prawns fed with the ascorbic acid deficient diet (0.44%).

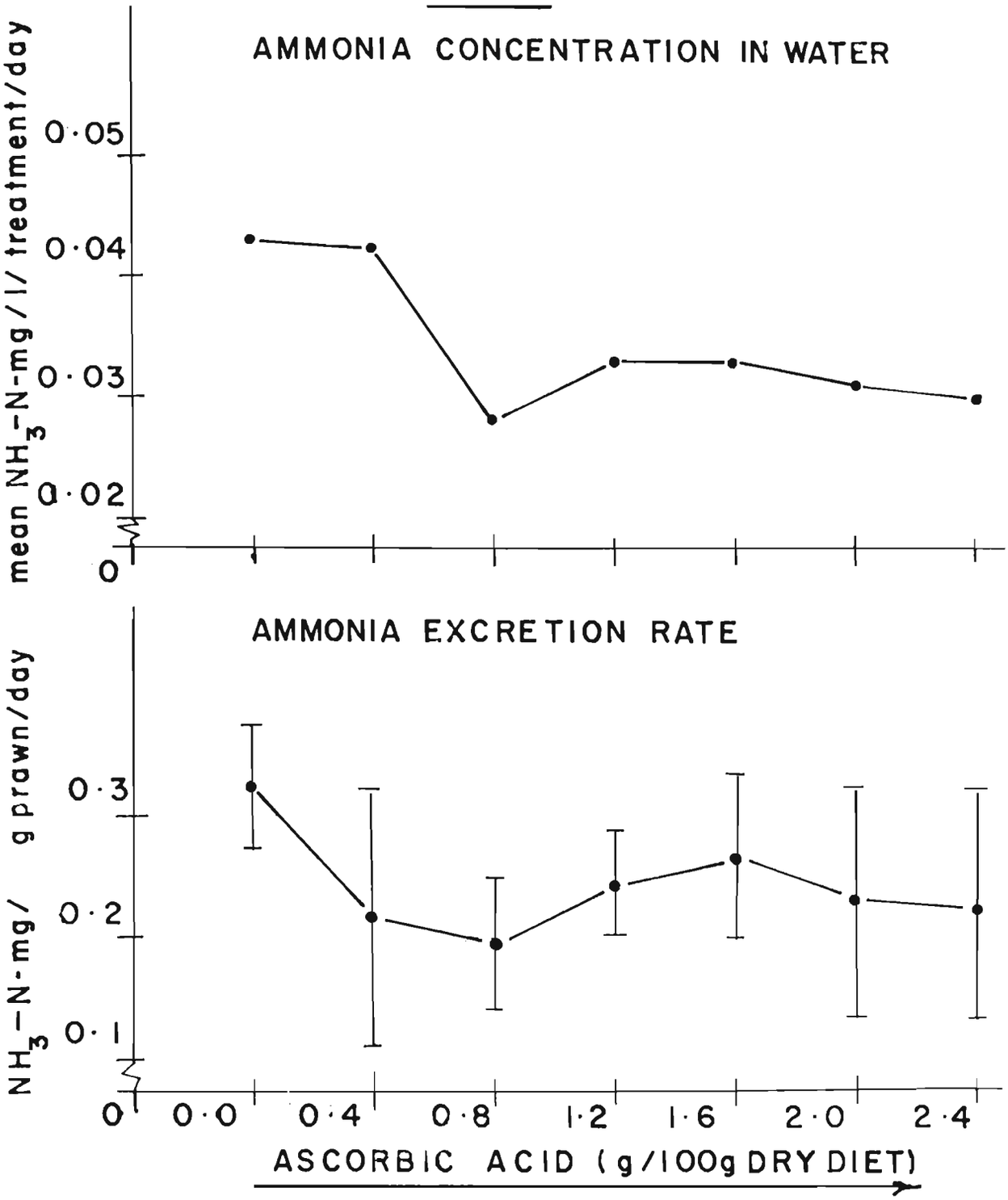
Wide variation in the phosphorus content was observed between prawns fed on the ascorbic acid deficient diet and those fed on graded levels of ascorbic acid in the diet (Fig. 29), indicating the significant ($P < 0.05$) influence of dietary ascorbic acid level on the phosphorus content of prawns. The highest phosphorus content was recorded in prawns fed on the diet with 1.2 g ascorbic acid (1.98%) and the lowest in prawns fed on the ascorbic acid deficient diet (0.98%). The prawns fed with 0.4 g of ascorbic acid in the diet had phosphorus content of about 1.66% which was significantly higher than that observed in the ascorbic acid deficient group, but significantly lower than all other groups, where phosphorus content varied narrowly between 1.82 and 1.98%. However, an increasing trend could be observed with increasing concentration of the vitamin in the diet upto 1.6 g, followed by a gradual decline with further increase in the vitamin concentration.

Ammonia Concentration In Water:

The concentration of ammonia in the water (Fig. 30) in which prawns were reared, varied insignificantly with respect to

Fig. 30. Ammonia concentration in seawater and ammonia excretion rate in prawns fed diets with different levels of ascorbic acid.

FIG.30


AMMONIA CONCENTRATION IN WATER
mean NH_3-N-mg/l/ treatment/day
0·05
0·04
0·03
0·02
0
AMMONIA EXCRETION RATE
NH_3-N-mg/ g prawn/day
0·3
0·2
0·1
0
0·0
0·4
0·8
1·2
1·6
2·0
2·4
ASCORBIC ACID (g/100g DRY DIET)

the treatments. The highest mean ammonia concentration was recorded in the treatment groups where prawns were fed on the ascorbic acid deficient diet (0.043 mg NH_4-N/l/d) as well as in those fed on the diet with 0.4 g ascorbic acid. In all other treatments the ammonia concentration ranged from 0.028 to 0.033 mg NH_4-N/l/day.

Ammonia Excretion Rates:

Experimental animals reared for 45 days and fed on diets containing different levels of ascorbic acid, including the ascorbic acid deficient diet were used for studying the ammonia excretion rates for 24 hrs, in order to ascertain if there were any significant differences in the ammonia excretion rates between treatments. The study showed that the experimental diets have highly significant ($P < 0.01$) effect on the ammonia excretion rates in prawn and that the prawns fed on the ascorbic acid deficient diet, excreted significantly ($P < 0.05$) higher ammonia than prawns fed on all other diets. The highest excretion rates (Fig. 30) in prawns fed on the ascorbic acid deficient diet (0.33 mg NH_4-N/ g prawn/day) was followed by prawns fed on diets containing 1.6 g ascorbic acid (0.27 mg NH_4-N/ g prawn/ day) and the lowest ammonia excretion rate was observed in the case of prawns fed on 0.8 g of ascorbic acid (0.19 mg NH_4-N/ g prawn/day). In all other treatment groups, the ammonia excretion rates ranged between 0.22 and 0.25 mg NH_4-N/ g prawn/day.

OBSERVATIONS

Molting:

The number of exuviae collected each day from the experimental tanks and the incidence of post-molt deaths were recorded, pooled and the average per treatment are shown in Table 16. In the case of prawns fed on the vitamin deficient diet, maximum number of exuviae was recorded during the first four weeks and during subsequent weeks the number of exuviae collected were relatively less, since the survival was reduced to about 50%. Among diets formulated with various levels of ascorbic acid and fed to prawns, considerable variation was observed from one treatment to the other in the number of exuviae collected. The maximum number (38) of exuviae were collected from the treatment with 0.8 g ascorbic acid and the minimum number (29) recorded in the treatment with ascorbic acid free diet.

Post-molt deaths (Table 16) were observed from the second week onwards and maximum numbers were recorded in the prawn groups fed on the ascorbic acid deficient diet. Usually in this groups, the prawns died within 24 hrs after molting. The number of post-molt deaths also increased, after the third week, in this group appreciably. In the case of treatments where prawns were fed on diets with different vitamin concentrations, the number of post-molt deaths differed significantl

TABLE 16: OBSERVATIONS IN PRAWNS FED WITH DIFFERENT EXPERIMENTAL DIETS

Concentration of L-ascorbic acid in the diet g/100 g dry diet	Mean nos. of molts recovered	Mean nos. of post-molt deaths	Texture of the body
0.00	33	15	SO
0.40	32	8	H
0.80	38	10	H
1.20	32	13	H
1.60	30	14	H
2.00	29	8	SO
2.40	30	13	SO

H - hard, So-soft

between each other, though maximum numbers were recorded at higher concentrations of 1.2 g and 1.6 g/100 g dry diet (13 Nos) compared to other dietary treatments. Post-molt deaths were considerably less in prawn groups fed on diets containing 0.4 g and 0.8 g ascorbic acid, indicating that most molted animals successfully survived.

Food Intake:

In the first two weeks, the feed intake did not vary much between treatment groups, and almost, all the quantity provided to the prawns were consumed. However, from the third week onwards, in the group fed on the ascorbic acid deficient diet, a decrease in food intake was observed and the amount of left-over food increased, as the number of days of experimentation prolonged. The animals also responded poorly, when feed was introduced in the medium. Similar responses were also observed, from the fourth week onwards, in the prawns fed on the diet with 2.4 g of ascorbic acid. However, in other treatments more or less, normal food intake was observed throughout the period of experiment and the prawns were attracted to the feeds as soon as the feed was introduced.

Behaviour Towards Light:

Table lamp light (1625 x 10^2 lux) when shown in the tanks, the prawns showed variable responses. The prawns fed on the

ascorbic acid deficient diet, from the second week onwards were observed to be most agitated to the light. Similar, responses were also observed in prawns fed on the diet with 2.4 g of ascorbic acid, from the fourth week onwards. The animals showed quick movements and took time to adjust to the photostimuli. In all other treatments, the prawns showed normal movements without showing much agitation throughout the experimental period.

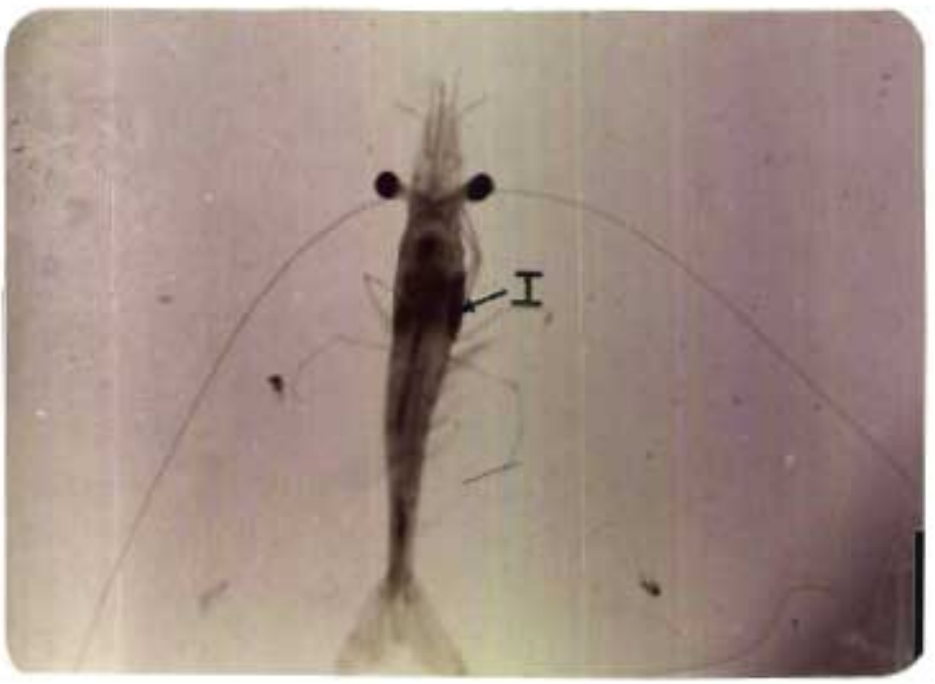
External Morphology:

During the 45 days of experimentation certain changes in the external morphology of prawns were observed in response to different dietary treatments. In the case of prawns fed on the ascorbic acid deficient diet, in two specimens the gill region was observed to show blackening after 20 days of experimentation as shown in Plate I . Within a week, the blackening intensified and one of the prawns died in 4 days. The other prawn survived till the end of the experiment and showed blackened gill and blackened lesions in the abdominal region as thick bands. These suspected diseased prawns with blackened gills were observed to show passive movements. Histological examination of these blackened gills could not be done due to poor fixation of the tissues.

At the end of 45 days, the surviving prawns in the ascorbic acid deficient groups showed soft, slippery texture,

PLATE 1. Juvenile *P. indicus*, showing blackening of gills due to ascorbic acid deficiency. I-infection site.

PLATE I



and had dark brownish spots densely spread on the upper margin of the rostrum and in the abdominal region. Similarly, dark spots were observed on the rostrum and abdominal region in prawns fed on the diet with 2.4 g of ascorbic acid. In all other treatment groups no specific morphological changes were observed, compared to a normal prawn.

Histology:

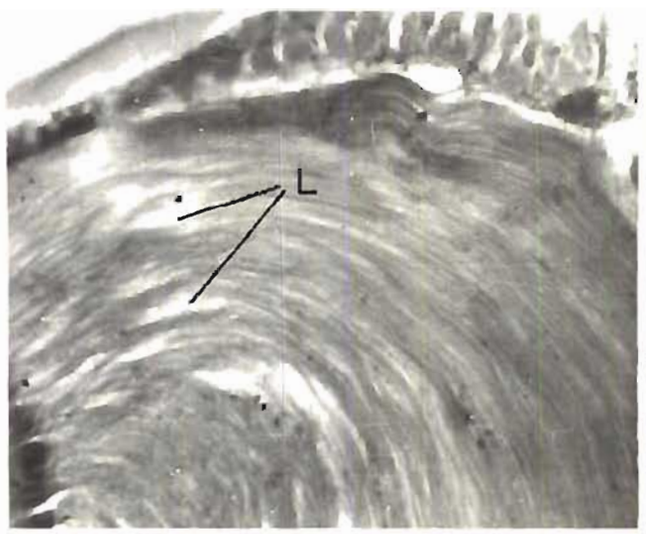
Histological examination of abdominal muscle and hepatopancreas showed clearly that ascorbic acid significantly influences the cellular structures. The normal muscle in cross section (Plate II, Fig. 4) appeared as distinct bundles, ensheathed by a thin membrane-endomysium. Similar structure, of the muscle was observed in almost all the ascorbic acid diets fed prawns. However, in the case of prawns fed with ascorbic acid-free diet the muscle fibres were scattered (Plate II, Fig. 2) giving an appearance of disorganization, which could have been as a result of degeneration of the endomysium,which in normal condition binds the muscle fibres into bundles. This degeneration of the endomysium and scattering of the muscle fibres clearly demonstrates the role of the ascobic acid in the maintenance of the cellular structures. However, in the longitudinal section no clear cut distinction could be made between the normal and affected muscle as a result of ascorbic acid deficiency (Plate II, Figs. 3 & 1).

PLATE II. Histological changes observed in the muscle of juvenile *P. indicus* fed with different concentration of ascorbic acid.

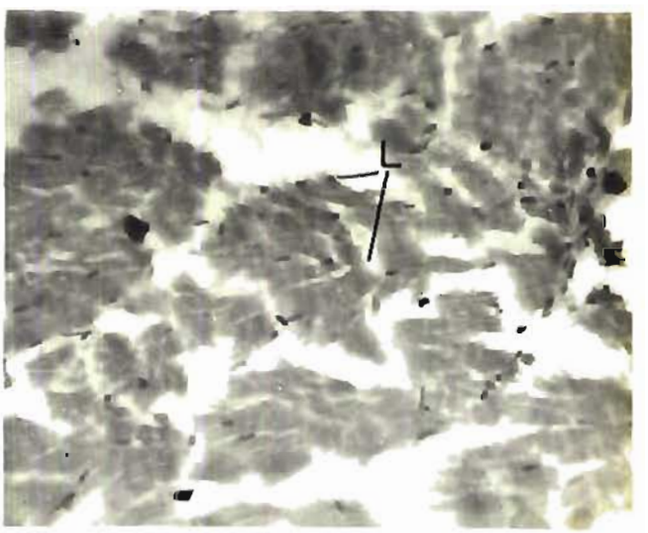
Fig. 1-2. Abdominal muscle showing lytic activity (L) due to ascorbic acid deficiency 50 X

Fig. 3-4. Normal abdominal muscle of prawns fed with ascorbic acid in the diet. 50 X

PLATE II



1



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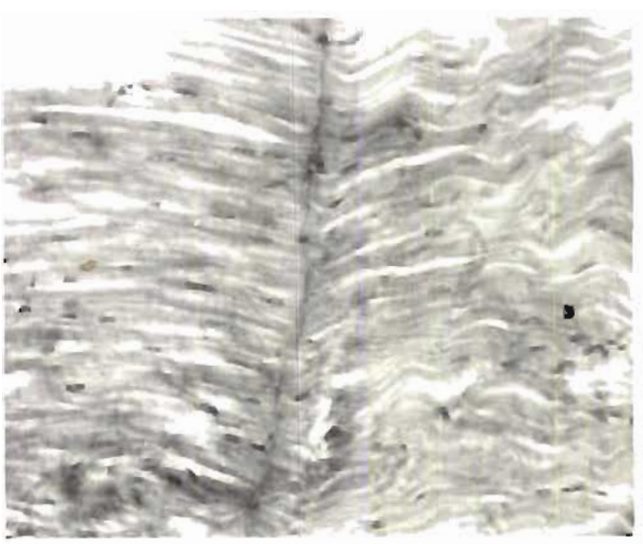
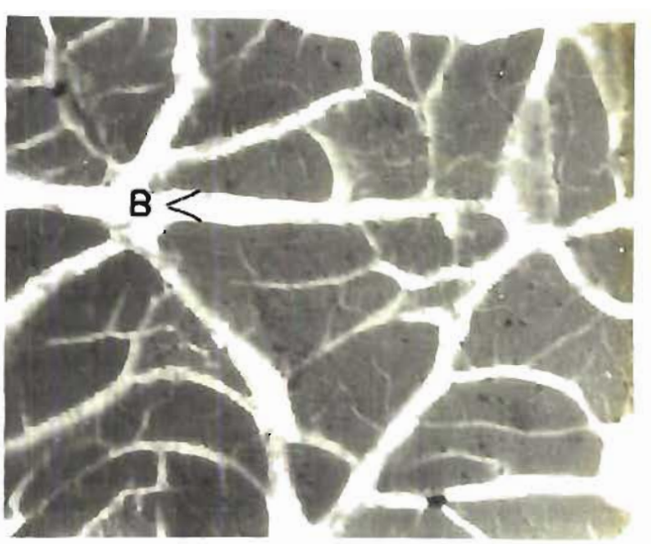


3



4

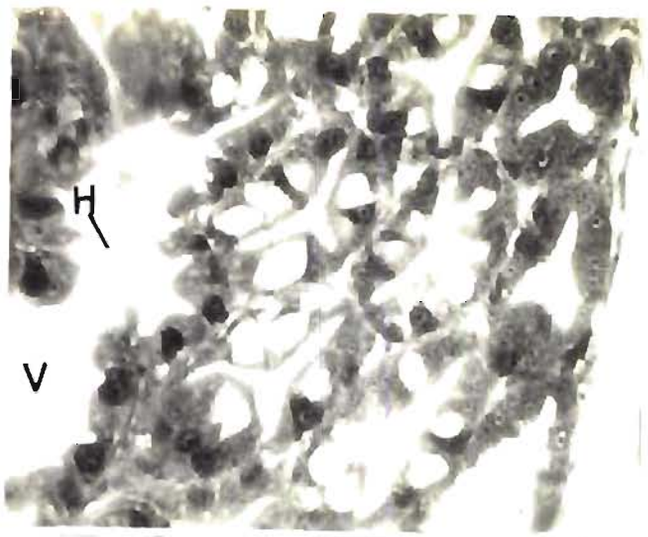
Similarly, the role of ascorbic acid in maintaining the cellular structures of the hepatopancreas is also evident from the study. In prawns fed with ascorbic acid free diet, the hepatopancreas was found to loose its integrity with the prolongation of the deficiency of ascorbic acid. The hepatopancreas sections of these prawns showed a general atrophy of the lobes (Plate III, Fig. 3) and a progressive appearance of connective tissue and hemocyte infiltration (Plate III, Fig. 1 to 3). Most of the tubules in these sections appear empty, with the cell contents of the tubule cells absorbed with only the ghost cell left behind. In some of the cells a number of pycnotic nuclei were observed to line the tubules. In contrast, the hepatopancreas removed from ascorbic acid fed prawns showed distinct tubules (Plate III, Fig. 6) exhibiting almost all the details. These sections showed distinct tubule cells with few numbers of vacuoles, surrounded by distinct tubular membranes. The intertubular spaces were also distinctly seen, unlike in the case of hepatopancreas of ascorbic acid deficient diet fed prawns. However, prawns fed with 2 g or more of ascorbic acid in the diet showed tubules with fewer number of vacuoles with tubular lumen much reduced almost to a star shape (Plate III, Fig. 4 & 5). Also in these sections progressive degeneration of the tubules was seen (Fig. 4 & 5) with number of the pycnotic nuclei increasing. Thus, hepatopancreas seems to be the target organ which is significantly affected by the ascorbic acid.

PLATE III. Histological changes observed in the hepatopancreas of juvenile *P. indicus* fed with different concentration of ascorbic acid.

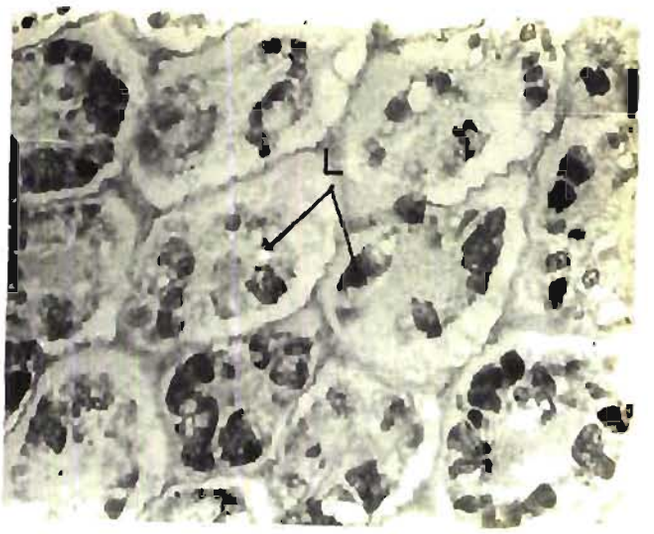
Fig. 1-4. Hepatopancreas tubules showing degeneration due in ascorbic acid deficient fed prawns. V-vacuole, H-hepatopancreas degenerating cellular tissues, ET-empty tubules, L-lysed tubules, PN-pyknotic nuclei

Fig. 5-6. Normal hepatopancreas tubules of ascorbic acid fed prawns.

PLATE III



1



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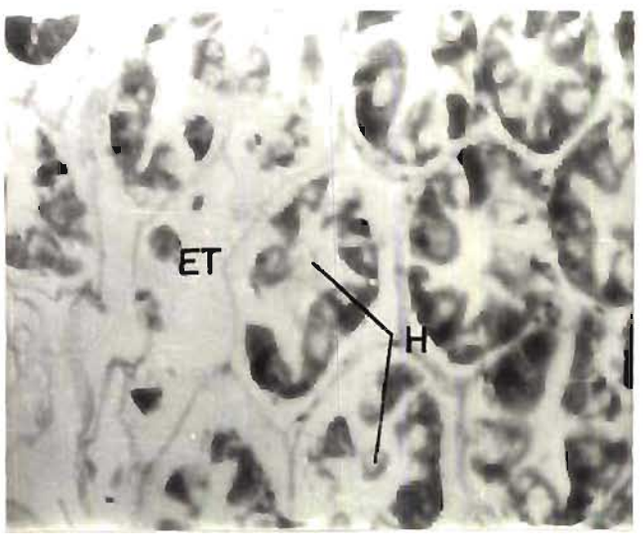

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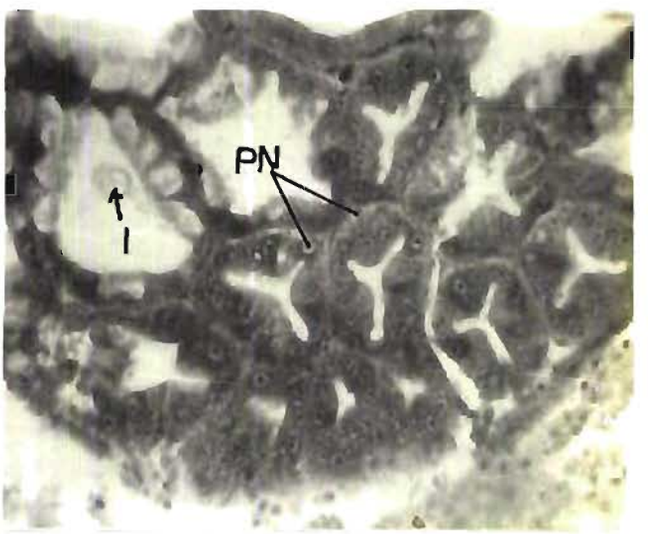

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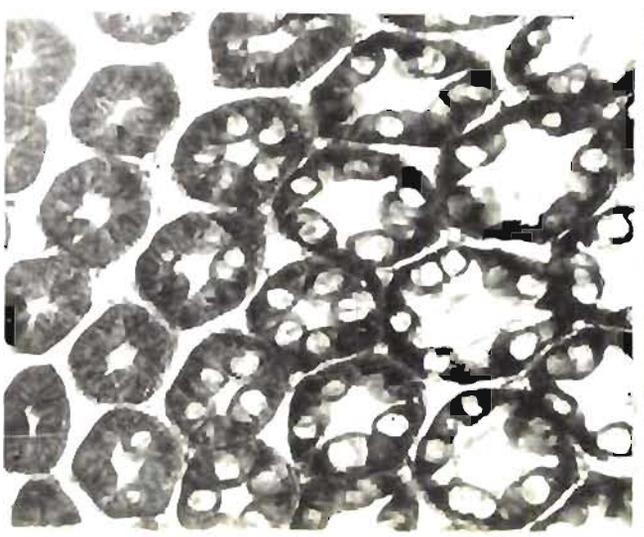

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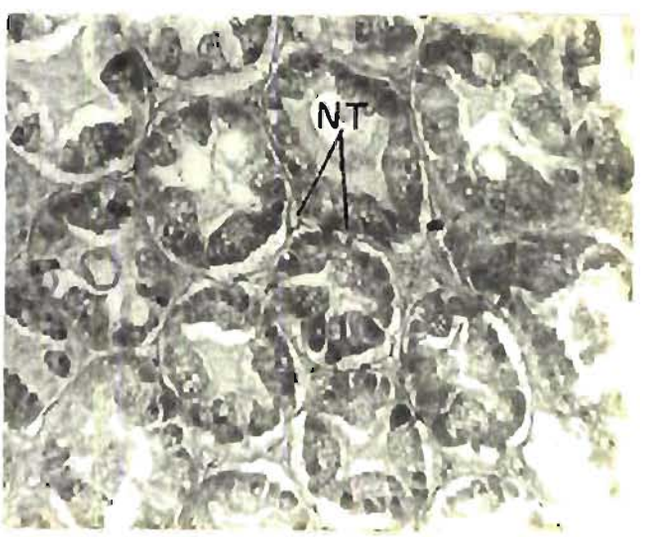


6

DISCUSSION

Among vitamins, ascorbic acid is a major biomolecule taking part in many of the biochemical reactions (Levin, 1976). Like most animal groups, crustaceans are incapable of biosynthesizing ascorbic acid (Gupta et al., 1972; Levin, 1976; Magarelli and Colvin, 1978; Magarelli et al., 1979) and therefore obtain this essential vitamin through the diet. Bacterial synthesis is also quite limited to meet its demand (Levin, 1976; Heinen, 1984).

Supplementation of ascorbic acid has been found to be essential in semi-purified and purified diets for cultivable species of crustaceans (Kitabayashi et al., 1971b; Guary et al., 1976; Magarelli et al., 1979; Heinen, 1984). However, when natural diets are fed, ascorbic acid supplementation may not be essential and may lead to hypervitaminosis according to New (1976a).

The results obtained during the present study relating to survival, growth, food conversion, body composition etc., show that for the early juveniles of P. indicus, dietary supplementation of ascorbic acid is essential and that concentrations between 0.4 g to 0.8 g/100 g dry diet is necessary for proper growth of the animal. The present findings, based on growth and survival, are comparable to that of Guary et al.(1976), who

reported that P. japonicus has a dietary requirement of 1-2 g of ascorbic acid/100 g diet.

The study clearly revealed the highly significant ($P < 0.01$) effect of dietary concentrations of ascorbic acid on the survival rate. Prawns fed on the diet deficient in ascorbic acid for six weeks showed very low survival rate (30%) compared to the prawns fed with ascorbic acid in the diet which ranged from 73 to 83%. Guary et al. (1976) also reported relatively poor survival in P. japonicus fed on ascorbic acid deficient diet, though they obtained relatively higher percent survival after 37 days of experiment. On the other hand, Magarelli et al. (1979) reported survival rates of 8% and 34% for P. californiensis and P. stylirostris, respectively, when ascorbic acid was deleted from the diet.

The effect of dietary deletion of ascorbic acid was prominent from the third week onwards, suggesting that the prawns were subsisting on ascorbic acid reserves present in the tissues for initial 2 weeks and thereafter on depletion of tissue ascorbic acid reserves, resulting in high mortality rates. Magarelli and Colvin (1978) found that the $T_{\frac{1}{2}}$ (half-life period) of ascorbic acid in P. californiensis and P. stylirostris to be 12.5 days and 3.5 days, respectively. They suggested that $T_{\frac{1}{2}}$ varies with species. So possibly the $T_{\frac{1}{2}}$ for juvenile P. indicus could be some where between 12-15 days, when the mortality rate in prawns

was relatively less; but beyond this half-life period of ascorbic acid, due to no further supplementation in the tissues through diet, mortality rate enhances.

While, the present study clearly demonstrates the essentiality of ascorbic acid in the diets of juvenile prawns, Deshimaru and Kuroki (1976b) observed that ascorbic acid is not required in the diets of P. japonicus. Yet, later studies have shown the need for ascorbic acid supplementation even in the diet of P. japonicus (Guary et al., 1976), P. stylirostris and P. californiensis (Magarelli et al., 1979) also.

Significantly, variations in survival were observed only between the treatment without ascorbic acid in the diet and those with graded levels of ascorbic acid in the diets; but no significant differences could be observed between the prawn groups fed on diets containing various concentrations of ascorbic acid. The concentrations selected for dietary formulations, probably, were relatively higher to bring about any marked variation in survival. Guary et al. (1976) reported increased survival rates with increasing concentrations of ascorbic acid in the diet with a maximum (94%) survival at 2 g/100 g dry diet for P. japonicus. However, in the present study, maximum survival was recorded (83.3%) with 0.4 g ascorbic acid/100 g diet, the minimum concentration used. Magarelli et al. (1979) reported maximum survival in P. californiensis at 1.2 g ascorbic acid/100 g dry diet (62%) and in P. stylirostris at 2.2 g/100 g dry diet (54%). Thus, the studies of Guary et al. (1976)

and Magarelli et al. (1979) and that of the present study, all highlight the need for ascorbic acid supplementation in the diet for maximum survival of prawns. These results also indicate the marked variations existing between species of prawns in the quantitative requirement of ascorbic acid in the diets.

Further, variations in growth were also observed with respect to the dietary ascorbic acid concentration, especially in treatment groups where ascorbic acid has been supplemented in the diet. Even though relatively higher growth (wet weight and dry weight) was recorded in prawns fed on the ascorbic acid deficient diet, the data cannot be considered for growth comparisons, because the growth attained is mainly due to the feeding of the dead prawns by the surviving ones, which alters the results considerably, since the study was done on group basis.

The percent gain in length, dry weight and wet weight were highest in prawns fed with 0.8 g/100 g dry diet of ascorbic acid, compared to other treatment groups. Increasing the ascorbic acid concentration in the diets beyond this level (0.8 g) did not promote growth. These observations in growth were similar to that reported in P. japonicus (Guary et al.,1976), and in P. californiensis and P. stylirostris (Magarelli et al., 1979). However, the decline in the percent gain in length and

weight at higher concentrations ($\geqslant$2 g) could be due to toxic effect of higher levels of ascorbic acid in the diet, as observed by New (1976a) and Heinen (1984).

Apart from survival and growth, specific food consumption (SFC), food conversion ratio (FCR) and protein efficiency ratio (PER) were also significantly influenced by ascorbic acid deletion or supplementation in the diet. Prawns fed diets without vitamin C or with excess ($\geqslant$1.2 g) vitamin, showed more food intake but less conversion. According to Baker *et al.* (1971) when ascorbic acid concentration in tissues reaches a peak level, the excess is excreted out. But till date no experimental proof has been established to ascertain whether crustaceans have the ability to get rid of excess of vitamin from their body (Heinen, 1984). However, from the poor growth and conversion ratio at high concentrations of ascorbic acid in the diets of prawns, it is apparent that hypervitaminosis induce stress in prawn, resulting in increased energy expenditure to get rid of the excess vitamin thereby affecting growth and food conversion inspite of high food intake.

Similar, results were obtained for PER values in the present study, wherein the highest PER was recorded at 0.8 g of ascorbic acid in the diet, which was significantly higher than the other treatment groups. This indicates that dietary proteins are more efficiently metabolized for synthesis

of tissue protein at this concentration level of ascorbic acid, whereas low PER values in other treatments indicate poor utilization of protein by the prawns.

The dietary levels of ascorbic acid also affected the chemical composition of the carcass of prawns. The moisture content in prawns showed fluctuations when ascorbic acid was deleted or given in excess of 0.8 g in the diet. The lowest moisture content was recorded in prawns fed on diets with 0.8 g of ascorbic acid wherein the growth and PER of the food were also maximum suggesting that prawns grow well at this concentration of vitamin in the diet. Higher moisture content in prawns from other treatments suggests that more of energy nutrients are utilised for enhanced energy needs, under dietary stress resulting in more water imbibition and poor deposition of organic and inorganic nutrients.

Ash content of prawns was also significantly influenced by the different levels of ascorbic acid in the diet, and the ash content showed a steady increase with increasing levels of ascorbic acid in the diet. Calcium, magnesium and phosphorus contents were found to be significantly lower in prawns fed on the ascorbic acid deficient diet.

Since molting is an essential physiological process in crustaceans and that during molting considerable amount of minerals are lost in the exuviae as well as these minerals

required for the synthesis of new cuticle, it can be expected that any imbalance in the uptake of these minerals can have significant influence on growth and body composition. Earlier studies have shown that uptake of calcium (Mahajan and Agarwal, 1980b) and phosphorus (Levin, 1976, New, 1976a) are affected by ascorbic acid in the diet. Besides at low concentrations of the vitamin, relatively higher numbers of post-molt deaths were observed during the present study and this may be due to inadequate concentration of minerals for synthesis of new cuticle. In comparision, post-molt deaths were relatively few in all other treatments. Perhaps, the calcium and phosphorus uptake in prawns seems to be unaffected by the different levels of ascorbic acid in the diet.

Conklin (1983) reported that under vitamin C deficiency, alkaline phosphatase activity was inhibited resulting in poor chitin synthesis and sclerotization of the epicuticle. Prawns have been reported to grow by the process of ecdysis (Passano, 1960) for which essential ingredients are required for chitin synthesis and sclerotization of the epicuticle. The increase in post-molt death in prawns fed on ascorbic acid deficient diets further may be due to above reasons.

Among the organic nutrients in the tissues of prawns, protein was observed to show a declining trend with increasing vitamin concentration in the diet. At higher concentration of

the vitamin in the diet, increased catabolism of protein probably occurs due to hypervitaminosis which may induce stress. This was also evident from the high ammonia excretion rate. Low ammonia exfretion rates in lower concentration of ascorbic acid fed prawns indicates probably protein mobilization from reserve areas is perhaps inhibited from being catabolized. Such inhibition of over oxidation process by ascorbic acid,by regulating mobilization of reserve materials was reported in plants (Saxena, 1969; Chinoy et al., 1970a; Chinoy and Saxena, 1971). These inhibition of over oxidation by ascorbic acid may be imbalanced in tissues when used in excess levels resulting to the breakdown of closely related metabolic pathways and thus poor growth may result as shown in the present study. Although, highest protein content was recorded in prawns fed on the vitamin C deficient diet, this cannot be considered for comparison because of low survival rate and high cannibalism rates, which significantly influence the results.

Lipid, being a major energy component in all living tissues, was found to be influenced by the ascorbic acid levels in the diet. At higher concentrations of ascorbic acid in the diet, the lipid level in the tissues gets markedly reduced suggesting that possibly lipids are mobilized freely from the reserve zones and were catabolized along with other energy nutrients. Poor lipid content in prawns fed with high ascorbic acid concentration in

the diet could also be due to higher lipolytic activity (Berthet, 1960; Levin, 1976) as shown in rats.

On the other hand, the high values of protein and lipid in prawns fed with 0.4 g ascorbic acid suggests that at this concentration of ascorbic acid, the over-oxidation of proteins and lipids is perhaps limited, as the movement of these energy nutrients from reserve depots is greatly restricted, as shown in plants (Saxena 1969; Chinoy et al., 1970a; Chinoy and Saxena, 1971).

Carbohydrate content in prawns was not significantly influenced by the levels of ascorbic acid in the diet, suggesting that eventhough ascorbic acid has prominent role in tissue metabolism by involving itself directly or indirectly (Levin, 1976); the lipid and protein metabolism seems to be influenced more than the carbohydrate metabolism.

Ascorbic acid has been reported to affect RNA synthesis (Price, 1966). Since ascorbic acid influence synthesis of collagen (Levin, 1976; Magarelli and Colvin, 1978; Lightner et al., 1979; Lightner, 1983) and activity of various enzymes (Levin, 1976); it can greatly affect the RNA content in tissues. In the present study though there was no significant difference between the RNA content in prawns fed on diets containing ascorbic acid ranging from 0.4 g to 1.6 g, a slight decline

in the RNA content was noticed as the concentration of ascorbic acid increased. This suggests that RNA synthesis is not influenced by ascorbic acid in the diet at these concentration. But the significantly low RNA content recorded in prawns, fed on diets with 2 g or more of ascorbic acid as compared to prawns fed on diets with less than 2 g of ascorbic acid, suggests that high concentration of ascorbic acid inhibits RNA synthesis. Low RNA synthesis reflects on lower protein content and poor growth. However prawns fed on the ascorbic acid deficient diet had relatively higher content of RNA, but the results are not comparable as very low survival was recorded at the end of the experiment due to cannibalism which significantly affected the result. Although, no significant variation in DNA conten of prawns was observed yet with increasing concentration of ascorbic acid, the DNA content in prawns showed a gradual decline, probably ascorbic acid at high concentration in the diet has inhibitory affect on DNA content. With increase in dry weight there was decrease in total RNA content indicating that protein synthesis is greatly affected by higher concentration of ascorbic acid. This is further supported by the lower protein content in prawns, accompanied by poor growth with the increase of ascorbic acid in the diet. The RNA-DNA ratio, also showed significant decline when ascorbic acid was added in concentration higher than 1.2 g, indicating poor RNA synthesis due to hypervitaminosis.

The ammonia excretion rate in prawns was also affected by the ascorbic acid concentration in the diet indicating the influence of ascorbic acid in protein metabolism. Prawns fed with ascorbic acid deficient diet, excreted significantly higher ammonia than the prawns fed with various concentration of ascorbic acid in the diet. The higher ammonia excretion in the former group of prawns, indicates increased metabolic rate probably to overcome the dietary stress as a result of avitaminosis and thereby more energy is utilized by catabolizing protein in keeping up the normal metabolic rate. Besides the metabolisable proteins may be catabolized due to relatively low activity of ascorbic acid activated enzymes involved in protein synthesis, especially those involved in collagen synthesis. The influence of ascorbic acid on enzyme activity has been well established (Levin, 1976).

Histological examination of muscle and hepatopancreas show distinct morphological changes occurring in these tissues of prawns by feeding with ascorbic acid deficient diet or with graded levels of ascorbic acid in diets. Prawns fed on the ascorbic acid deficient diet showed marked vacuolization of hepatopancreas tubules as a result of autolysis. Only the remains of autolysation were seen, with the connective tissue stroma of the tubule remaining intact. Most of the cells in the peripheral region were pyknotic with the central region of tubule occupied with tissue debris and remnants. This

vacuolization in the hepatopancreas tubules, possibly occurs as a result of release of proteolytic enzymes which might start digesting the tissue as a result of stress induced by ascorbic acid deficiency. As ascorbic acid has been known to regulate the movement of energy nutrients from storage organs, (Saxena, 1969, Chinoy et al., 1970a; Chinoy and Saxena, 1971) and also has tendency to reduce the surface tension of various macro and micro molecules present in tissues (Levin, 1976); probably under deficiency of ascorbic acid, the tissue may be losing these properties resulting in vacuolisation of tissues. On the other hand, the tubular cells of hepatopancreas in prawns fed on ascorbic acid containing diets were distinct and well defined with a centrally placed nucleus surrounded with cytoplasm. There were no large inter-tubular spaces which were seen in the hepatopancreas of ascorbic acid deficient diet fed prawns. Apart from the structural changes in hepatopancreas, ascorbic acid deficient diet fed prawns showed empty spaces in the muscular region as a result of lytic activity under deficiency conditions. Regions where the tissue was damaged was found to be infected with bacteria. Similarly the gills in few specimens of prawns fed on ascorbic acid deficient diet, showed darkened bands.

CONCLUSIONS

The present study in early juveniles of *P. indicus* clearly showed the essentiality of ascorbic acid in the diet of prawns. From the study, it is quite evident that deletion or supplementation of vitamin C has significant influence on survival rate. Even on supplementation of lecithin in the diet, which was shown to enhance survival (Conklin *et al.*, 1978), the mortality could not be reduced. Most of the deaths recorded in vitamin deficient diet treatments were as a result of post-molt deaths. Histological examination of prawns suggests that under deficiency of vitamin C, lytic activity of tissue in the muscle and hepatopancreas becomes prominent, resulting in the breakdown of cellular machinery and increased susceptibility to pathogenic bacterial infection (Lovell, 1973), making the prawn incapacitated, resulting ultimately to death. Post-molt deaths are usually high in these treatment groups. The present study shows that poor calcium and phosphorus uptake in prawns is partially responsible for most post-molt deaths, engineered by the dietary ascorbic acid deficiency.

Further, the present finding indicate that ascorbic acid concentrations ranging between 0.4 and 0.8 g of ascorbic acid/ 100 g dry diet is preferable in the diets of juvenile prawns. However, the requirement may decline with increase in age (Mahajan and Agarwal, 1980a) or influence of other parameters

(Terroine, 1953; Kanazawa et al., 1970; Kutsky, 1973; Levin, 1976; Hilton et al., 1977; Millikin, 1982).

However, in the present study, the requirement of relatively high concentration of ascorbic acid is justifiable under the present temperature and other environmental conditions, as it has been reported that higher dosages are required at higher temperatures (Tseitina, 1965; Siddique et al., 1972; Scott, 1975) and to compensate the loss through leaching of the vitamins from the diet (Milton et al., 1977, Halver, 1982; Heinen, 1984).

The present recommended levels of ascorbic acid for juvenile P. indicus are also well supported by low specific food consumption and conversion, high protein efficiency ratio, protein deposition, ash deposition, along with high growth and survival. Histological examination of the tissues also clearly shows that the musculature and hepatopancreas are well developed in these prawns unlike the observations made in ascorbic acid free fed prawns or high dosages of ascorbic acid fed prawns. The activity of the animals were normal, unlike the other treatment groups.

CHAPTER-V

CHOLINE REQUIREMENT

INTRODUCTION

Choline in biological tissues exists, both in free and combined state, in the form of lecithin, acetylcholine and in certain plasmogens and sphingomyelins. In biological tissues it functions as a methyl donor and as a lipotropic and anti-haemorrhagic factor. In addition to these, in methylated state as acetylcholine, it functions as an important neuro-transmitter (Griffith and Nye, 1954; West et al., 1966).

One of the earliest reported deficiency symptoms of choline was in rats, where it causes the development of fatty liver (Mookerjea, 1971). Other deficiency diseases in higher organisms have been reported in the excretory organs as a result of haemorrhagic degeneration (Griffith and Wade, 1939; McLaren et al., 1947a). In birds, perosis has been reported as a result of choline deficiency and reversed only by administering methyl donors in the form of choline or betaine (Jukes, 1941; Pike and Brown, 1975). The haemorrahgic diseases have been assumed to be caused by the poor acetylcholine concentration in the organs and a low concentration of blood clotting factor.

Choline deficiency symptoms in animals can be judged from poor growth rate, food conversion, and by impaired fat metabolism. Halver (1957, 1969) and Coates and Halver (1958) reported increased gastric emptying time in salmon fed on

choline deficient diets. Choline deficiency in rainbow trout resulted in anemia and kidney degeneration (McLaren et al.,1947a). In the crustacean, Oniscus asellus, it was found that in the absence of methyl donors, longevity decreased from 90 to 28 days without affecting the weight (Beerstecher et al., 1954b; Fisher, 1960).

In crustaceans only meagre information is available on its role and requirement. However, lecithin (phosphatidyl choline) is found to be an essential component in the diet of crustaceans (New, 1976a; Boghen and Castell, 1980; Conklin et al., 1983) which indicates that choline has role in the metabolism of crustaceans. In Cancer pagurus and Astacus astacus, choline has been quantitatively measured and reported to be 1 µg/g in the whole body, excepting in muscle and ganglia, where it is about 30 µg/g (Carayon Gentil and Gautrelet, 1938). Similarly, in the American lobster (Homarus americanus) trimethylamine oxide (TMO), a strong organic base distributed in the tissues, has been extracted and found to be having choline as precursor molecule (Bilinski, 1961). Bilinski(1961) reported that crustaceans require choline during the formation of TMO. Low levels of TMO were recorded in the choline deficient diet fed animals, indicating that Homarus is unable to synthesize choline. Certain species like Moina (D'Abramo and Baum, 1981) Artemia (Provasoli and D' Agostino 1962, 1969; D'Agostino, 1980) and P. japonicus (Kanazawa et al., 1976) have

been found to require this vitamin. Choline is required by all insects (Dadd, 1970), and evidence so far indicate that it may be one for the crustaceans as well (Heinen, 1984).

Quantiative requirements of choline in diets have been worked out for few species of crustaceans. In the case of Moina macrocopa it was estimated to require about 750-850mg/100 g of particulate diet (D'Abramo and Baum, 1981) for maximum survival and growth. This value is appreciably high for these micro-crustaceans when compared to that of P. japonicus which require about 45 mg choline/100 g dry diet (Kanazawa et al., 1976). However, in another study, Deshimaru and Kuroki (1979) demonstrated no requirement of this vitamin by P. japonicus. Choline requirement in animals is affected by the level of other dietary constituents. It was reported that with increasing cystine content in the diet and with increasing food intake, the choline requirement increases. It has also been reported that high fat and low protein diets increase choline requirements which if not met, leads to cirrhosis of the liver (Jukes, 1952). Due to its hygroscopic nature, thermolability and other characteristics, the studies on choline requirement had been restricted. In the case of P. indicus no study has been done so far to ascertain its essentiality, requirement and deficiency symptoms and hence the present study was carried out.

MATERIAL AND METHODS

Choline requirement of juvenile prawns was studied using isonitrogenous and isocaloric purified diets with vitamin free casein as protein source. Graded levels of choline chloride was used in the diets (Table 19). An additional diet was also prepared in which both choline chloride and lecithin were deleted. All the diets were adjusted to 100% dry diet by using α-cellulose. Diet preparation, feeding level and schedules were similar to that described in earlier Chapters (I and III).

Experimental set up, rearing of animals before and during the experimental study, and maintenance and monitoring of the environmental conditions were similar to that reported in earlier chapters. Table 17 gives the mean environmental conditions and the initial length and weight of the animals used for the study.

To evaluate the response of various diets, the parameters considered and methods adopted for their determination were same as described in earlier Chapters (I, II and IV). Data were statistically analysed following the procedures reported in Chapter I.

TABLE 17: ENVIRONMENTAL PARAMETERS AND STOCKING SIZE OF JUVENILE PRAWNS

Parameter	Mean values
Temperature (°C)	27.4 ± 1.0
Salinity (‰)	21.65 ± 1.67
pH	7.5 ± 0.3
Ammonia concentration in the water (NH_4-N mg/l/d)	0.028 ± 0.0035
Initial length (mm)	17.48 ± 0.47
Initial weight (mg)	45.0 ± 0.0013

TABLE 18: DIETARY COMPOSITION OF EXPERIMENTAL DIETS WITH GRADED LEVELS OF CHOLINE CHLORIDE

Ingredient	g/100 g							
Choline chloride	0.0*	0.0	0.25	0.50	0.75	1.00	1.50	2.00
α-Cellulose	2.00	2.00	1.75	1.50	1.25	1.00	0.50	0.0

* Lecithin deleted

RESULTS AND OBSERVATIONS

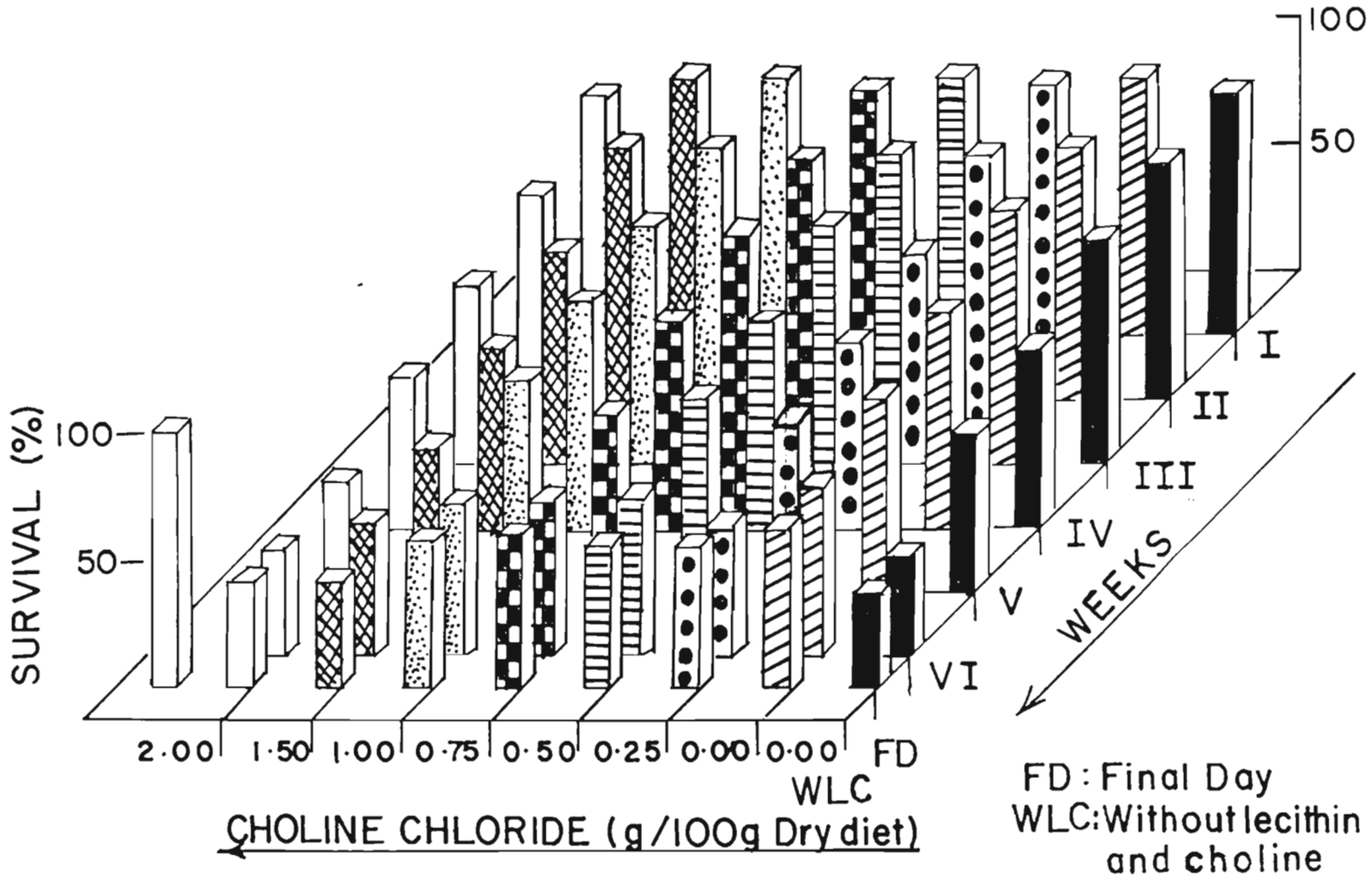
With a view to determining dietary requirements for choline for juvenile *Penaeus indicus*, an experiment was carried out using purified diets containing graded levels of choline chloride (0 to 2 g/100 g dry diet). Along with these dietary treatments, one additional treatment, with three replicates, was also kept, in which juvenile prawns were fed on a diet without both choline and lecithin. The results of the experiment are presented here.

Survival:

The survival rate of prawns (Fig. 31) was significantly ($P < 0.01$) affected by the diets fed to them. The survival rate was significantly lower ($P < 0.01$) in prawn groups fed on the diet deficient in both choline and lecithin (44.4%) compared to other diets. Interestingly, the diet deficient in choline, but with lecithin gave good survival rates (64.4%), almost comparable to the higher survival (66.7%) recorded with 0.75 g of choline in the diet. The survival rates were also significantly ($P < 0.05$) lower in prawn groups fed on diets containing 1.5 g and 2.0 g of choline when compared to lower concentrations of choline. In all other treatment groups, the survival ranged between 55.5 and 57.8%. A comparison of the survival rate of prawns, fed on the diet without lecithin and choline, with that of the choline deficient diet, but with lecithin, showed that

Fig. 31. Weekly percent survival of prawns fed diets with different levels of choline chloride.

FIG.31


SURVIVAL (%)
100
50
100
50
I
II
III
IV
V
VI
WEEKS
2·00
1·50
1·00
0·75
0·50
0·25
0·00
0·00
FD
WLC
CHOLINE CHLORIDE (g/100g Dry diet)
FD : Final Day
WLC: Without lecithin and choline

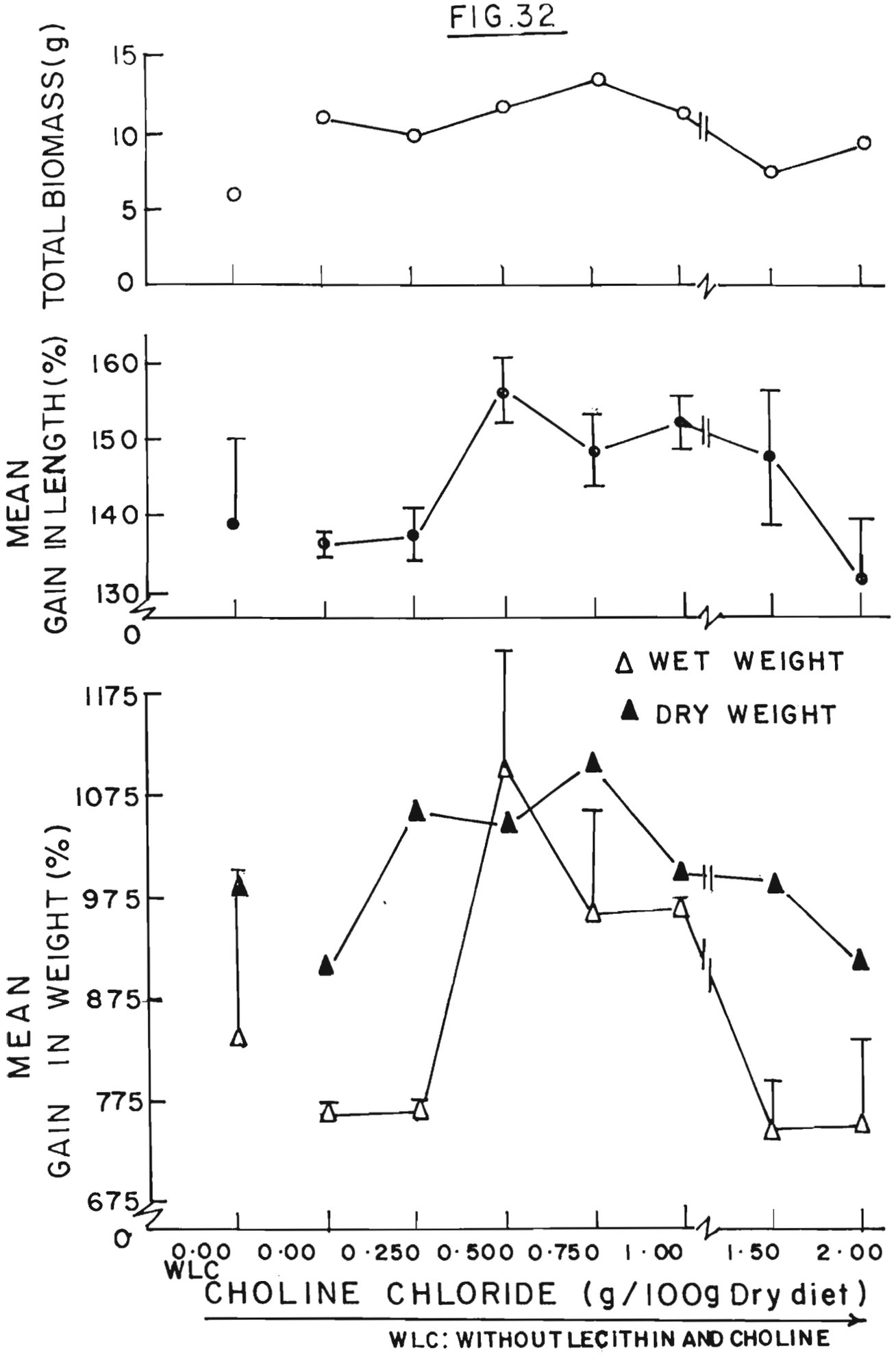
lecithin inclusion in the diet offsets the choline requirement of prawns.

Growth:

Data on the mean percent gain in length, wet weight and dry weight are shown in Fig. 32. Analysis of variance of the data resulted in a significant F value ($P < 0.05$) for percent gain in length and highly significant ($P < 0.01$) F value for mean percent gain in wet weight and dry weight, indicating the significant influence of the various diets on growth. While the highest mean percent gain in length (156%) and wet weight (1102%) were observed in prawns fed on the diet with 0.50 g choline, the highest mean percent gain in dry weight (1107%) was recorded in prawns fed on the diet with 0.75 g choline chloride. The lowest mean percent gain in length (132%) was obtained in prawns fed on the diet with 2.0 g choline, but this was not significantly different from the mean percent gain in length recorded in prawns fed on the diet deficient in choline (136%), diet with 0.25 g choline (138%) or the diet deficient in both choline and lecithin (139%). There were also no significant differences between the mean percent gains in length of prawns fed on diets containing 0.5 (156%), 0.75 (145%) or 1.0 g (152%) choline chloride.

Fig. 32. Percent gain in length and weight, and total biomass (g) in prawns fed diets with different levels of choline chloride.

FIG.32


MEAN TOTAL BIOMASS(g)
15
10
5
0
MEAN GAIN IN LENGTH(%)
160
150
140
130
0
Δ WET WEIGHT
▲ DRY WEIGHT
MEAN GAIN IN WEIGHT(%)
1175
1075
975
875
775
675
0
0·00
WLC
0·00
0·250
0·500
0·750
1·00
1·50
2·00
CHOLINE CHLORIDE (g/100g Dry diet)
WLC: WITHOUT LECITHIN AND CHOLINE

The mean percent dry weight gain of prawns fed on the diet with 0.75 g choline was significantly ($P < 0.05$) higher (1107%) than that recorded in all other groups of prawns. The prawns fed on diets with 0.25g and 0.5 g choline chloride also showed significantly ($P < 0.05$) higher mean percent dry weight gains (1055% and 1049%, respectively) compared to prawns from all other treatment, with the exception of prawns fed on the diet containing 0.75 g choline. The diet deficient in choline, however, gave significantly ($P < 0.05$) low percent gain in mean dry weight (907%) compared to all other groups of prawns. There were no significant differences between the mean percent dry weight gains of prawns fed on diets containing choline chloride concentrations ranging from 1.0 g to 2.0 g.

Both the mean percent wet weight and dry weight gains were relatively more in prawns fed on the diet deficient in both choline and lecithin, compared to those fed on the diet deficient in choline and diet with 2 g choline. The high cannibalism, as well as devouring of dead prawns by the surviving ones in treatment fed on the diet deficient in both choline and lecithin is the main reason for the increased weight gain.

Specific Food Consumption (SFC):

Specific food consumption in prawns was significantly ($P < 0.01$) influenced by the different diets (Fig. 33). Prawn

groups fed without both choline and lecithin in the diet ha significantly ($P < 0.05$) lower (2.14%) SFC than prawn groups fed on the choline deficient diet and those fed with choline concentration higher than 0.75 g in the diet. Similarly, significantly ($P < 0.05$) low SFC values were recorded in treatment groups of prawns fed with 0.25 g and 0.5 g of choline in diets compared to the SFC values of prawns fed with either the choline deficient diet or with 0.10 g or more of choline in the diet. The highest SFC was recorded in prawns fed on the diet with 2 g of choline (4.96%) and the lowest in prawns fed on the diet with 0.5 g of choline (1.77%).

Food Conversion Ratio (FCR):

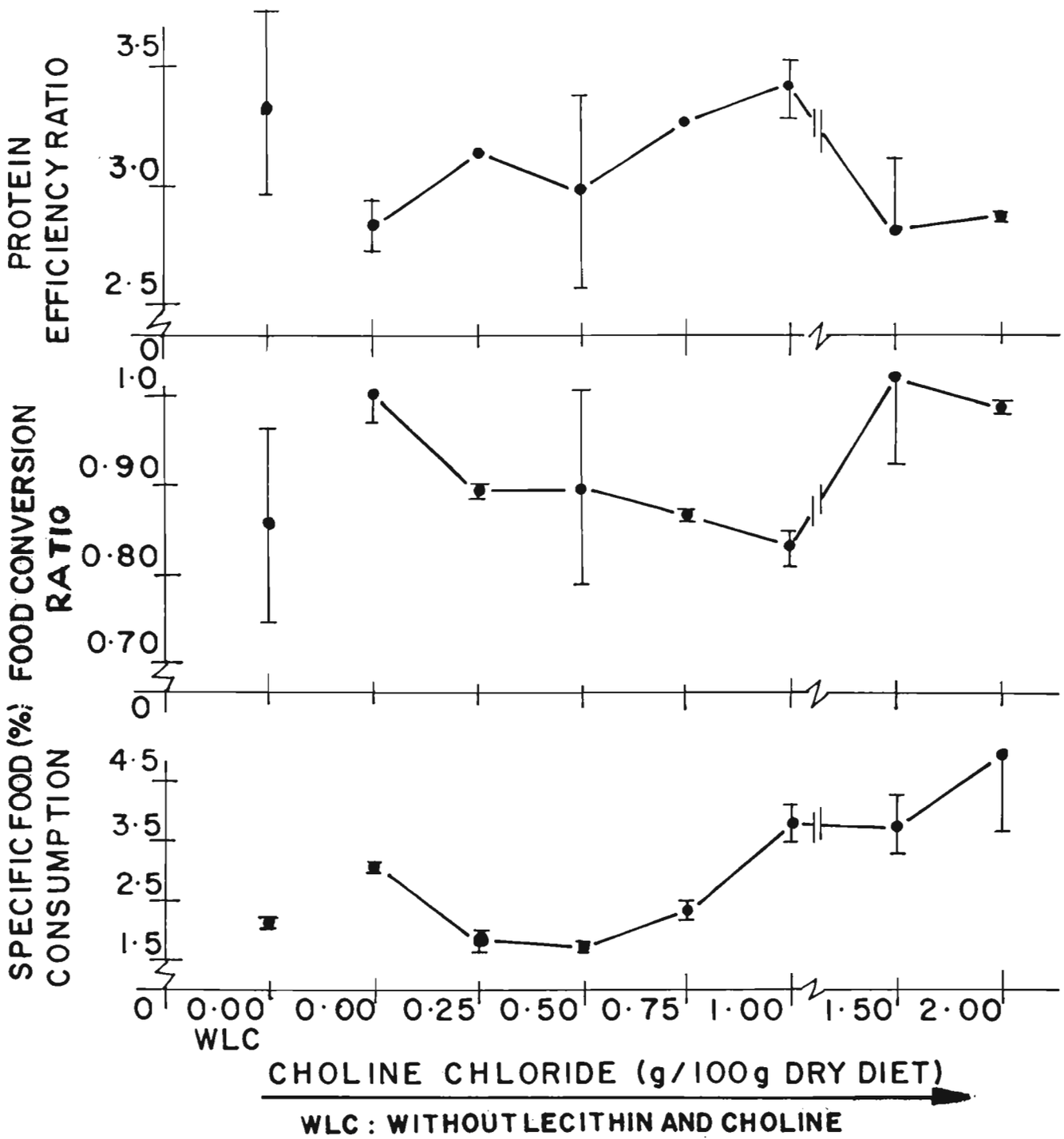
As shown in Fig. 33, food conversion ratio did not show any specific trend. Statistical analysis of the data also did not show any significant effect of the experimental diets on the FCR. The maximum FCR was recorded with 1.5 g of choline (1.02) and minimum with 1.0 g of choline (0.83) in the diet. In all other treatment groups, the FCR ranged between 0.86 and 1.0. Prawns fed on the diet without lecithin and choline, showed slightly lower FCR value (0.86) than those fed without choline in the diet (1.0); but the observed difference was not significant.

Protein Efficiency Ratio (PER):

Protein efficiency ratios (Fig. 33) recorded from the various treatments also did not show any specific trend, with

Fig. 33. SFC, FCR, and PER for diets with different levels of choline chloride.

FIG. 33


PROTEIN EFFICIENCY RATIO
3·5
3·0
2·5
0
FOOD CONVERSION RATIO
1·0
0·90
0·80
0·70
0
SPECIFIC FOOD (%) CONSUMPTION
4·5
3·5
2·5
1·5
0
0·00 WLC
0·00
0·25
0·50
0·75
1·00
1·50
2·00
CHOLINE CHLORIDE (g/100g DRY DIET)
WLC : WITHOUT LECITHIN AND CHOLINE

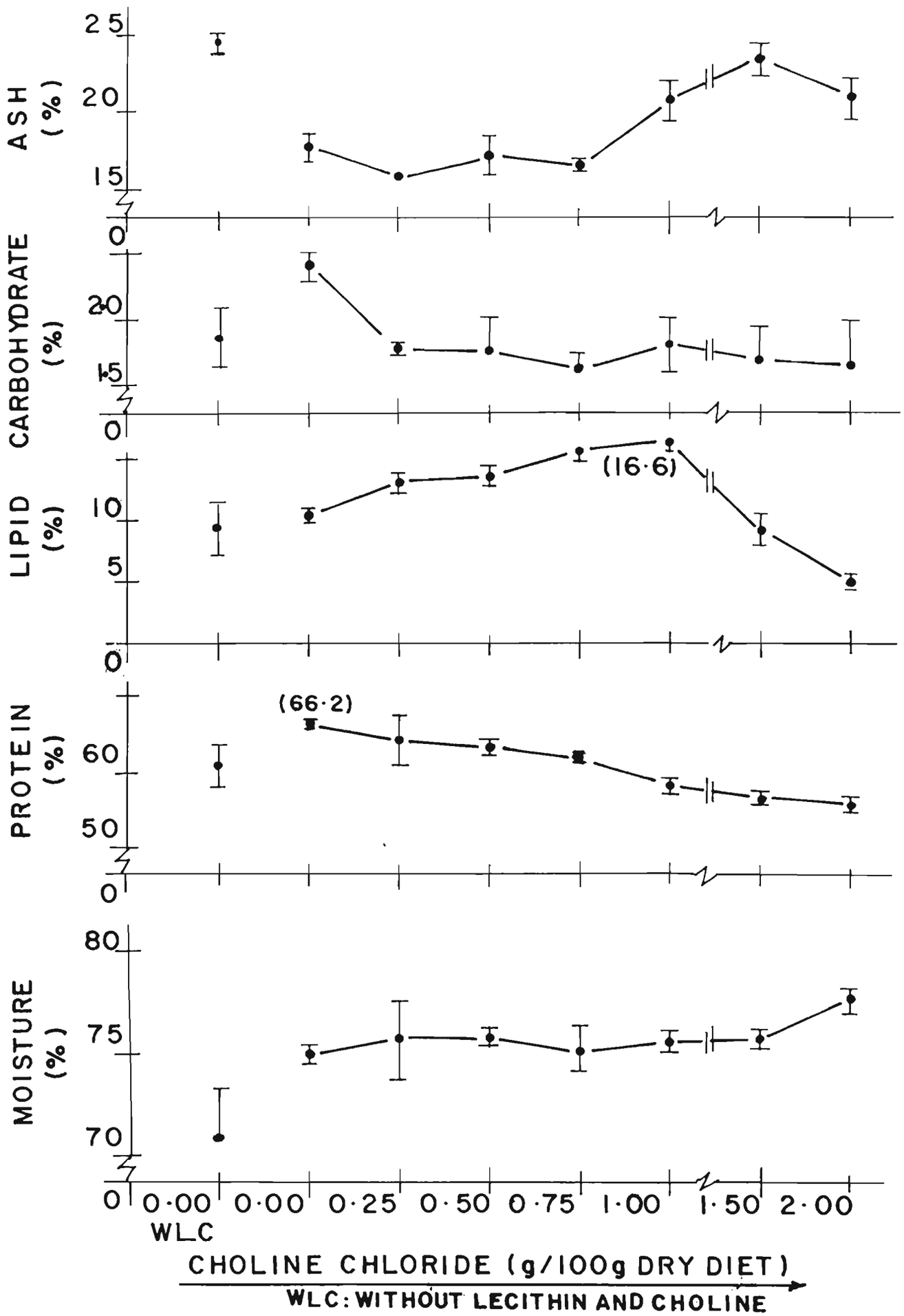
increasing choline concentration in the diets. There were also no significant differences between the PER obtained from the various treatments. The highest PER (3.33) was recorded from the treatment where prawns were fed on a diet containing 1.0 g of choline and the lowest PER with the diets containing 1.5 g of choline (2.74) and 2 g of choline (2.76). The prawns fed on the diet without choline and lecithin recorded relatively high PER (3.28) compared to most other treatment groups. In all other treatments the PER did not show much difference, but ranged from 3.06 to 3.2.

Biochemical Composition:

The moisture, ash, protein and lipid contents of prawns from various treatments are presented in Fig. 34. Analysis of variance of the data showed that the diets significantly ($P < 0.01$) influenced all these parameters. The highest moisture content (76.5%) was recorded in prawns fed on the diet with 2 g of choline and lowest (71.2%) in prawns fed without lecithin and cholinein the diet. In prawns from other treatment groups, the moisture content varied insignificantly between 74 and 75.7%. However, no specific trend could be observed in the moisture content when diets containing increasing concentrations of choline was fed to the prawns (Fig. 34). However, the highest ash content was recorded (Fig. 34) in prawns fed on the diet without choline and lecithin (24.4%) and the lowest in prawns fed on the diet with 0.25 g of choline (15.8%). The prawns

Fig. 34. Biochemical composition of prawns fed diets with different levels of choline chloride.

FIG. 34


ASH (%)
25
20
15
0
CARBOHYDRATE (%)
20
15
0
LIPID (%)
10
5
0
(16·6)
PROTEIN (%)
60
50
0
(66·2)
MOISTURE (%)
80
75
70
0
0·00
WLC
0·00
0·25
0·50
0·75
1·00
1·50
2·00
CHOLINE CHLORIDE (g/100g DRY DIET)
WLC: WITHOUT LECITHIN AND CHOLINE

fed on the diet without lecithin and choline, and those fed on diets containing more than 0.75 g of choline had significantly ($P < 0.05$) higher ash than prawns fed on diets with 0.75 g and lesser concentrations of choline.

The protein content in prawns was observed to decrease with increasing concentrations of choline in the diets (Fig. 34 The prawns fed on diets containing higher dosages of choline (≥ 0.1 g) had significantly ($P < 0.05$) less protein content, compared to prawns fed with lower concentrations of choline in the diets. The maximum protein content (66.2%) was observed in prawns fed on the choline deficient diet and the minimum (53.1%) in prawns fed on the diet containing 2.0 g of choline.

The lipid content in prawns, shown in Fig. 34, was significantly affected ($P < 0.01$) by the experimental diets. The lipid content showed an increasing trend with increasing concentration of choline up to 1 g and thereafter declined sharply with further increase in concentration of the vitamin. The lipid content was significantly ($P < 0.05$) higher in prawns fed on diets containing choline concentrations of 0.25 g, 0.5 g, 0.75 g and 1 g when compared to that found in prawns from other treatments. The lowest lipid was found in prawns fed on the diet with 2g of choline (5.3%). Prawns fed on diets deficient in both choline and lecithin or choline recorded, relatively, lower lipid contents (9.2 and 10.6%, respectively) compared to the prawns fed on diets with choline

ranging from 0.25 to 1 g (13.0 to 16.6%). It is interesting to note that high concentrations of choline in the diet markedly affects the lipid content in prawns.

No significant effect of the diets was observed on the carbohydrate content (Fig. 34) of prawns, though slight differences were observed between the carbohydrate contents of prawns fed on the various diets. The prawns fed on the diet deficient in choline and lecithin had relatively lower carbohydrate content (1.85%) than those fed on the diet deficient in choline (2.4%).

The RNA content in prawns (Fig. 35) was also significantly ($P < 0.05$) affected by the diets. Significant ($P < 0.05$) differences were observed between the RNA content of prawns fed on the diet without choline and that of prawns fed at higher concentrations (≥ 1.5 g) of choline in the diets. The highest RNA content was recorded in prawns fed on the diet deficient in lecithin and choline (2.39 µg/mg); but this was not significantly different from the RNA content in prawns fed on the diet with 1.5 g and 2 g of choline (2.07 µg/mg and 2.22 µg/mg, respectively). The lowest RNA content was found in prawns fed on the diet with 1 g of choline (1.68 µg/mg).

The dry weight/total RNA ratio (Fig. 35) was not significantly affected by the experimental diets. However, with the increasing concentration of choline in the diet, the ratio increased up to 1 g of choline in the diet and thereafter showed

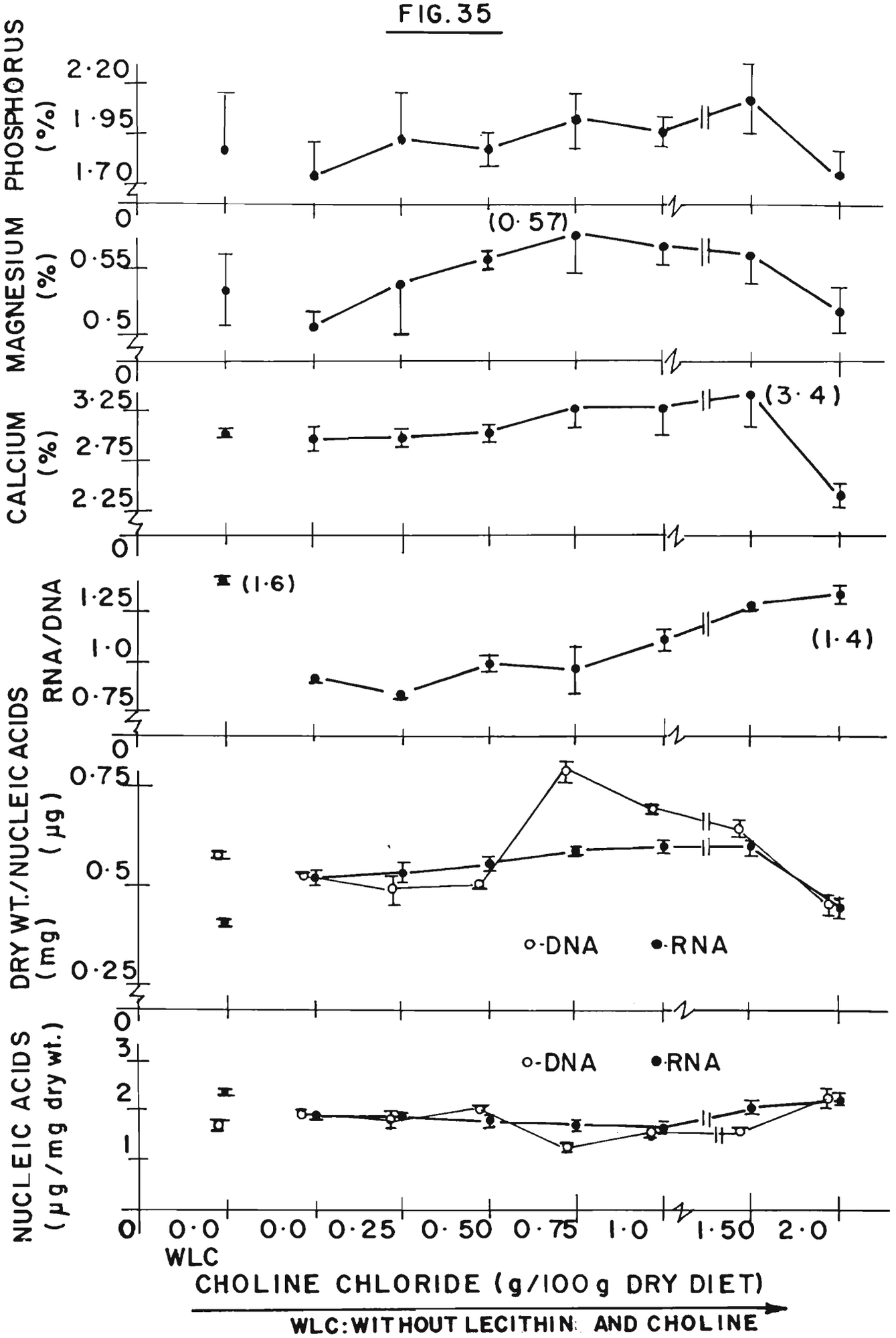
a steady decline. In the lecithin and choline deficient diet fed prawns, the ratio (0.42) was relatively less compared to that obtained with all other diets.

The DNA content of prawns (Fig. 35) showed significant ($P < 0.05$) variation with the increasing concentrations of choline in the diets. The prawns fed on diets with 0.75 g, 1 g and 1.5 g of choline chloride had significantly ($P < 0.05$) higher DNA contents compared to that of prawns from other treatments. However, no specific trend could be observed in DNA with increasing levels of vitamin in the diet. Similarly, dry weight/total DNA ratio also did not show any specific trend (Fig. 35). However, significant ($P < 0.05$) effect of the diets on the dry weight/DNA ratio was observed. The prawns fed on diets containing higher concentrations (0.75 g, 1 g and 1.5 g) of choline had significantly ($P < 0.05$) higher ratios compared to that of prawns from other treatments.

The RNA/DNA ratio (Fig. 35) in prawns was also significantly ($P < 0.01$) influenced by the diets fed to them. The diet without choline and lecithin gave significantly ($P < 0.05$) higher RNA/DNA ratio than diets containing different levels of choline. Also the prawns fed with high concentrations of the vitamin in the diets (> 0.75 g) showed significantly higher ($P < 0.05$) RNA/DNA ratios compared to that of prawns fed on diets containing lower concentrations of the vitamin. The highest ratio was recorded in prawns fed on the diet deficient in both choline and lecithin

Fig. 35. Biochemical composition of prawns fed diets with different levels of choline chloride.

FIG. 35


PHOSPHORUS (%)
2·20
1·95
1·70
0
MAGNESIUM (%)
(0·57)
0·55
0·5
0
CALCIUM (%)
3·25
2·75
2·25
0
(3·4)
RNA/DNA
(1·6)
1·25
1·0
0·75
0
(1·4)
DRY WT./NUCLEIC ACIDS (mg) (µg)
0·75
0·5
0·25
0
o-DNA
•-RNA
NUCLEIC ACIDS (µg/mg dry wt.)
3
2
1
0
o-DNA
•-RNA
0 0·0 0·0 0·25 0·50 0·75 1·0 1·50 2·0
WLC
CHOLINE CHLORIDE (g/100 g DRY DIET)
WLC: WITHOUT LECITHIN AND CHOLINE

(1.6) and the lowest in prawns, fed on the diet containing 0.5 g (0.86) of choline. In all other treatment groups, RNA/DNA ratio ranged between 0.99 to 1.29. However, with increasing concentration of choline in the diet, the ratio showed a declining trend upto 0.5 g choline and thereafter it increased with further increase in choline concentration in the diet.

The various diets fed to the prawns also had significant ($P < 0.05$) effect on the calcium content of prawns between most of the treatments. However, among the treatment groups, prawns fed on the diet with 2 g of choline had significantly ($P < 0.05$) lower calcium content. While, the highest calcium content was recorded in prawns fed with 1.5 g of choline (3.4%), the lowest was recorded in prawns, fed with 2 g of choline (2.3%) in the diet. With increasing levels of choline in the diet, the calcium content showed an increase upto 1.5g and thereafter it sharply declined with further increase in choline concentration in the diet. In all the other treatments, the calcium content ranged from 2.98 to 3.3%. Prawns fed on the diet without lecithin and choline had slightly higher (3.02%) calcium content, than that of prawns fed without choline (2.98%) in the diet; but the observed difference was statistically not significant.

The magnesium content in prawns was not significantly affected by the diets (Fig. 35) and also it did not show any significant variation with the increasing dosages of choline in the diet. The magnesium content in prawns from various treatments ranged between 0.50 and 0.58%.

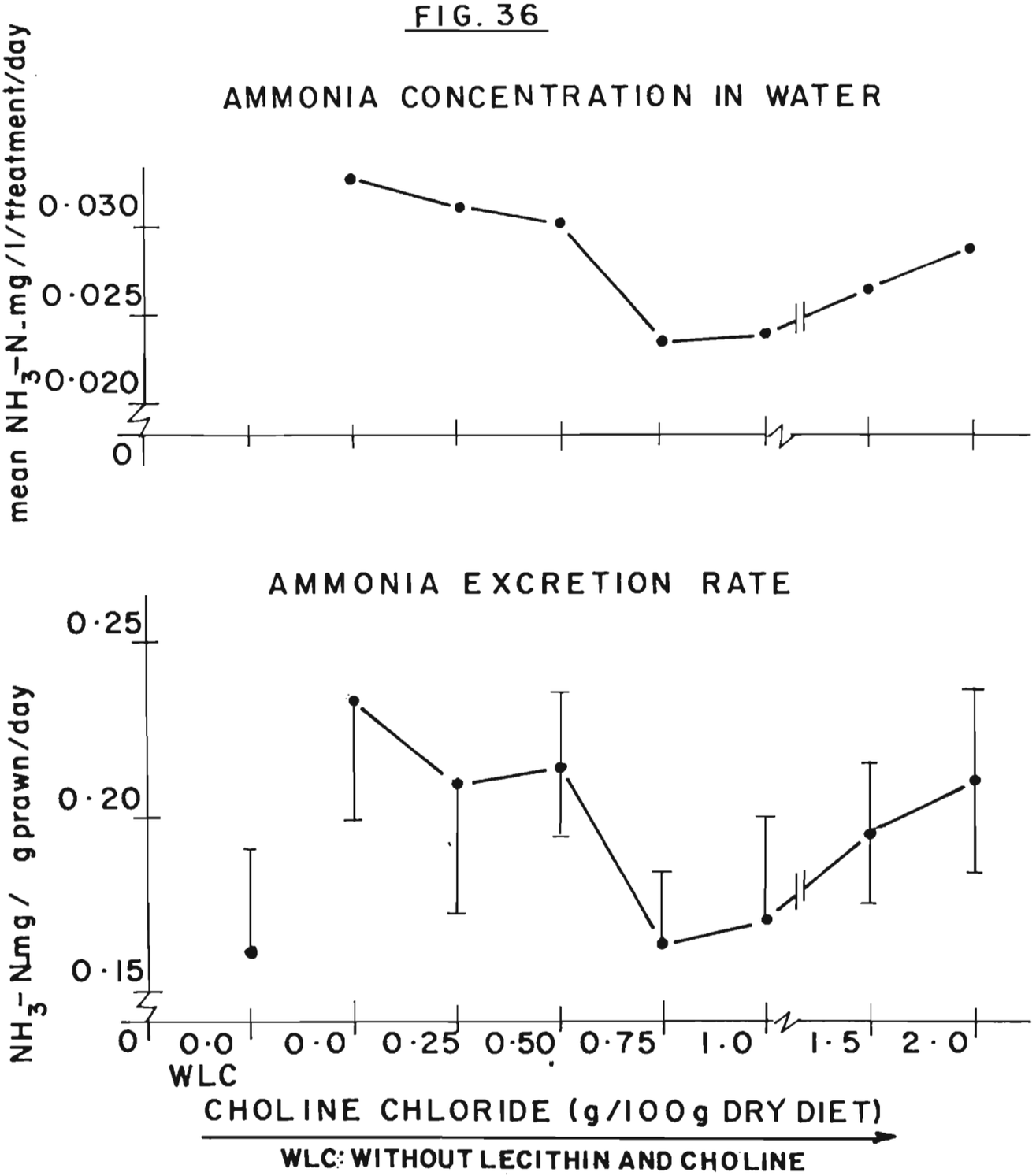
The phosphorus content in prawns (Fig. 35) was significantly ($P<0.05$) affected by the various diets fed to them. It increased with increasing concentrations of choline in the diet upto 1.5 g and thereafter declined sharply with further increase in dietary choline concentration. The prawns fed on diets with 0.75 g to 1.5 g of choline had significantly ($P<0.05$) high phosphorus, compared to all other treatment groups. While the highest phosphorus content was recorded in prawns fed on the diet with 1.5 g of choline (2.26%), the lowest phosphorus content was found in prawns fed on the diet with 2 g of choline (1.51%).

Ammonia Concentration In the Water:

Ammonia concentration in the water was determined from each of the replicate of the treatments to see the effect of dietary choline on the excretion rates. The mean ammonia concentration in each treatment, expressed as mg NH_4-N/l of sea water/day as shown in Fig. 36. In the treatment in which the prawns were fed on the diet deficient in choline and lecithin, the lowest ammonia concentration was recorded. The ammonia concentration was relatively more in the treatment where prawns were fed on the choline deficient diet (0.033 mg/l/d) compared to that in other treatments. Among other treatments, the ones with diets containing 0.75 g and 1 g of choline, showed relatively low ammonia concentration (0.024 mg/l/d).

Fig. 36. Ammonia concentration in seawater and ammonia excretion rate in prawns fed diets with different levels of choline chloride.

FIG. 36


AMMONIA CONCENTRATION IN WATER
mean NH_3-N mg/l/treatment/day
0·030
0·025
0·020
0
AMMONIA EXCRETION RATE
NH_3-N mg / g prawn/day
0·25
0·20
0·15
0
0·0
WLC
0·0
0·25
0·50
0·75
1·0
1·5
2·0
CHOLINE CHLORIDE (g/100g DRY DIET)
WLC: WITHOUT LECITHIN AND CHOLINE

Ammonia Excretion Rates In Prawns:

Experimental prawns, fed on the various diets for 45 days, were selected for determining the ammonia excretion rates. There were no significant differences between the excretion rates of prawns from various treatments (Fig. 36). However, it showed a gradual decrease with increasing dietary choline concentrations upto 0.75 g/100 g diet and thereafter showed an increase with further increase in the choline concentration in the diet. The highest excretion rate was observed in prawns fed on diets deficient in choline (0.23 mg/ g prawn/d) and the lowest in prawns fed on the diet with 0.75 g choline (0.16 mg/ g prawn/d). In all other treatments, ammonia excretion rates varied between 0.17 and 0.21 mg/ g prawn/dy.

O B S E R V A T I O N S

Molting

Molting frequency was studied based on the number of exuviae collected during the experimental study. The number of exuviae (Table 19) recovered from groups fed on the choline and lecithin deficient diet were higher (37 nos) than those fed with only the choline deficient diet (33 nos). During the second half of the experimental study, the exuviae from the former treatment

groups were not consumed by the cohabitors and mostly the exuviae were recovered completely unlike in the latter case. The maximu number of exuviae were recovered from the treatment with 1.5 g of choline (52 nos), which was followed by prawns fed with 0.75 g of choline (47 nos). In all other treatment groups, the number of exuviae recovered ranged from 37 to 41, excepting the treatment group of prawns fed with 2 g choline where it was lowest (32 Nos).

The occurrence of post-molt deaths varied from treatment to treatment and the treatment with the diet deficient in both choline and lecithin recorded higher post-molt deaths (15 nos), compared to the treatment with choline deficient diet (8 Nos). However, among prawn groups fed with graded levels of choline in the diet, the highest number of post-molt deaths occurred in diets containing 1.5 g(16 Nos) choline and the lowest in 0.75 g (11 nos) and 0.25 g(11 nos) choline. In all other treatment groups, the number of post-molt deaths recorded were almost same (14-15 nos).

Food Intake:

Experimental animals during the first two weeks did not show any significant variation in feed intake between treatments. However, aversion towards food was evident in different treatments from the subsequent weeks. The prawns fed on the diet deficient in both choline and lecithin showed poor food intake from the second week onwards, as the left-over food increased with the

TABLE 19: OBSERVATIONS IN PRAWNS FED WITH DIFFERENT EXPERIMENTAL DIETS

Concentration of choline chloride in the diet g/100 g dry diet	Mean nos. of molts recovered	Mean nos. of post-molt deaths	Texture of the body
0.00*	37	15	H
0.00	33	8	H
0.25	37	11	SO
0.50	39	15	H
0.75	47	10	H
1.00	41	14	H
1.50	52	16	SO
2.00	32	14	SO

H - hard, SO - soft.

* Lecithin deleted

experimental period extending. By the fourth week, prawns showed poor attraction towards food and the left-over food was also quite high. Prawns fed on the choline deficient diet consumed almost the entire ration supplied till the fourth week, but, from the fifth week onwards left-over feed was more and some animals were showing aversion towards the feed. However, in other treatments, variation in feed intake was not marked.

Behaviour Towards Light:

Table lamp light (1625 x 10^2 lux), when shown to the experimental tanks, the prawns showed distinct behaviour towards the light. Initially, no changes in behaviour pattern was observed towards light in different treatment groups and in all cases the prawns showed normal behaviour. However, from the third week onwards, prawns fed on the diet deficient in both choline and lecithin, and those fed on the choline deficient diet showed passive movements in response to light. By the end of the fourth week, the animals poorly responded towards light and even on hitting the walls of aquaria or disturbing the water column did not evoke any quick positive responses in these animals. The prawns fed on diets containing various concentrations of choline responded normally to light during the first two weeks. However, variations appeared from the third week onwards in different treatments. Prawns fed with choline concentrations between 0.25 g and 0.75 g in the diet showed normal movements towards light, throughout the 45 days of experiment. However

prawns fed with more than 0.75 g of choline in the diet showed variations in movements from third week onwards and were easily excited in response to table lamp light or when the water column was disturbed. These in-coherent movements in certain cases resulted in death of certain prawns.

Histology:

Prawns fed with the choline lecithin deficient diet and those fed with graded levels of choline in the diet, showed marked variations in the histological details of muscle, nerve cord and hepatopancreas. When both choline and lecithin were deleted from the diet, a slow process of degeneration of the muscle fibres resulted in prawns. Though in the longitudinal section (Plate IV, Fig. 1), degeneration was not prominent; a section passing through the darkened region of the abdominal muscle (as seen externally) revealed accumulation of dense black bodies, probably bacteria. However, these sections did not show any other specific variations in the cellular details with that of sections of muscles of prawns from other treatment groups.

The sections of the nerve cord, however, showed relatively marked variations between various prawn groups. It was observed that in prawns fed on the choline and lecithin deficient diet (Plate IV, Fig. 3 & 4), the nerve cord showed degeneration of the neural mass with a number of empty regions (Fig. 3). However, no degeneration of the neural mass was observed

PLATE IV. Histological changes observed in the muscle and nerve cord of juvenile prawns fed with different concentration of choline.

Fig. 1. Degenerating abdominal muscle of prawns fed on both choline and lecithin deficient diet.

Fig. 2. Normal muscle of prawns fed with both choline and lecithin in the diet.

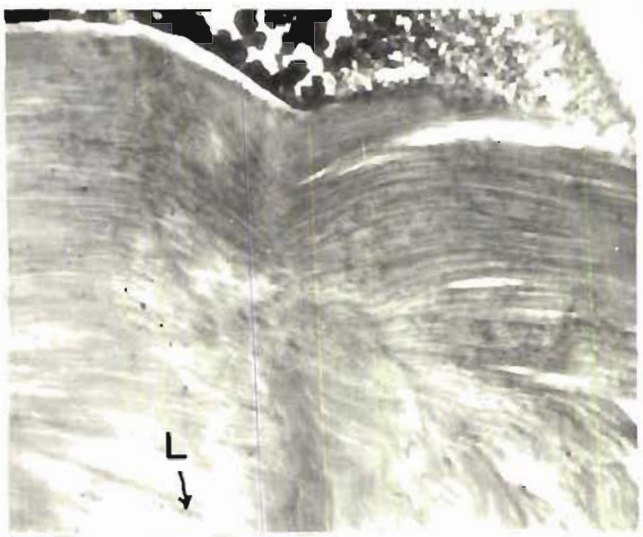
Fig. 3. Transverse section of nervous tissue of prawns fed with choline and lecithin deficient diet showing lytic activity(L) 50 X.

Fig. 4. Transverse section of nervous tissue at higher magnification 100 X.

Fig. 5. Transverse section of normal nervous tissue of prawn fed with both choline and lecithin in the diet 50 X.

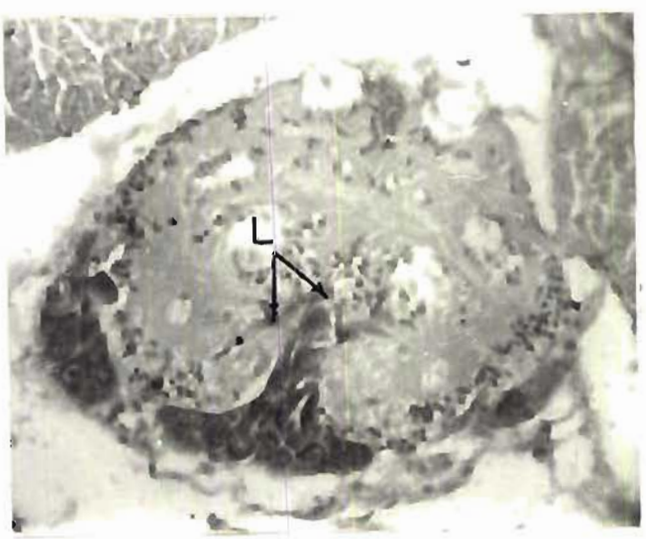
Fig. 6. Transverse section of normal nervous tissue at higher magnification. 100 X.

PLATE IV



1

2



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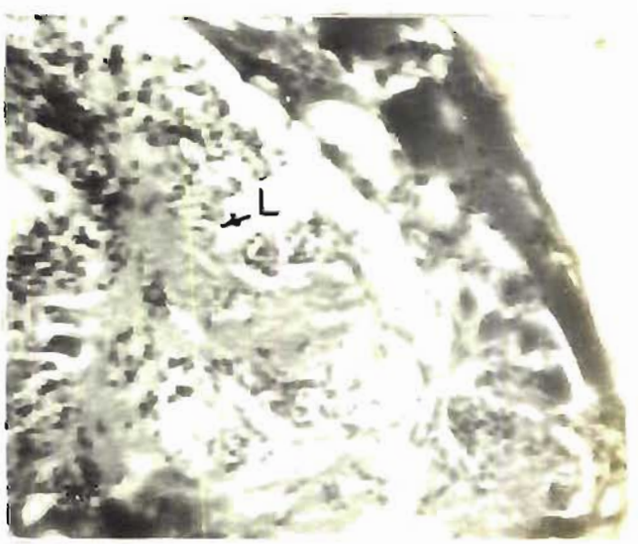

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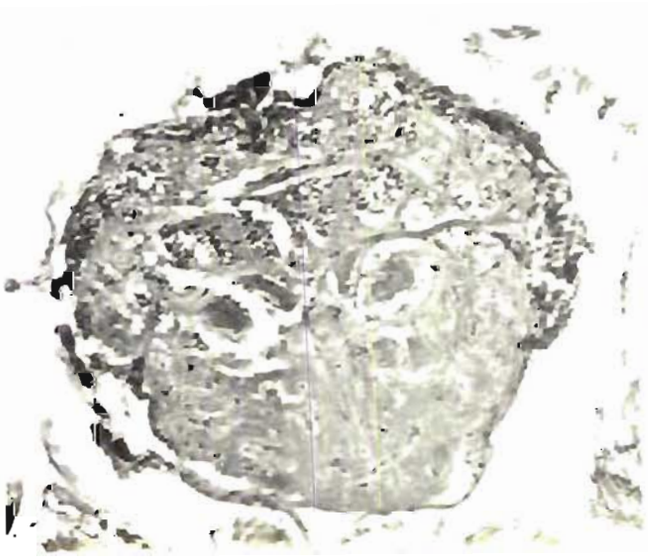


5

6

in prawns from other treatments (Plate IV, Fig. 5 & 6). Nissil bodies were also seen prominently spread within the nerve cord.

Choline deficiency or supplementation in the diet also significantly affected the hepatopancreas cellular details (Plate V, Fig. 1 to 3). When both choline and lecithin were deleted from the diet a general atrophy of the hepatopancreas tubules were observed with most of the tubules, exhibiting (Fig. 1 & 2) complete degeneration. In certain regions of the sections there was dense accumulation of the cellular contents in the proximal end of tubule lumen, leaving the rest of the tubule cells vacant. On the other hand, in prawns from other treatments, the hepatopancreas section showed normal polygonal to circular tubules with prominent tubule cells. There were a number of vacuoles interspersed in these tubule cells.

D I S C U S S I O N

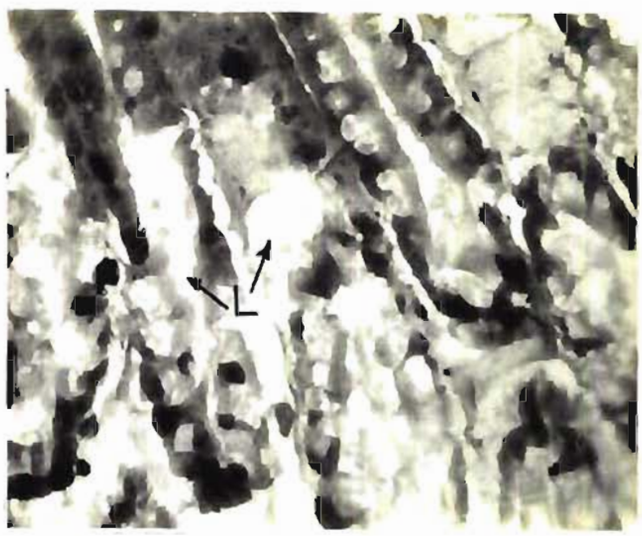
Choline has important functions in the general metabolism of animals viz., as methyl donor in many of the metabolic reactions as lipotropic agent (Best et al., 1946) and as neurotransmitter in the form of acetylcholine (Griffith and Nyc, 1954; West et al., 1966). The vitamin has been reported as a general requirement for insects (Dadd, 1970) and evidences so far indicate that it may be essential one for the crustaceans as well (Bilinski, 1964; D'Abramo and Baum, 1981; Heinen, 1984). Dietary source of

PLATE V. Histological changes observed in the hepatopancreas of juvenile prawns fed with different concentration of choline.

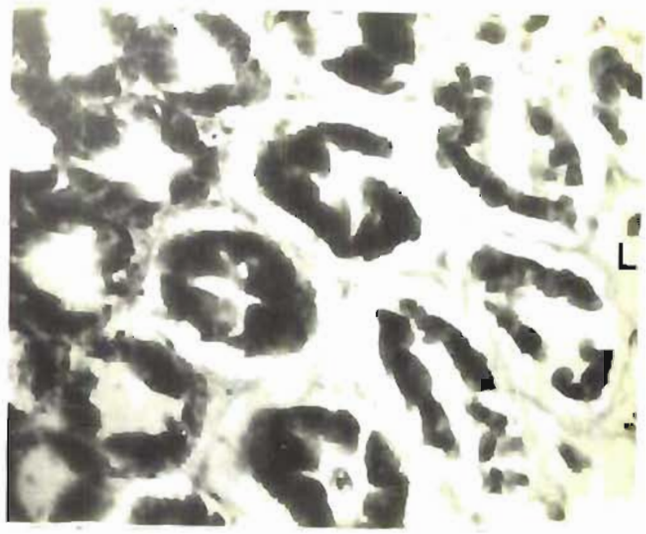
Fig. 1-2. Hepatopancreas tubules of prawns showing degeneration due to both choline and lecithin deletion from the diet.

Fig. 3. Normal hepatopancreas tubules of prawns fed with choline and lecithin in the diet.

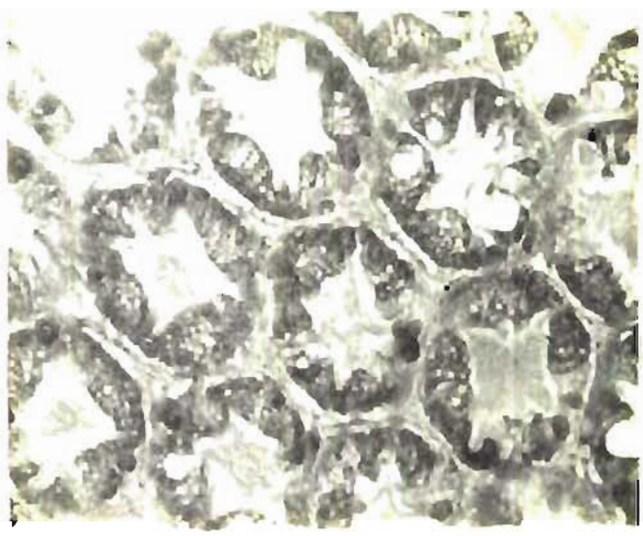
PLATE V



1



2



3

choline also comes from phospholipids (lecithin) which improve the survival rate markedly in crustaceans (Conklin, 1980 Kanazawa, 1983).

In the present study, survival of juvenile Penaeus indicus was significantly influenced by the diets fed to them, with the prawn groups fed on the diet deficient in both choline and lecith and those containing choline concentrations of 1.5 g or more, showing relatively poor survival. The poor survival in the former case may be due to the absence of methyl donors in the form of choline or its derivative-lecithin. The relatively higher survival rate with the choline deficient diet, but with lecithin, indicates that lecithin can offset the requirement of choline, if adequate levels of lecithin is added in the diet. The presence of phospholipid (lecithin) in the diet has been shown to improve survival rate in some crustaceans (Kanazawa et al., 1979; D'Abramo and Baum, 1981). More recently, Conklin et al. (1983) found that soy lecithin efficiently satisfies the requirement of an essential dietary factor for lobster, the absence of which leads to high mortality rates due to the inability of lobsters to successfully extricate themselves from their exoskeleton during molting (Bowser and Rosemark, 1981). Although no symptoms could be observed during the present study, the data for post-molt deaths indicate that deficiency of both choline and lecithin in the diet results in high mortality, probably due to the inability of the prawns to

recoup themselves from the losses in organic and inorganic nutrients during molting. However, deficiency of choline and lecithin could not severely affect the survival rate because of the presence of the sulphur amino acid, methionine (in casein) which has been reported to be another important methyl donor, as well as a precursor molecule for synthesis of choline (Jukes, 1952), and it is assumed that at least a part of this amino acid could have been used for choline synthesis.

The low survival rates occurring in treatment groups fed with 0.25 g or 0.5 g choline could not be explained precisely, but for the reason that they showed almost uniform weekly survival rate upto the third week; and only during fourth and fifth week, there was a spurt in post-molt deaths, for unknown reasons, resulting in poor survival at the end of 45 days of experimental study. However, prawns fed with 0.75 g of choline, recorded high survival and the number of exuviae recovered were also high, indicating that most likely the dietary requirement of choline for these prawns could be around this concentration. On the other hand, the poor survival recorded in prawn groups fed on diets with more than 0.75 g of choline suggests that high concentration of choline may be detrimental for survival. Earlier studies have also shown that high concentration of choline in the diet affects the survival of Artemia (Provasoli and Shiraishi, 1959) and Penaeus japonicus (Deshimaru and Kuroki, 1979).

Growth of prawns was also significantly influenced by the different dietary concentrations of choline. While the highest gain in wet weight was obtained at 0.5 g of choline; the highest gain in dry weight was obtained at 0.75 g choline. These results indicate that the dietary requirement of choline for maximum growth may range from 0.5 g to 0.75 g and that higher concentrations of choline in the diet may affect the survival and growth in prawns significantly. Although, no significant difference was observed between the growth of prawns fed on the diet deficient in both choline and lecithin and diet deficient in only choline, the survival was significantly low in the former group and the growth achieved in these prawns is mainly due to the consumption of dead prawns by cohabitors. Kanazawa (1983) demonstrated that phospholipids containing choline, exerts positive effect on growth and survival of P. japonicus. D'Abramo and Baum (1981) also reported from the studies of M. macrocapa that lecithin contributes to increased growth rates. The present findings in P. indicus also supports the above observations.

Based on their studies on P. japonicus, Kanazawa et al. (1979) reported that the type of lecithin used in the diet significantly influence the survival rate. D'Abramo (1981) reported that soy-lecithin was one of the best type of lecithin for lobsters as this contains phosphatidylcholine, which has an important role in the synthesis of lipoprotein (D'Abramo et al.,

1981). In the present study soy-lecithin was used for formulation of diets and therefore, the enhanced growth and survival rates in the treatments, where prawns were fed with the choline deficient diet is justified. However Deshimaru and Kuroki (1979) reported in P. japonicus that choline is dispensable and disagreed with the results of Kanazawa et al. (1976), who reported that choline deficient diet produce poor growth and high mortality. In the present study, diets containing lecithin 3% and choline chloride more than 0.75 g when fed to juvenile prawns resulted in reduced growth and survival rates, indicating that higher concentrations of choline in the diet may affect the metabolism and thereby growth of the prawns.

The data for specific food consumption, FCR and PER also indicate, the significant influence of the vitamin on these parameters. Although, the prawns fed on the diet with 0.75 g choline grew well with relatively low food intake, the utilization of the food and dietary protein seems to be more efficient in prawns fed on the diet with 1 g of choline. However, the high SFC, FCR and low PER in prawns fed on diet containing choline concentrations of 1.5 and 2 g/100 g dry diet, indicates the detrimental effect of excess vitamin dosage on food and protein utilization.

Biochemical composition of the carcass of prawns shows that choline concentration in the diet significantly influences prawn's metabolism. Moisture content was significantly lower

in prawns fed on the diet deficient in choline and lecithin as compared to prawns fed on diets with various concentrations of choline. This indicates that deficiency of choline does significantly affect the level of nutrients deposition.

The prawns fed on diets with 1 g or more of choline had significantly high ash and significantly low protein and lipid contents compared to prawns fed with less than 1 g choline. These prawns were also found to be highly active, molted faster than the others and in most treatments, higher post-molt deaths were recorded. These observations indicate that possibly high concentrations of choline may have some influence on the molting behaviour, resulting to faster mobilization and uptake of inorganic constituents.

On the other hand, the various nutrients in prawns fed with less than 1 g choline showed normal deposition, and normal behavioural activities. Yet, distinct abnormal behaviour was observed in the case of prawns fed on diets deficient in choline and lecithin. Although the body compozition did not show marked changes compared to choline fed prawns at lower concentrations, the passive activity of prawns during the experimentation, indicates the possible role of choline or lecithin in the transmission of stimuli. As it is known that acetylcholine is an important neural transmitter and choline is the precussor molecule for acetylcholine, the inactivity of these prawns can be attributed to the deficiency of methyl donors in the form of

choline or its derivative lecithin (Griffith and Nyc, 1954; West et al., 1966).

Besides, histological studies of muscles, nerve and hepatopancreas of prawns fed without choline and lecithin in the diet showed higher lytic affected areas than that of only choline deficient diet fed prawns. The lysis affected areas in various tissues clearly demonstrate the involvement of choline or its derivative in the general maintenance of the body through its involvement as an important methyl donor in various metabolic reactions. But no marked variations were observed in the various tissues in prawns fed with various concentrations of choline, eventhough biochemical composition of prawns significantly varied between treatments indicating that choline might have important role in the maintenance of structural integrity of various tissues in prawns.

Thus, it appears that the concentration of inorganic and organic constituents in the tissues can be affected by stress induced by dietary deficiency, resulting in physiological ionic imbalances, disturbances in normal neuromuscular stimuli transmission and in the poor formation of phospholipids (Levin, 1976) which are integral constituents in the biomembranes. Thus, the uptake, repletion and depletion of these constituents especially inorganic ions form an important aspect of study in animals when fed with different dietary constituents.

Amongst the organic constituents, protein and lipid contents are important characteristics in determining the possible physiological activity undergoing in the prawns fed with different concentration of choline. The data in the present study shows clearly that when both choline and lecithin are deleted from the diet, the prawns record significantly high protein content than most other prawns fed with choline in the diet. Similarly, prawns when fed with only lecithin in the diet record higher protein content. However, the RNA-DNA ratio shows insignificant values compared to other treatments indicating that the protein synthesis is notmuch affected by the deletion of choline and lecithin alone from the diet. However, the higher protein content in these prawns may be due to subdued physiological activities as indicated by the low ammonia excretion rates and passive movements observed in these prawns. So, for the general body maintenance, possibly breakdown of lipids and carbohydrates takes place in these prawns.

On the other hand, prawns fed with choline in the diet showed characteristically a declining trend in protein content in contrast to the increasing trend of RNA-DNA ratio, with increasing concentration of choline in the diet. However, the ammonia excretion rates in these did not show any specific trend, indicating that possibly at higher concentrations of choline in the diet, the proteins are catabolised faster for

energy purpose as a result of hyperactivity. But at lower concentrations (less than 1 g) of choline in the diet, the proteins are utilized more for growth than for energy production, and so the prawns exhibited normal activity to various stimuli.

On the other hand, choline concentration in the diets has been found to affect the lipid content in prawns significantly. In other animals, choline deficiency has been reported to affect the synthesis and transport of triglycerides (Mookerjea, 1971) and choline supplementation in the diet results in reduced fat and cholesterol contents of livers (Best et al., 1946). In the present study, a gradual rise in lipid content was observed with the increase in dietary supplementation of choline upto 1 g and thereafter a sharp decline was obtained in the lipid content, with further increase in the concentration of the choline in the diet. Decline in lipid content at higher concentrations of dietary choline has also been reported in chicks (Jukes, 1952) and this decline in lipid could be because of reduction in fat and cholesterol contents of the liver (Best et al., 1946).

Ogino et al. (1970b) in carps showed that with increasing concentrations of choline, accumulation of neutral fats results in the hepatopancreas. So, it is likely that in the present study also, with increasing concentration of choline in the diet, neutral fats are accumulated more in the hepatopancreas and for this reason the total lipid in prawns fed with 1 g choline was highest, closely followed by 0.75 g choline in the diet. However, when both choline and lecithin or choline alone was

deleted from the diet, low lipid content in prawns results probably due to the interference in the synthesis of triglycerides as observed by Jukes (1952) and Mookerjea (1971) in other animals.

Histological examination of muscle, nerve and hepatopancreas of prawns fed on the diets deficient in both choline and lecithin, and only choline showed marked degeneration of these tissues. The hepatopancreas in either cases was found to be affected by the lytic activity, resulting in emptying of number of hepatopancreas cells with the cell membrane alone remaining in these ghost cells. Probably, the breakdown of protein - phospholipid complex in biomembranes results in the damages of these tissues. The involvement of choline in the metabolism of lipids particularly that of phospholipids is well established by Jukes (1952), from radioactive studies. So in the present study under choline deficiency, the phospholipid content of the tissues might have been affected resulting in the breakdown of biomembrane linkages and thus causing damage to the tissues.

C O N C L U S I O N S

The present study shows that choline is an essential vitamin in the diet and that the preferable level of choline in the diet could be about 0.75 g/100 g dry diet, since the prawns were observed to show highest survival and growth, relatively low food conversion ratio and high concentrations of organic nutrients deposition. Yet, amongst the other tested vitamin levels, 0.5 g and 1.0 g choline also supported good growth and food efficiency, even though the survival rate was slightly lower than the above group of prawns. On the other hand, higher concentrations of ($\geqslant$ 1.0 g) choline significantly affect growth and food efficiency, protein utilization and body composition. However, lecithin supplementation shows improved growth and survival in choline deficient diet fed prawns, suggesting that lecithin has some role in influencing the growth and survival.

It is evident, from the present study that deficiency of lecithin along with choline, affects the metabolism of the prawn, resulting in high mortality rate by the third week. Similar observations have been made in other crustaceans by Conklin (1980), Kanazawa (1983), D'Abramo and Baum (1981) D'Abramo *et al.* (1981) and they conclude that crustaceans have a requirement for lecithin for good survival and growth. In the present study, improved growth and survival on

supplementation of lecithin in choline deficient diet fed prawns gives an indication that lecithin can partially offset the requirement for choline in prawns, since these prawns can derive choline from lecithin.

The preferable levels of choline observed in the present study seems to be within the ranges reported by others for various crustacean species. D'Abramo and Baum (1981) showed a requirement some where in between 750-850 mg choline/100 g dry particulate diet for Moina macrocopa and they presume that the requirement was same as that for insects, about 150-900 mg/100 g diet (Dadd, 1970). They admit that the value was quantitatively higher than the requirement of about 45 mg/100 g reported by Kanazawa et al. (1976) for P. japonicus and attribute this wide variation to the accelerated growth rates associated with the warm (26°C) culture temperature. If temperature has a dominant role in determining the quantitative requirement of choline for a species, then the present findings will hold good for the juvenile prawns, as they were reared at relatively higher water temperatures(28-30°C). However, in dietary formulation for various experimental studies in P. japonicus, Kanazawa and his group (Kanazawa et al., 1970, 1976; Villegas and Kanazawa, 1980) use choline chloride ranging from 120 to 300 mg/100 g dry diet in their vitamin mixtures. So comparing the habitat of the two species, P. japonicus and P. indicus, the present findings for the latter species holds good because of the higher rearing temperatures.

According to Deshimaru and Kuroki (1979), choline is dispensable in prawns (P. japonicus) and contradicted the findings of Kanazawa et al. (1976). But the present study amply supports the findings of D'Abramo and Baum (1981), Kanazawa (1983) and Heinen (1984) that choline is essential and indispensable, if lecithin is not one of the dietary components.

CHAPTER-VI

THIAMINE REQUIREMENT

INTRODUCTION

Late in nineteenth century, Eijkman (1897)(cited, Goldsmith 1964)for the first time identified polyneuritis 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, characterised 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, Schneberger (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 Spies (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 (Krampitz, 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 (Fisher, 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 Moina, 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 shows 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, 1976a;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 upto 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

Parameter	Mean values
Temperature (°C)	28.6 ± 0.961
Salinity (‰)	21.60 ± 1.86
pH	8.02 ± 0.56
Ammonia concentration in the water (NH_4-N mg/l/d)	0.015 ± 0.0035
Initial length (mm)	18.0 ± 0.84
Initial weight (mg)	33.6 ± 0.0032

TABLE 21: DIETARY COMPOSITION OF EXPERIMENTAL DIETS WITH GRADED LEVELS OF THIAMINE HYDROCHLORIDE

Ingredient	g/100 g							
Thiamine Hydrochloride	0.0*	0.0	0.002	0.004	0.006	0.008	0.01	0.015
α-Cellulose	0.020	0.020	0.018	0.016	0.014	0.012	0.010	0.005

*Carbohydrate deleted

RESULTS AND OBSERVATIONS

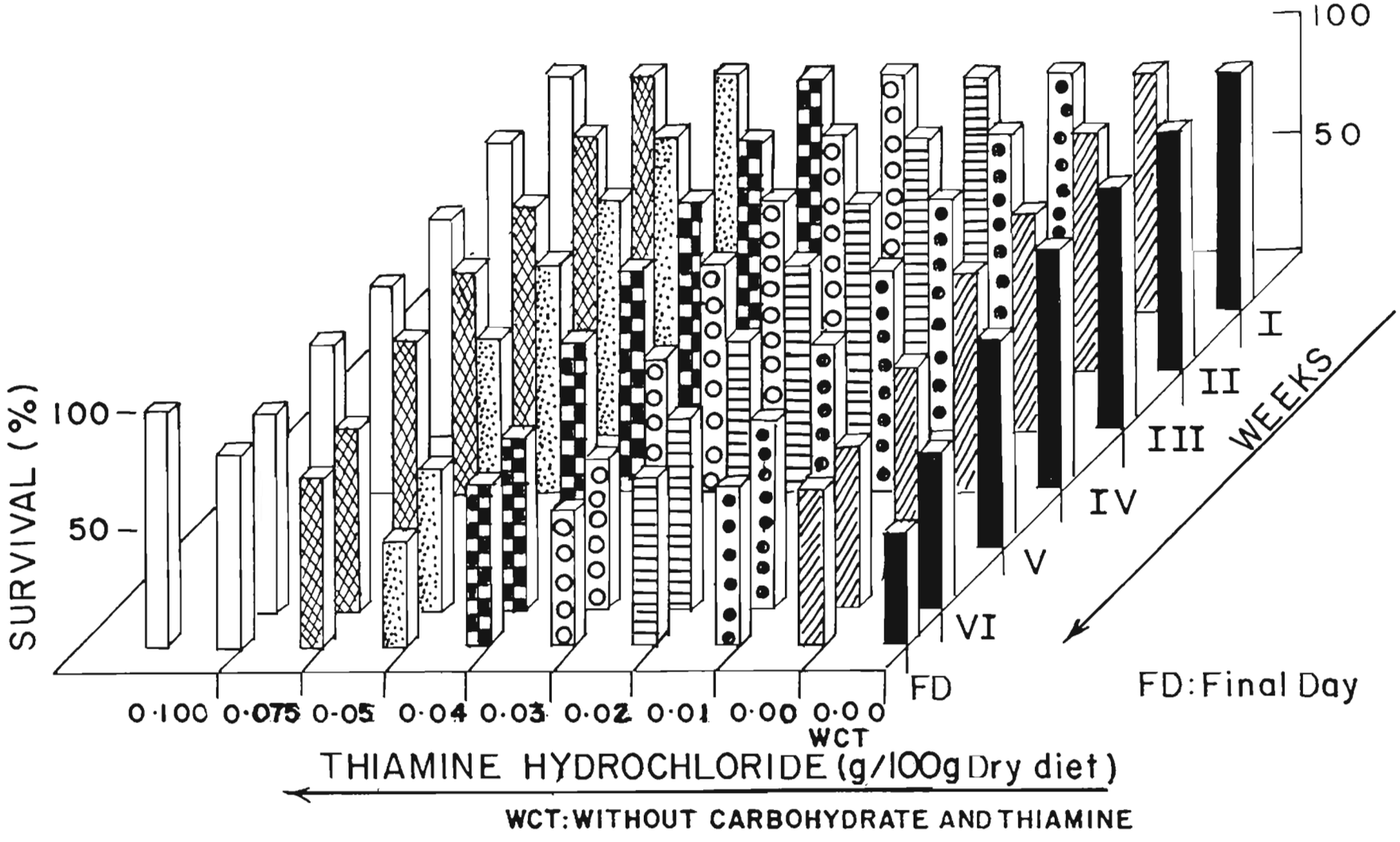
To determine the tentative dietary requirement of thiamine for juvenile *P. indicus*, an experimental study was conducted using purified diets containing graded levels of thiamine (0.0 - 0.1 g/100 g dry diet) and the results as well as significant observations are presented here.

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


100
50
SURVIVAL (%)
100
50
I
II
III
IV
V
VI
WEEKS
FD
FD: Final Day
0·100 0·075 0·05 0·04 0·03 0·02 0·01 0·00 0·00
WCT
THIAMINE HYDROCHLORIDE (g/100g Dry diet)
WCT: WITHOUT CARBOHYDRATE AND THIAMINE

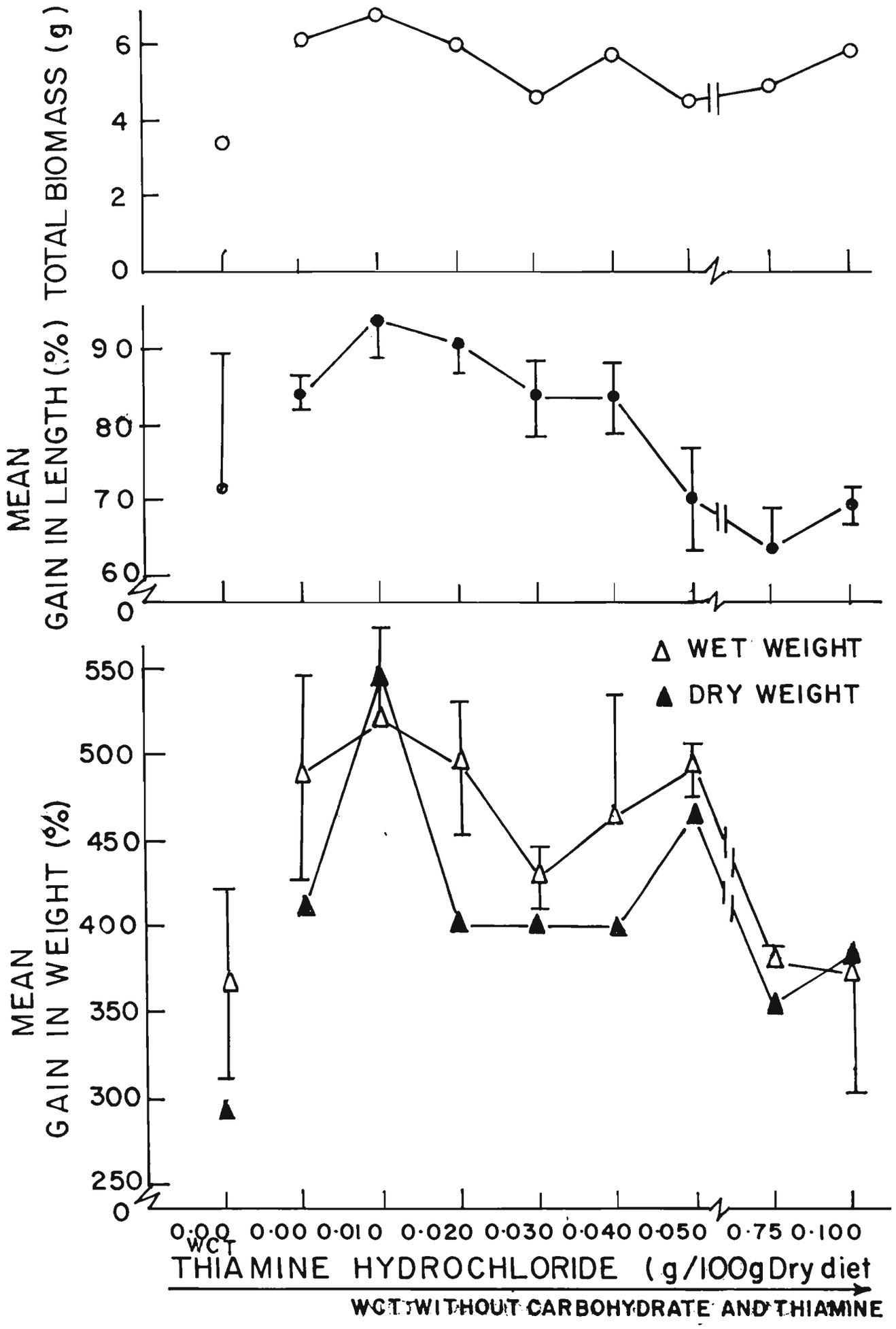
Growth:

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 ($\geqslant 0.075$ 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 ($\geqslant 0.075$ 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.

FIG. 38


MEAN TOTAL BIOMASS (g)
0
2
4
6
MEAN GAIN IN LENGTH (%)
0
60
70
80
90
MEAN GAIN IN WEIGHT (%)
0
250
300
350
400
450
500
550
Δ WET WEIGHT
▲ DRY WEIGHT
0·00 WCT
0·00 0·010 0·020 0·030 0·040 0·050 0·75 0·100
THIAMINE HYDROCHLORIDE (g/100g Dry diet
WCT: WITHOUT CARBOHYDRATE AND THIAMINE

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.

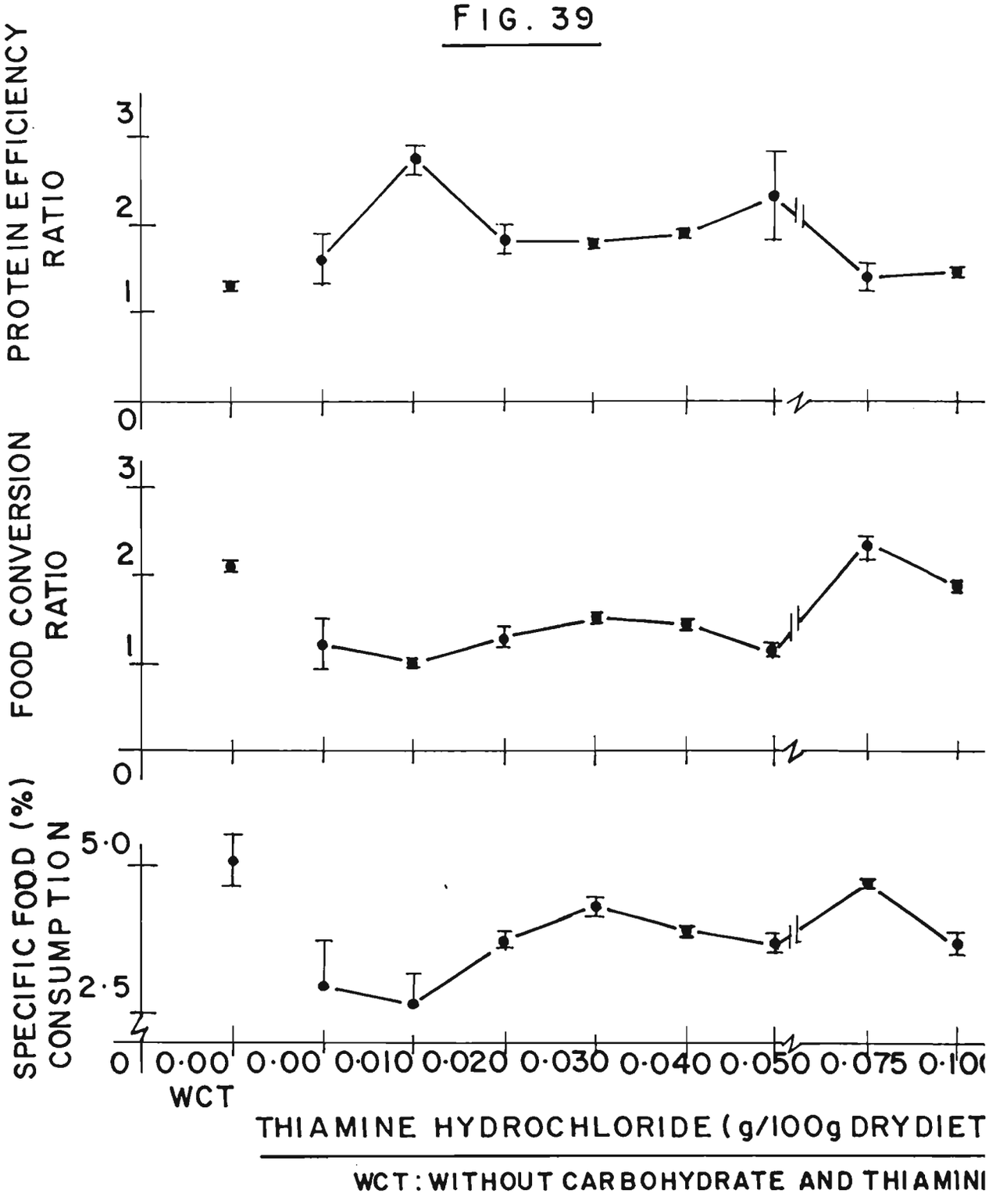
The experimental diets also had highly significant ($P < 0.01$) influence on the mean percent dry weight gain of prawns (Fig. 38). The highest percent gain in dry weight was observed in prawns fed on the diet with 0.01 g of thiamine (545.6%) and the lowest in prawns fed on the diet without carbohydrate 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



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 these 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:

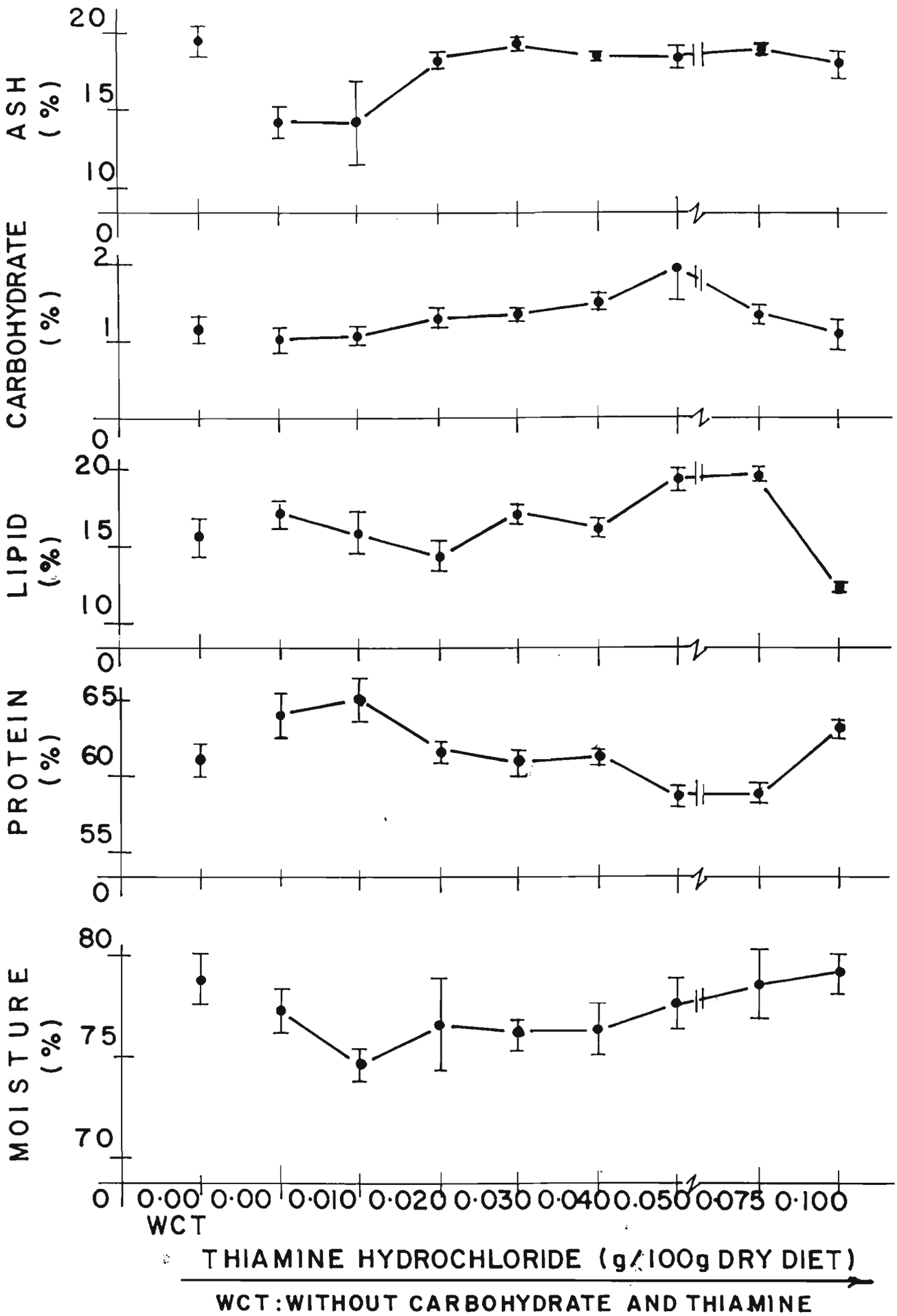
The moisture, ash, protein, lipid and carbohydrate contents of prawns recorded after the experiment are shown in Fig. 40. Analysis of variance of the data showed that the dietary thiamine level significantly ($P < 0.05$) influence the moisture, ash, protein and lipid contents.

The moisture content was significantly ($P < 0.05$) higher in prawns fed on diets with thiamine concentration of 0.075 and 0.10 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 78.8%.

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.

FIG. 40


ASH (%)
20
15
10
0
CARBOHYDRATE (%)
2
1
0
LIPID (%)
20
15
10
0
PROTEIN (%)
65
60
55
0
MOISTURE (%)
80
75
70
0
0·00
0·00
0·010
0·020
0·030
0·040
0·050
0·075
0·100
WCT
THIAMINE HYDROCHLORIDE (g/100g DRY DIET)
WCT: WITHOUT CARBOHYDRATE AND THIAMINE

of 0.01 g or more had 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 upto 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

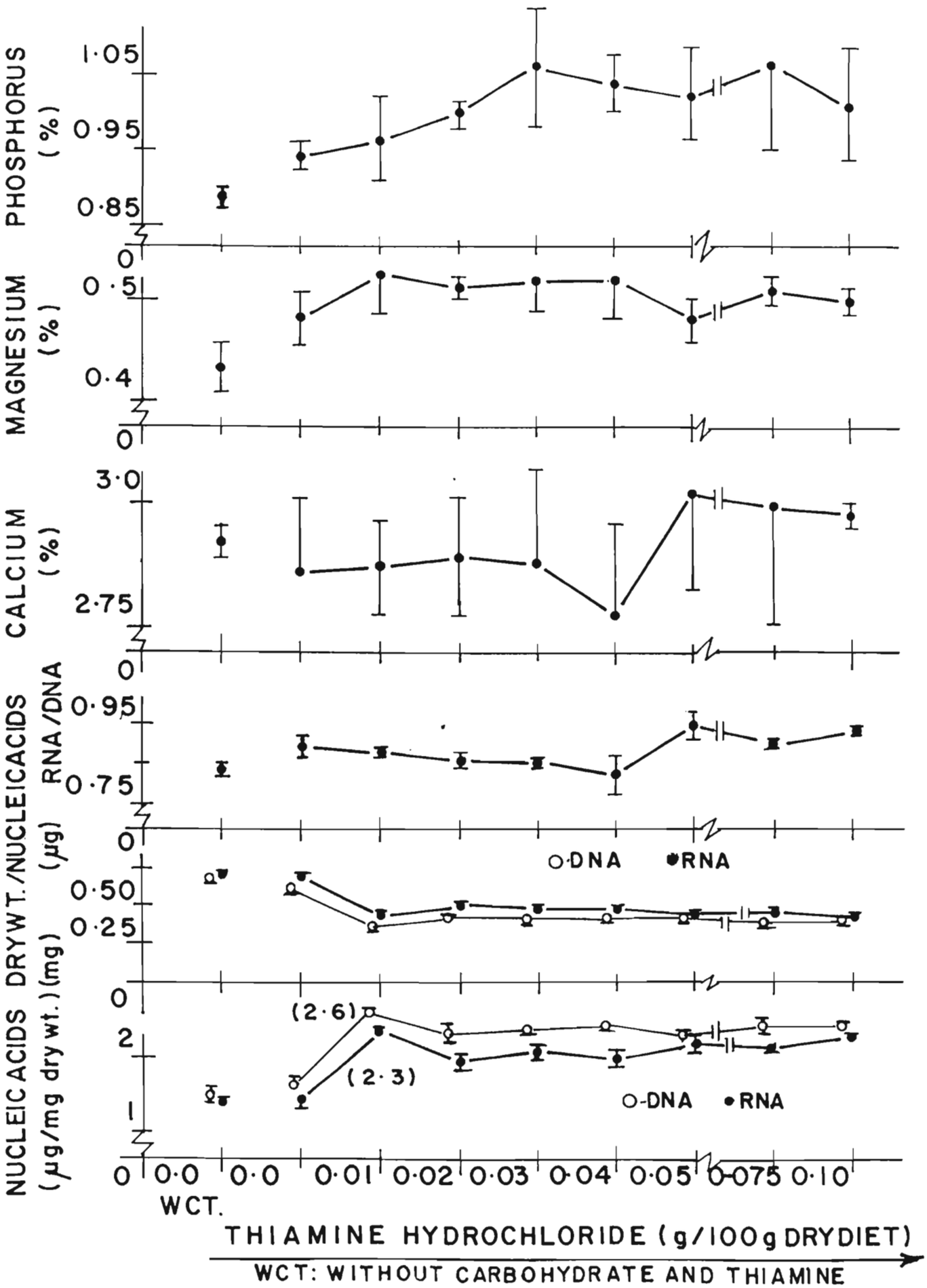
thiamine. The maximum RNA content was recorded with 0.01 g of thiamine (2.3 µg/mg) and the minimum in prawns fed on the diet without both carbohydrate and thiamine (1.4 µg/mg).

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 th re

Fig. 41. Biochemical composition of prawns fed diets with different levels of thiamine hydrochloride.

FIG. 41


PHOSPHORUS (%)
1·05
0·95
0·85
0
MAGNESIUM (%)
0·5
0·4
0
CALCIUM (%)
3·0
2·75
0
RNA/DNA (µg)
0·95
0·75
0
○-DNA ●RNA
0·50
0·25
0
(2·6)
(2·3)
2
1
○-DNA ●RNA
NUCLEIC ACIDS DRY WT./NUCLEICACIDS (µg/mg dry wt.)(mg)
0 0·0 0·0 0·01 0·02 0·03 0·04 0·05 0·075 0·10
WCT.
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 dist 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 22. AMMONIA CONCENTRATION IN SEAWATER HELD IN EXPERIMENTAL AQUARIA

Concentration of thiamine hydrochloride g/100 g dry diet	Mean ammonia concentration in seawater, mg/l/d
0.0*	0.011
0.0	0.024
0.01	0.013
0.02	0.014
0.03	0.014
0.04	0.013
0.05	0.014
0.075	0.015
0.10	0.017

*Carbohydrate deleted.

O B S E R V A T I O N S

Molting:

There were differences in the number of exuviae collected from various treatments (Table 23). Prawns in treatment wit 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 cohabitors. 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 23: OBSERVATIONS IN PRAWNS FED WITH DIFFERENT EXPERIMENTAL DIETS

Concentration of thiamine hydrochloride in the diet g/100 g dry diet	Mean nos.of molts recovered	Mean nos. of post-molt deaths	Texture of the body
0.00*	13	11	SO
0.00	22	9	H
0.002	35	14	H
0.004	36	11	H
0.006	21	13	H
0.008	32	13	H
0.010	31	19	H
0.015	17	8	H
0.020	18	7	H

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 (1625×10^2 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 upto 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.

D I S C U S S I O N

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 the diets 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 suggests 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 insignificant, 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 Heinen (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 probabl 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 (Conklin and Provasoli, 1977). This may be due to negative feed back 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 PER 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, FCR and PER 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, [illegible] due to the partial utilization of tissue carbohydrates, lipids and proteins, higher moisture and ash contents occurred. The percent ash content in prawns increased significantly when dietary levels

of thiamine was above 0.01 g, indicating that hypervitaminosis. (>0.02g) 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 suggests 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.

C O N C L U S I O N S

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, PER 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.

CHAPTER-VII

PYRIDOXINE REQUIREMENT

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

Pyridoxine (vitamin B_6) is an essential vitamin, required by all animal species, so far studied. This vitamin is widely distributed in almost all the natural products, principally as complexes of proteins, such as pyridoxal phosphate, which is an active coenzyme (Sinclair, 1953). It was first defined by György (1934) as that part of the vitamin B complex, essentially required for curing a specific dermatitis developed by rats fed on a vitamin-free diet. Pyridoxine was first isolated by Keresztesy and Stevens (1938), and subsequently in the same year by four independant researchers including György (1938) and by Lepkorsky (1938).

Pyridoxine is highly soluble in water and insoluble in organic solvents. Since it is a base, it easily forms hydrochloride, and this form is widely preferred for dietary preparations because of its thermostability (Halver, 1953). The active form of vitamin B_6 is the coenzyme pyridoxal phosphate (PLP), which is also sometimes referred to as codecarboxylase or co-transaminase.

The coenzyme pyridoxal phosphate participates in enzymatic systems in amino acid decarboxylation, transamination, recemization, desulfhydration of methionine and cystein,

deamination of hydroxy amino acids (Cori and Illingworth, 1957) and in a variety of miscellaneous transformations. PLP functions as a coenzyme for 22 or more transaminases, occurring in the body (Chow, 1964) and in the decarboxylation of 5-hydroxytryptophan to produce serotonin (Halver, 1972) and it activates desulfhydrase in converting cysteine to pyruvic acid. It is also involved in the synthesis of porphyrin, γ-aminolevulinic acid and RNA (Halver, 1972).

Witten and Holman (1952) and Sato(1970) suggested that the vitamin is essential for the metabolism of unsaturated fatty acids, specifically in the conversion of linoleic acid to arachidonic acid. However, Muellar(1964) considers that an effect of the vitamin on fatty acid metabolism is probably indirect and still others suggest no relationship between vitamin B_6 and essential fatty acids metabolism(Johnston et al., 1961; Williams and Scheier, 1961).

Vitamin B_6 deficiency has been shown to affect the amino acids and protein metabolism (Axelrod et al., 1945) in higher organisms. Also, a number of genetic diseases involving vitamin B_6 dependant enzyme systems have been reported (György, 1971; Mudd, 1971; Brown, 1972). In rats, it has been shown that there is a relationship between pyridoxine and essential fatty acids in preventing some of the diseases associated with the deletion of either of thecompounds individually or together from the diet (Witten and Holman, 1952).

Sure and Esterling (1949) studied the effect of high-protein and low-fat diet, but deficient in pyridoxine in rats and reported poor gain in weight, less deposition of fat, ash and protein than their control pair mates. Similarly, Quackenbuch et al. (1942); Sherman (1950) and Carter and Phizackerely (1951), showed relationship between pyridoxine and fat metabolism. In some studies on rats, it has been shown that under pyridoxine deficiency, vitamin B_{12} absorption is impaired (Yeh and Chow, 1959) and Na^+, K^+ electrolyte balance in the blood sera is disturbed (Chow, 1964).

In aquatic species, Tunison et al. (1943) were the pioneers in identifying the importance of pyridoxine and reported the quantitative requirements for some of the fishes. Subsequently, deficiency studies in fish, such as trout (McLaren et al., 1947a) and salmon (Halver, 1953) were carried out. When pyridoxine deficient diets were administered to salmon, acute condition develops in 14-21 days and the entire population dies after 28 days, even when fed with 50% or more protein at a water temperature of 12-15°C. The deficiency symptoms observed in these fish were epileptic type fits, general nervous disorders, hyperirritability, alteration in the control of melanophores, and edema in the peritoneal cavity (Halver, 1953 ; 1957., Coates and Halver, 1958)

The quantitative requirement of pyridoxine has not been widely studied in fish. The lack of specific deficiency

symptoms has been a distinct handicap for the precise work. The predominant number of attempts to measure the requirements for pyridoxine rely upon rates of growth of animals at the same level of the vitamin or at several increasing levels, under conditions of uncontrollable food consumption permitting normal growth. Brown and Sturtevant (1949) advocated a requirement of 1 mg of pyridoxine/kg of diet in rats, but Mills (1943) found a requirement of 2 mg/kg of food for rats irrespective of ambient temperatures. Hogan et al. (1941), Briggs et al. (1942) and Kradzer et al. (1947) reported 2.75-3.0 mg/kg of diets for chicks. In fishes, the requirement of pyridoxine ranges from 5-20 mg/kg dry diet (Halver, 1972). Though in crustaceans few works have been done (Deshimaru and Kuroki, 1979) as to the requirement, yet most nutritionists supplement their diets with pyridoxine (New, 1976a) ranging from 60-100 mg/kg dry diet (Kanazawa et al., 1970; Deshimaru and Shigueno, 1972) or 10-50 mg/kg dry diet (Forster and Beard, 1973; Deshimaru and Kuroki, 1974a).

The intimate association of pyridoxine with various phases of amino acids metabolism implies that the pyridoxine requirement of an animal should be greater on a high protein than on a low protein diet (Cerecedo and Foy, 1944). Significantly, food intake remains unaltered as the protein level in the diet of rats increase from 15-60%, but the pyridoxine requirement decreased (Miller and Baumann, 1945; Morgan et al.,

1946). However, supplementation of moderately high protein diets by cystine (Page and Gingras, 1947), methionine (Carecedo et al., 1948), lysine, glutamic acid and histidine (Beaton, 1954) increased the pyridoxine requirements.

The few studies so far made in crustaceans, indicate that higher dosages of pyridoxine in the diet has detrimental effect, as in Artemia (Provasoli and Shiraishi, 1959), Moina (Conklin and Provasoli, 1977) and P. japonicus (Deshimaru and Kuroki, 1979). Some studies have reported slower growth rate in crustaceans (P. japonicus) in terms of gain in length under pyridoxine deficiency (Heinen, 1984), as compared to the controls. Thus, even though studies on the requirement of the vitamin are scanty, it has been visualized that crustaceans have dietary requirement of the vitamin (Heinen, 1984). The present study was carried out to determine the dietary requirement of pyridoxine for juveniles of P. indicus, as there is no information on the pyridoxine requirement of the species.

MATERIAL AND METHODS

Pyridoxine requirement was studied using isonitrogenous purified diets, with vitamin-free casein as protein source. Pyridoxine was added in graded levels in the purified diet as shown in the Table 25. The total content of vitamin mixture

TABLE 24: ENVIRONMENTAL PARAMETERS AND STOCKING SIZE OF JUVENILE PRAWNS

Parameter	Mean values
Temperature (°C)	29.1 ± 0.9
Salinity (‰)	20 ± 2.5
pH	7.65 ± 0.8
Ammonia concentration in the water (NH_4-N mg/l/d)	0.0112 ± 0.0018
Initial length (mm)	22.4 ± 1.209
Initial weight (mg)	73.1 ± 0.01

TABLE 25: DIETARY COMPOSITION OF EXPERIMENTAL DIETS WITH GRADED LEVELS OF PYRIDOXINE HYDROCHLORIDE

Ingredient	g/100 g							
Pyridoxine hydrochloride	0.00	0.01	0.02	0.03	0.04	0.04	0.10	0.15
α-Cellulose	0.15	0.14	0.13	0.12	0.11	0.10	0.05	0.00

in each of the diet was adjusted using α-cellulose.

Experimental set up, environmental conditions, rearing of prawns before and during experimental study were same as carried out for earlier experimental studies. Table 24 shows the data for environmental factors and the initial length and weight of animals used for the study.

The ingredient composition of the diet, method of preparation and feeding level and schedules were similar to what has been described in earlier Chapters (I &II). The parameters studied in the pyridoxine requirement, methods adopted for the determination and statistical analysis of the data were similar to those described in earlier Chapter (I).

R E S U L T S A N D O B S E R V A T I O N S

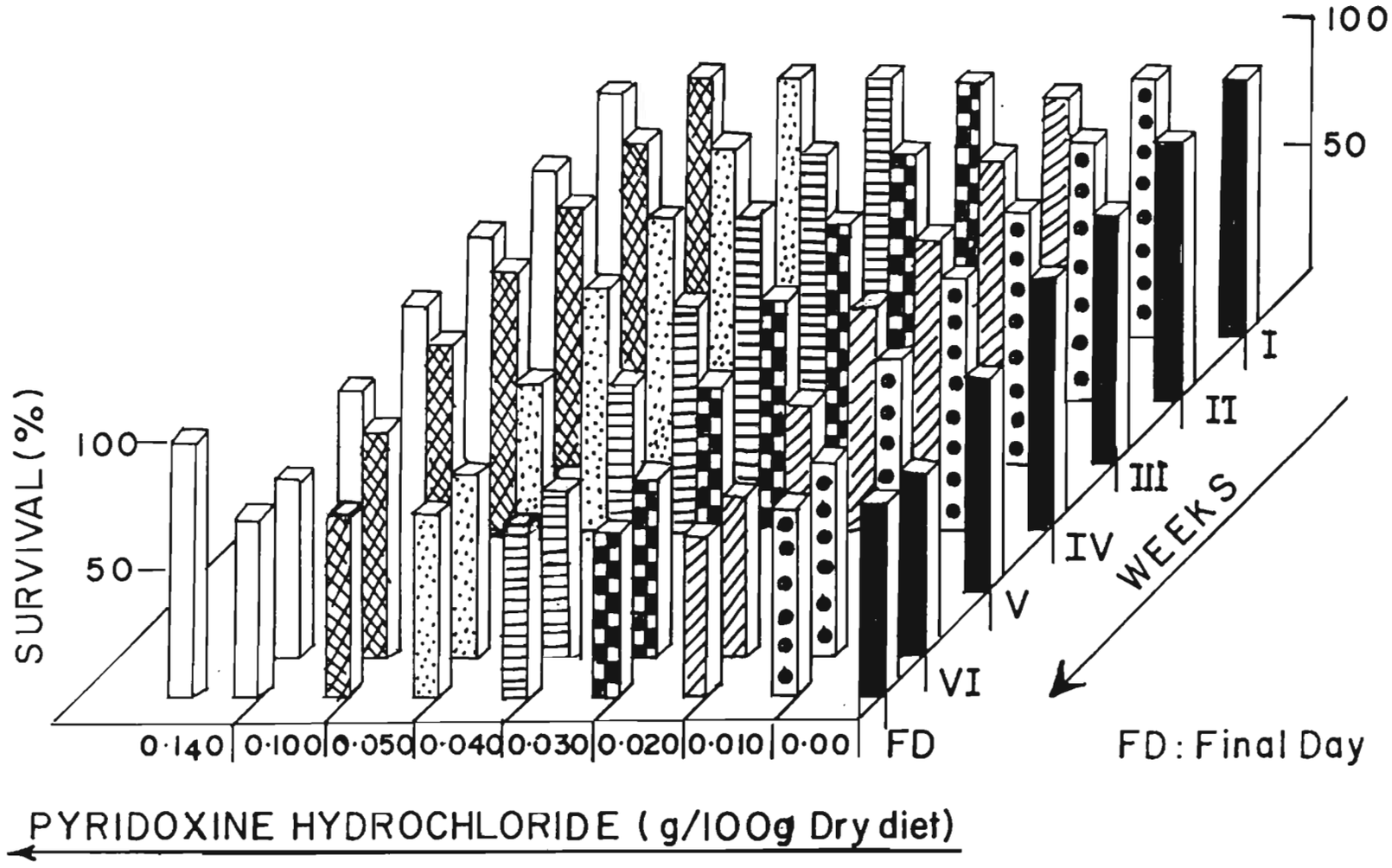
The results of the experiment conducted to determine the dietary requirements of pyridoxine for juvenile P. indicus, using graded levels of pyridoxine hydrochloride (0.0 to 0.15g/ 100 g dry diet) in the purified diets are presented here.

Survival:

Although slight differences in the survival rate existed between prawns fed on diets with different concentrations of pyridoxine; pyridoxine deletion or supplementation in the diet, as such did not have any significant effect on the survival (Fig. 42). The highest percent survival was observed

Fig. 42. Weekly percent survival of prawns fed diets with different levels of pyridoxine hydrochloride.

FIG. 42


100
50
SURVIVAL (%)
100
50
I
II
III
IV
V
VI
WEEKS
0·140
0·100
0·050
0·040
0·030
0·020
0·010
0·00
FD
FD : Final Day
PYRIDOXINE HYDROCHLORIDE (g/100g Dry diet)

in the treatment with pyridoxine deficient diet (73.3%) and the lowest in treatments with 0.02 g and 0.03 g of pyridoxine (62.2%). In all other treatment groups, percent survival ranged between 66.7 and 68.8%.

Growth:

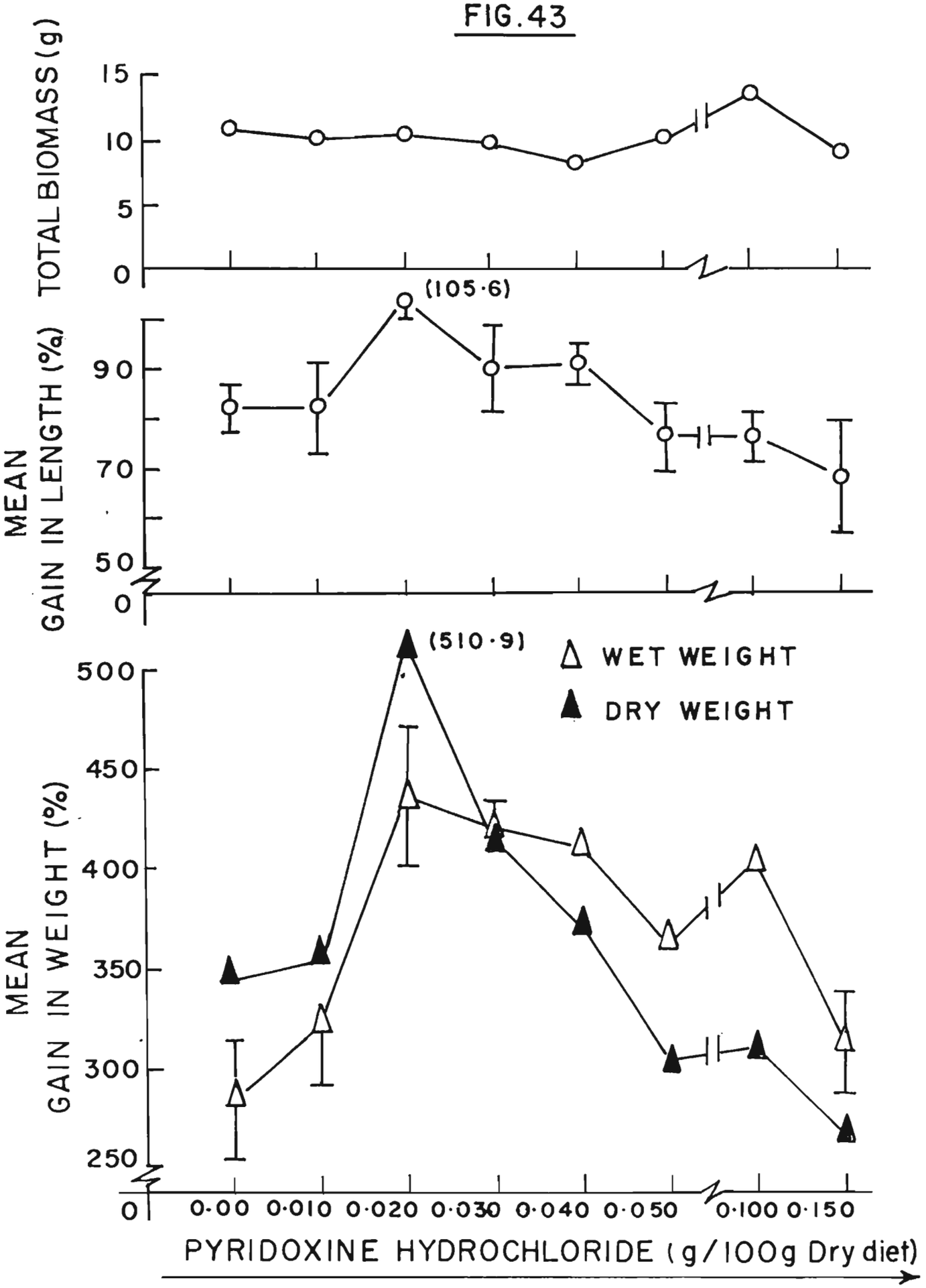
The growth of prawns was significantly ($P<0.05$) affected by the dietary concentrations of pyridoxine. Among the treatments prawns fed on the diet with 0.02 g pyridoxine had significantly ($P<0.05$) higher mean percent gain in length, wet weight and dry weight. Growth of prawns increased with dietary concentrations of pyridoxine upto 0.02g and thereafter, a steady decline was observed with further increase in dietary concentration of the vitamin. The maximum percent gain in length (10.56%), wet weight (436.1%) and dry weight (511%) were observed in prawns fed on the diet with 0.02 g of pyridoxine.

Significant ($P<0.05$) differences in mean percent gain in length of prawns were observed (Fig. 43) between diets with more than 0.05 g of pyridoxine and those diets containing less than 0.05 g of pyridoxine. Although, the minimum mean percent gain in length was recorded at 0.15 g of pyridoxine (68.1%), it was not significantly different from that recorded with 0.05 g (76.7%) and 0.10 g (76.9%) pyridoxine.

The mean percent gain in wet weight of prawns fed on diets with 0.02 g of pyridoxine, 0.03 g of pyridoxine (418.4%) and

Fig. 43. Percent gain in length and weight, and total biomass (g) of prawns fed diets with different levels of pyridoxine hydrochloride.

FIG.43


TOTAL BIOMASS (g)
15
10
5
0
MEAN GAIN IN LENGTH (%)
(105·6)
90
70
50
0
MEAN GAIN IN WEIGHT (%)
(510·9)
WET WEIGHT
DRY WEIGHT
500
450
400
350
300
250
0
0·00 0·010 0·020 0·030 0·040 0·050 0·100 0·150
PYRIDOXINE HYDROCHLORIDE (g/100g Dry diet)

0.04 g of pyridoxine (410.9%) were significantly ($P < 0.05$) higher than that of prawns from other treatments (Fig. 43). Similarly, the mean percent gain in wet weights observed in prawns fed on diets with 0.01 g (320.9%) and 0.15 g (311.2%) of pyridoxine were significantly lower than that of prawns fed with 0.05 g (360.2%) and 0.10 g (401.67%) of pyridoxine. The prawns fed without pyridoxine in the diet recorded lowest mean percent gain in weight (284.1%) and significantly ($P < 0.05$) differed from almost all the other treatment groups.

The mean percent dry weight gain was highest in prawns fed on diet with 0.02 g pyridoxine (511%) and lowest in prawns fed on diet with 0.15 g pyridoxine (267%). These two treatment groups showed significant ($P < 0.05$) differences with most other groups (Fig. 43). Diets with 0.05 g (303.5%) and 0.01 g (309.5%) produced almost same percent dry weight gains and did not show any significant differences with the pyridoxine deficient diet (343.7%) and diets with pyridoxine concentrations of 0.01 g (355.8%) and 0.04 g (371.9%). However, prawns fed with 0.03 g of pyridoxine (413.3%) recorded relatively high percent gain in dry weight compared to most other treatment groups.

Specific Food Consumption (SFC):

No significant influence of dietary levels of pyridoxine was observed on the specific food consumption in prawns (Fig.44). The SFC was highest in prawn groups fed with diets containing 0.15 g of pyridoxine (3.97%) and lowest in prawns fed on the

diet with 0.03 g of pyridoxine (3.25%). In all other treatment groups, the SFC ranged between 3.27 and 3.96%.

Food Conversion Ratio (FCR):

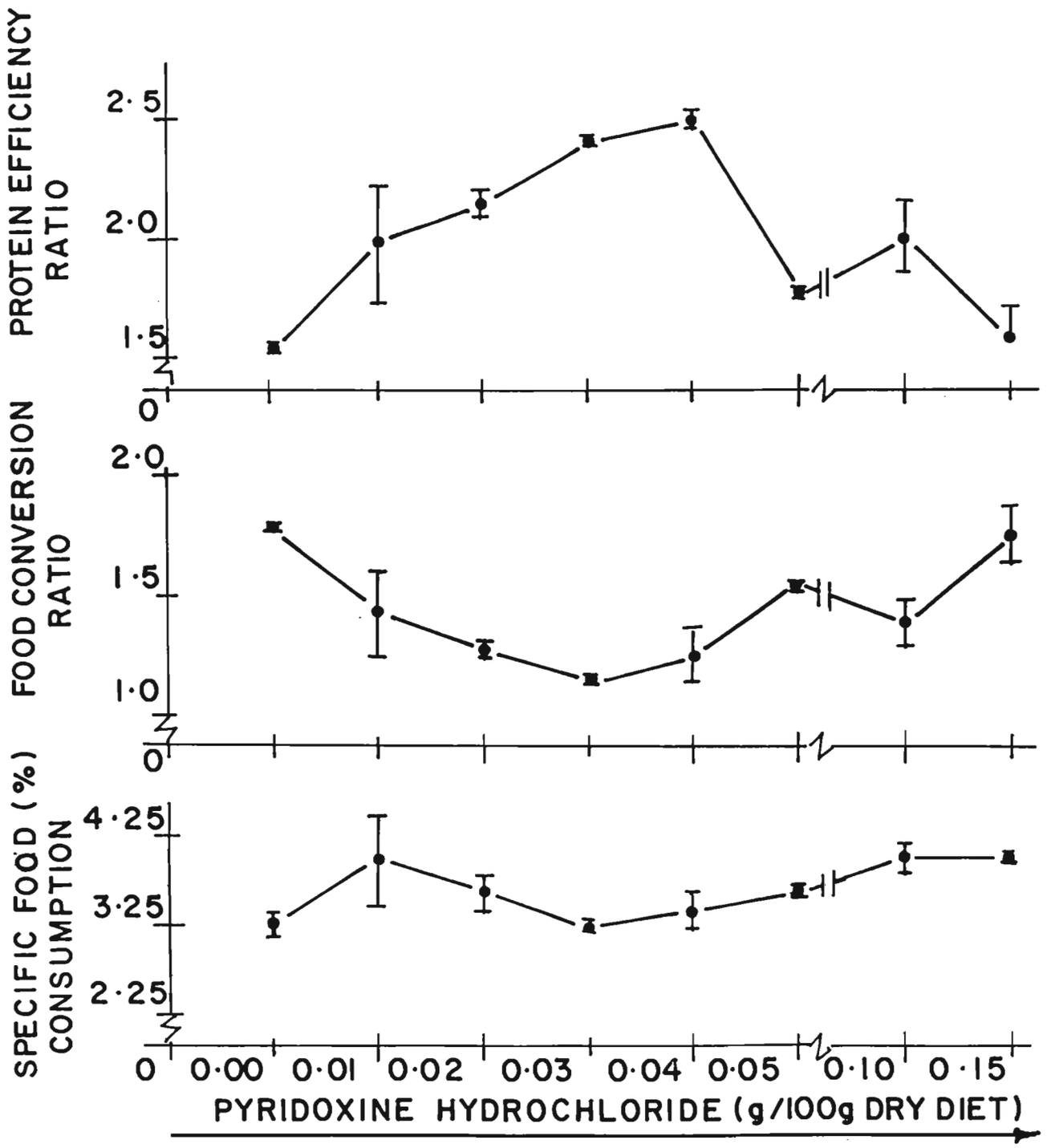
Food conversion ratios (Fig. 44) were also not significantly influenced, by the dietary concentrations of pyridoxine. However, the FCR was highest in prawns fed without pyridoxine in the diet (1.79), closely followed by prawns fed with 0.15 g of pyridoxine (1.75). It was lowest in prawns fed with 0.03 g of pyridoxine in the diet (1.14). In all other treatment groups, the FCR ranged between 1.24 and 1.55. A declining trend in FCR was observed with increasing concentration of pyridoxine up to 0.03 g and showed an upward trend with further increase in concentration of the vitamin.

Protein Efficiency Ratio (PER):

Protein efficiency ratios (PER) were significantly ($P < 0.05$) influenced by the concentrations of pyridoxine in the diets (Fig.44) Diets with 0.04 g and 0.03 g pyridoxine recorded significantly ($P < 0.05$) higher PER values (2.49 and 2.42, respectively) than most other treatment groups. The lowest PER was recorded in prawns fed on the pyridoxine deficient diet (1.54), closely followed by prawns fed with the diet containing 0.15 g of pyridoxine (1.59). These values of PER were significantly ($P < 0.05$) lower than that observed in most other treatments. In all other treatment groups, the PER ranged between 1.78 and 2.15, but did not show any significant variations between them.

Fig. 44. SFC, FCR and PER for diets with different levels of pyridoxine hydrochloride.

FIG. 44


PROTEIN EFFICIENCY RATIO
2·5
2·0
1·5
0
FOOD CONVERSION RATIO
2·0
1·5
1·0
0
SPECIFIC FOOD (%) CONSUMPTION
4·25
3·25
2·25
0
0·00
0·01
0·02
0·03
0·04
0·05
0·10
0·15
PYRIDOXINE HYDROCHLORIDE (g/100g DRY DIET)

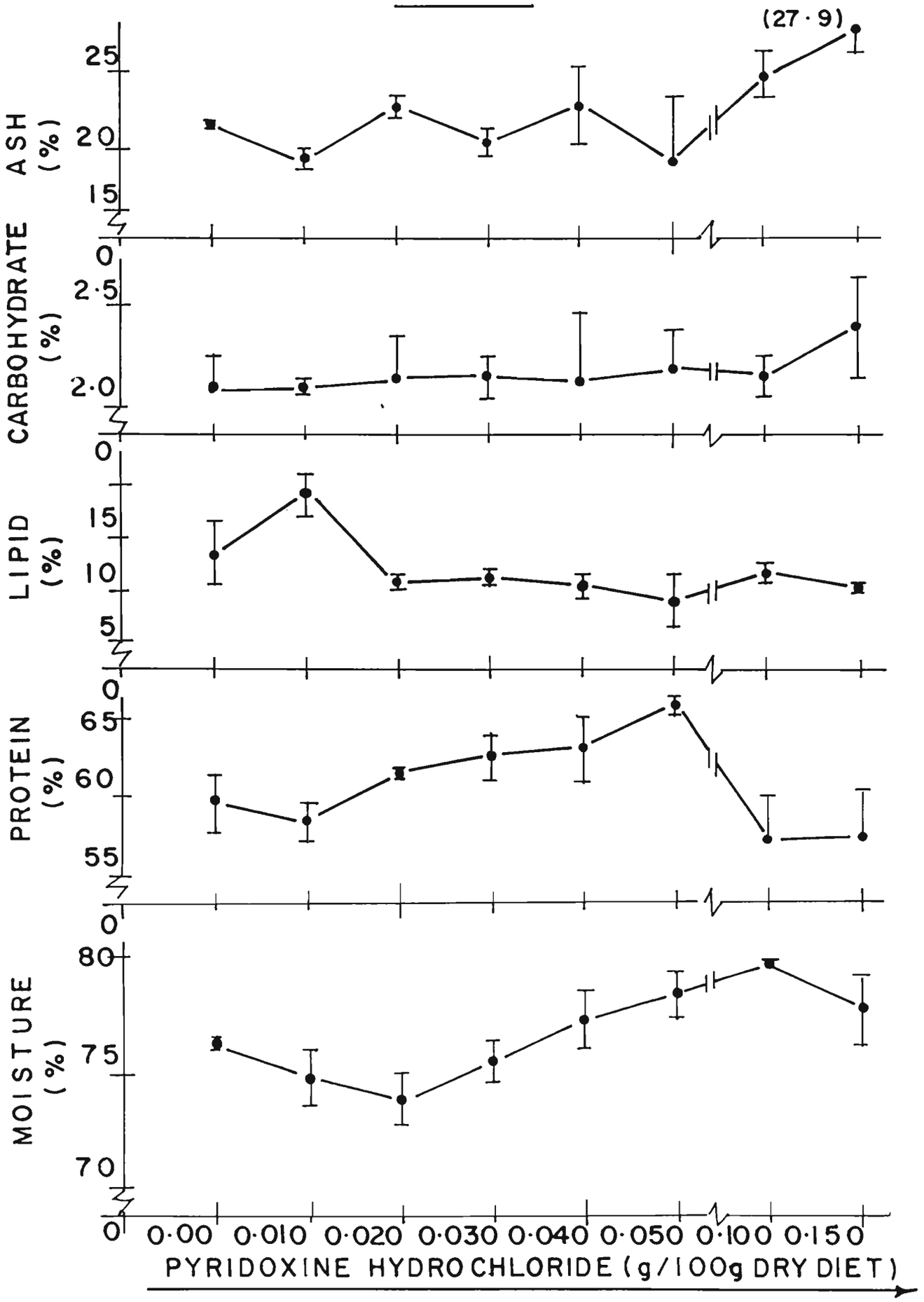
Biochemical Composition:

The diets containing various concentrations of pyridoxine also significantly ($P < 0.05$) influenced the moisture, ash, protein and lipid content of prawns (Fig. 45). Prawns fed on diets containing pyridoxine concentrations of 0.01 g and 0.02 g had significantly ($P < 0.05$) lower moisture content than most other groups. While the maximum moisture content was recorded in prawns fed on the diet containing 0.10 g of pyridoxine (79.8%), the minimum occurred in prawns fed on the diet with 0.02 g of pyridoxine (73.9%). The ash content was highest in prawns fed on the diet with 0.15 g of pyridoxine (27.9%) and lowest in prawns fed on diets with 0.01 g (19.3%) and 0.05 g (19.3%) pyridoxine. However, the ash contents in prawns fed on the pyridoxine deficient diet and those fed on diets with 0.02 g (22.8%), 0.03 g(20.5%) and 0.04 g(22.9%) of pyridoxine did not differ significantly from each other, but were relatively lower than that in prawns fed on the diet with 0.1 g of pyridoxine (24.9%).

The protein content in prawns increased with increase in concentration of pyridoxine in the diet upto 0.05 g and thereafter showed an abrupt decline, indicating significant ($P < 0.01$) effect of pyridoxine concentrations. The protein content in prawns fed on diets with 0.04 g and 0.05 g pyridoxine was significantly ($P < 0.05$) higher than that of prawns from other treatments. No significant differences were observed in the protein contents between the other treatment

Fig. 45. Biochemical composition of prawns fed diets with different levels of pyridoxine hydrochloride.

FIG. 45


(27·9)
ASH (%)
25
20
15
0
CARBOHYDRATE (%)
2·5
2·0
0
LIPID (%)
15
10
5
0
PROTEIN (%)
65
60
55
0
MOISTURE (%)
80
75
70
0
0·00 0·010 0·020 0·030 0·040 0·050 0·100 0·150
PYRIDOXINE HYDROCHLORIDE (g/100g DRY DIET)

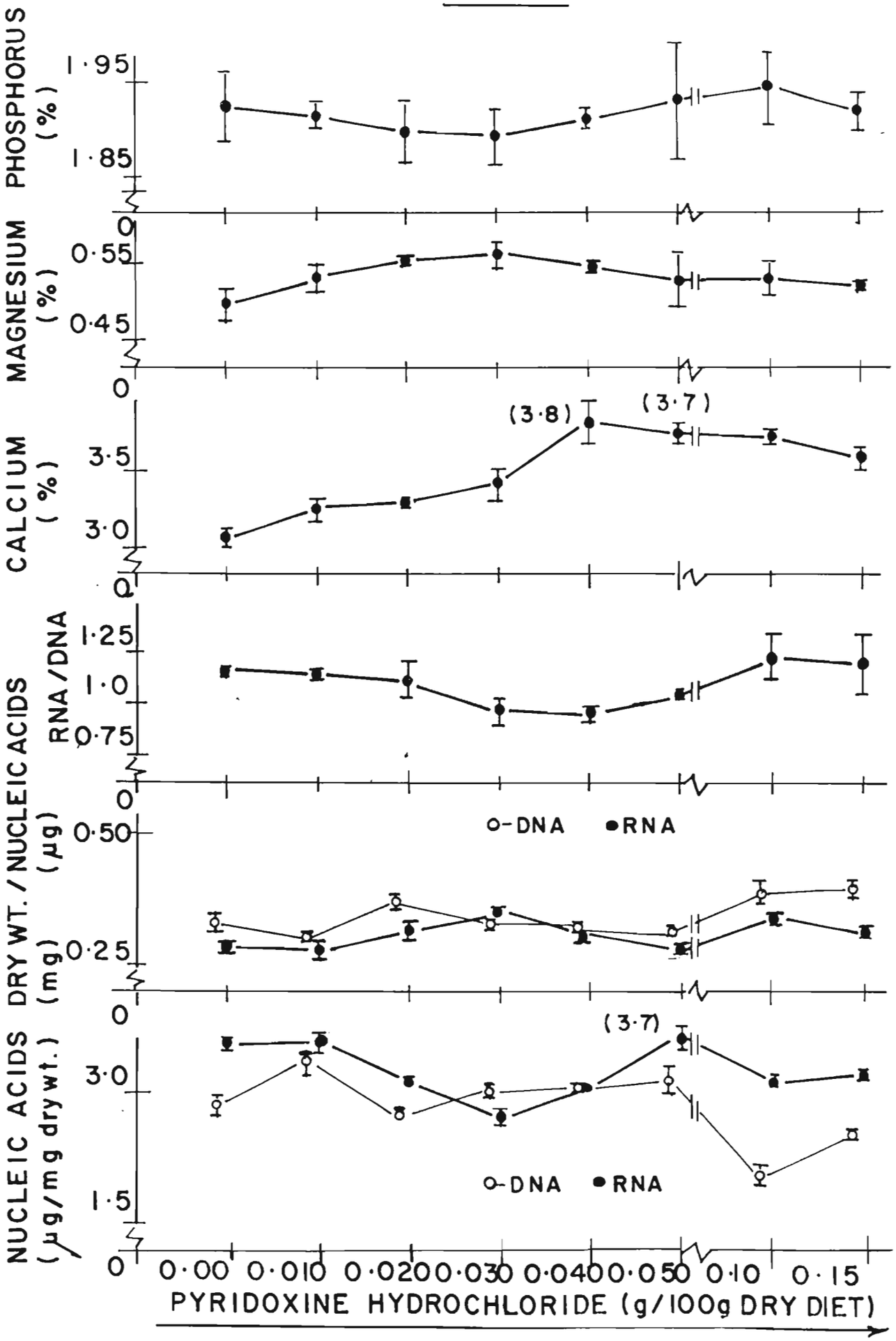
groups. The protein was found to be maximum in prawns fed on the diets with 0.05 g (65.9%) and 0.04 g pyridoxine (63.9%) and minimum in prawns fed diet with 0.10 g of pyridoxine (57.3%).

The lipid content in prawns showed (Fig. 45) a sharp increase by the inclusion of 0.01 g of pyridoxine in the diet; but further increase in the concentration of dietary pyridoxine resulted in abrupt decline in the lipid level. The highest lipid content was recorded in prawns fed with 0.01 g of pyridoxine (19.1%) in the diet, which was significantly ($P < 0.05$) higher than that observed for all other diets. In treatment groups with pyridoxine concentration higher than 0.01 g, the lipid levels non-significantly ranged between 9 and 11.7%. The prawns fed on the pyridoxine deficient diet had relatively higher lipid levels (13.5%) as compared to the above groups. Thus, the concentrations of pyridoxine in the diet had highly significant ($P < 0.01$) influence on the lipid levels in prawns. The carbohydrate content in prawns (Fig.45) from various treatments ranged from 2.1 to 2.4 and there were no significant differences between treatments.

The RNA content in prawns was significantly ($P < 0.05$) influenced by the different dietary concentrations of pyridoxine (Fig. 46). However, no consistent trend was observed, due to the tremendous fluctuations in values between treatments. However, the values ranged between 2.74 and 3.7 µg/mg

Fig. 46. Biochemical composition of prawns fed diets with different levels of pyridoxine hydrochloride.

FIG. 46


PHOSPHORUS (%)
1·95
1·85
0
MAGNESIUM (%)
0·55
0·45
0
CALCIUM (%)
(3·8)
(3·7)
3·5
3·0
0
RNA/DNA
1·25
1·0
0·75
0
DRY WT. / NUCLEIC ACIDS (µg) (mg)
o-DNA ●RNA
0·50
0·25
0
NUCLEIC ACIDS (µg/mg dry wt.)
(3·7)
3·0
1·5
0
o-DNA ●RNA
0·00 0·010 0·020 0·030 0·040 0·050 0·10 0·15
PYRIDOXINE HYDROCHLORIDE (g/100g DRY DIET)

in prawns. The dry weight/total RNA ratio (Fig. 46) was not significantly affected by the pyridoxine concentration in the diet, and the ratio ranged from 0.27 to 0.35.

The DNA content in prawns, like the RNA was significantly ($P < 0.05$) influenced by the dietary levels of pyridoxine (Fig. 46). The highest DNA content was recorded at 0.01 g of pyridoxine (3.39 µg/mg) and the lowest at 0.10 g of pyridoxine (2.04 µg/mg). In all other treatment groups, the DNA levels varied insignificantly between 2.54 and 3.17 µg/mg. The dry weight/total DNA ratio was significantly ($P < 0.05$) influenced by the pyridoxine level in the diet. The prawns fed on the diet with 0.15 g of pyridoxine, showed the highest ratio (0.39) which was closely followed by 0.10 g (0.38) and 0.02 g (0.37) pyridoxine. The prawns fed with 0.01 g of pyridoxine (0.29) showed the lowest ratio (0.29). The ratio showed an increase upto 0.02 g of pyridoxine in the diet and thereafter remained almost constant up to 0.10 g, though a slight rise was observed with further increase in pyridoxine concentration in the diet.

The RNA/DNA ratio (Fig. 46) in prawns was also significantly ($P < 0.05$) affected by the pyridoxine levels in the diet. Thehighest ratio was observed in prawns fed with 0.1 g pyridoxine in the diet (1.24) and the lowest in prawns fed with 0.1 g pyridoxine in the diet (0.95). The RNA/DNA ratio showed a declining trend with increasing concentrations

of pyridoxine in the diets up to 0.05 g, but prawns fed with very high concentrations of pyridoxine in the diet had relatively higher RNA/DNA ratio.

Calcium, magnesium and phosphorus contents in prawns (Fig. 46) were not significantly influenced by the concentration of pyridoxine in the diet. However, minor variations in calcium content were observed, with the highest calcium level at 0.04 g pyridoxine (3.82%) and the lowest in prawns fed diet without pyridoxine (3.06%). The calcium content in prawns showed an upward trend with increasing pyridoxine level in the diet up to 0.04 g and thereafter, declined gradually with further rise in the concentration of the vitamin. The magnesium content in prawns ranged between 0.49% and 0.56%. It increased with the increase in pyridoxine in the diet upto 0.03 g level, and thereafter decreased gradually, with further rise in pyridoxine concentration. The phosphorus content in prawns ranged between 1.89 and 1.95%. The variations observed in the phosphorus levels were marginal and insignificant.

Ammonia Concentration in Water:

Ammonia concentration in water from the experimental tanks, was determined twice weekly and the results expressed as mean ammonia concentration (mg/l/day) are shown in Table 26. Analysis of variance of the data showed that the ammonia excretion in prawns was significantly influenced by the dietary concentrations of pyridoxine. The highest mean ammonia

TABLE 26: AMMONIA CONCENTRATION IN SEAWATER HELD IN AQUARIA

Concentration of pyridoxine in the diet g/100 g dry diet	Mean ammonia concentration in seawater mg/l/d
0.00	0.007
0.01	0.011
0.02	0.013
0.04	0.0125
0.05	0.011
0.10	0.011
0.15	0.11

concentration was observed in treatments with 0.02 g and 0.03 g (0.013 mg/l/d) pyridoxine, whereas, the lowest was recorded in the treatment without pyridoxine (0.066 mg/l/d). There was a steady increase in ammonia concentration up to 0.02 g of pyridoxine in the diet followed by a decrease with further increase in pyridoxine level in the diet.

O B S E R V A T I O N S

Molting:

The number of exuviae and post-molt deaths recorded from the treatments during the experimental period are given in the Table 27. The maximum number of exuviae collected was in the treatment with 0.05 g of pyridoxine (30 nos) and the minimum in treatment with 0.10 g of pyridoxine in the diet (17 nos). In all other treatment groups, the number of exuviae ranged between 22 and 28 nos, with relatively more numbers in treatmen with lower concentrations of pyridoxine (< 0.10 g). Post-molt deaths were relatively more in prawn groups fed on diets with lower concentrations of pyridoxine (less than 0.1 g). However, at 0.1 g and more of pyridoxine, post-molt deaths were relative less. Examinations of the dead prawns from the above treatment showed that they were mostly in the intermolt stage or in pre-molt stage. On the other hand, the dead prawns from treatment with lower concentration of pyridoxine, were usually soft in texture and of the post-molt stage.

TABLE 27: OBSERVATIONS IN PRAWNS FED WITH DIFFERENT EXPERIMENTAL DIETS.

Concentration of pyridoxine hydrochloride in the diet g/100 g dry diet	Mean nos.of molts recovered	Mean nos. of post-molt deaths	Texture of the body
0.00	28	10	SO
0.01	27	12	H
0.02	26	11	H
0.03	25	10	H
0.04	26	11	H
0.05	30	10	H
0.10	17	4	SO
0.15	22	5	SO

H - hard, SO - soft

Food Intake:

During the initial 2 weeks, the food offered was almost completely ingested by prawns in all the treatments. However, from the fifth week onwards, slight variation in food intake was observed in treatment groups fed without pyridoxine and those fed with more than 0.05 g of pyridoxine in the diet. The left-over food was relatively more in these experimental tanks as compared to that of prawns fed with other diets. In treatment with more than 0.05 g of pyridoxine, prawns showed an aversion towards food. However, no significant variations were observed in other treatment groups, which showed almost consistent food intake and attractability to food, throughout the experimental period.

Behaviour Towards Light:

Prawns in the various treatments showed distinct responses towards light (1625 x 10^2 lux). In response to a sudden flash of light, prawns fed on a diet deficient in pyridoxine and those on a diet with more than 0.1 g of pyridoxine, showed agitated and incoherent movements from the fourth week onwards. On the other hand, prawns fed on diets with pyridoxine concentrations between 0.01 and 0.05 g, showed temporary evasive responses towards light, but adjusted to the stimulus quickly and behaved normally. Disturbances of the water column or hitting the side of the experimental tanks, induced quick

responses in prawns fed with a diet deficient in pyridoxine or with pyridoxine level more than 0.1 g in the diet. Such prawns showing agitated movements and convulsions, died in a few hours time.

External Morphology:

No specific changes in external morphology of prawns could be observed on feeding with the experimental test diets. However brownish spots were observed on the abdomen, gills and rostrum. The prawns fed with the pyridoxine deficient diet and those fed with more than 0.1 g of pyridoxine, had dense distribution of spots on the rostrum, abdomen and gills. Some spots were also observed in the rostrum and abdominal regions in prawns fed with 0.01 g, 0.02 g and 0.03 g of pyridoxine in the diet.

D I S C U S S I O N

Pyridoxine has been reported to be an indispensable vitamin for all forms of animals (Sinclair, 1953; Mitchell, 1964; Dadd, 1983), because of its role in the metabolism of amino acids and protein, enzymatic reactions (Cori and Illingworth, 1957), synthesis of mRNA (Halver, 1972) and in a variety of miscellaneous transformations.

Among crustaceans, its essentiality and requirements have been reported for Artemia (Provasoli and Shiraishi, 1959),

Moina (Conklin and Provasoli, 1977), P. japonicus (Deshimaru and Kuroki, 1979) and more recently for juvenile M. rosenbergii (Heinen, 1984). In the present study also, juvenile P. indicus, has shown a dietary requirement for pyridoxine. Thus, the present observations support the view (Heinen, 1984) that in crustaceans dietary supplementation of pyridoxine is essential.

In the present study, although significant differences were observed between the growth of prawns from different treatments, survival was not significantly affected, when pyridoxine deficient diet or diets containing graded concentrations of pyridoxine were fed to the prawns. This is in contrast to the observations made by Deshimaru and Kuroki (1979) in P. japonicus and more recently by Heinen(1984) in juvenile M. rosenbergii. In P. japonicus, prawns fed without pyridoxine in the diet showed relatively poor survival rates of 56% after 12 weeks and M. rosenbergii showed 33% for same number of weeks. The relatively higher survival obtained in the present study may be due to the shorter duration of the experiment (45 days), thereby the tissue stores of the vitamin may not have been fully exhausted to induce mortality. It is also probable that the microbes present in the digestive tract could have contributed to the vitamin needs of the animal for maintenance. However, lengthening of the experiment to few more weeks would have resulted in more conclusive observations.

According to Halver (1969), carnivorous fishes (salmonids and ictalurids) exhaust body pyridoxine stores rapidly when fed with pyridoxine deficient diets. The entire population was observed to be wiped off in 28 days. Since P. indicus is an omnivorous species, high survival in the pyridoxine deficient diets in the present study, suggests that dietary pyridoxine reserves may not be exhausted so efficiently as in carnivorous fishes. However, lower survival rate in pyridoxine diet fed prawns suggests that high dosages may have inhibitory effect on survival as found in Artemia (Provasoli and Shiraishi, 1959); Moina (Conklin and Provasoli, 1977) and P. japonicus (Deshimaru and Kuroki, 1979).

Eventhough, no significant differences could be observed on the survival rate of prawns, significant differences were observed between treatments in growth, feed consumption, food conversion, protein efficiency ratio and carcass chemical composition. From the observations, it is clear that among the concentrations tested, 0.02 g is the preferable level of pyridoxine hydrochloride in the diet of juvenile prawns. The concentration of 0.02 g pyridoxine hydrochloride given above is however, higher than the recommended concentration level (0.012 g) for P. japonicus (Deshimaru and Kuroki, 1979). However, from the high survival at 0.01 g and high growth at 0.02 g pyridoxine concentrations in the diet, it can be expected that the optimum concentration of pyridoxine required in the diet of juvenile P. indicus may lie within the range of 0.01 g to 0.02 g. These suggested dosages are tentative

as the requirement of pyridoxine can be significantly affected by a variety of factors, of which the protein concentration in the diet is very important. In finfish, Halver (1980) has reported increased requirement of pyridoxine with the increase in protein concentration in the diet.

Specific food consumption (SFC) did not show any significant variation among different treatments like the survival rate, but the FCR and PER were significantly ($P < 0.05$) influenced by the pyridoxine concentrations in the diet. The highest FCR and lowest PER in prawns fed on the pyridoxine deficient diet, indicate that pyridoxine deficiency affects the utilization of the ingested food and protein. However, low FCR and high PER at 0.02 g of pyridoxine suggests that the prawns efficiently utilize the food and protein for maximum growth at this concentration. Similarly, at high concentrations (≥ 0.05 g) high FCR and low PER, accompanied by poor growth, suggests that over-dosages of pyridoxine in the diets of prawns results in inhibitory effect on the utilisation of food.

Biochemical composition of carcass of prawns fed with different pyridoxine levels, reflects to some extent on the role of pyridoxine in the deposition of various organic and inorganic constituents in the tissues of prawns. The dietary levels of pyridoxine had significant ($P < 0.05$) influence on the moisture, protein and lipid contents of *P. indicus*, suggesting the active involvement of pyridoxine in the cellular metabolism,

which has also been reported in other animals (Sure and Esterling 1949; Beare et al. 1953; Chow, 1964; Cowey and Sargent, 1972; Halver, 1972, 1982).

Juvenile prawns fed with 0.02 g of pyridoxine in the diet showed the highest growth and lowest moisture content, suggesting that at this concentration of pyridoxine, maximum organic and inorganic nutrients are deposited. The significantly higher protein content, the RNA content, dry weight/RNA ratio, dry weight/DNA ratio and the RNA/DNA ratio in prawns at this level, suggests that protein synthesis is efficiently carried out in the prawn tissues resulting in higher growth. The involvement of pyridoxine in protein metabolism has been widely reported (McCoy and Columbini, 1972). So the present findings of good growth and higher protein deposition in prawns fed on diets with 0.02 g pyridoxine suggests the active role of pyridoxine in various metabolic cycles as in other animals. At optimal dietary dosages of the vitamin, the enzyme catalysed reactions perhaps are at their peak performance resulting in growth. However, in the present study, at 0.05 g/100 g of pyridoxine, the protein deposition and the RNA-DNA contents were also high, but the growth was poor, probably due to energy diversion to metabolic functions, rather than for growth. These prawns also showed high SFC and FCR values and low PER value, which indicates that poor growth was inevitable under these circumstar The present observations also support the view that high dosages of pyridoxine results in poor feed intake (Halver, 1972).

Thus, prawns fed with more than 0.05 g of pyridoxine in the diets showed poor food intake, poor growth and poor protein deposition indicating probable hyper-vitaminosis.

The prawns grown under the pyridoxine deficient diet, showed poor growth and lower protein content than prawns fed with different concentrations of the vitamin in the diets. Similar, observations were also made in higher vertebrates (Sure and Esterling, 1949, Beare et al., 1953) and these authors suggested that the assimilated protein is utilized for energy purposes rather than for growth as the metabolic rate in animals was found to be high under pyridoxine deficiency. Axelrod et al. (1945) and Mitchell (1964) reported that pyridoxine deficiency profoundly affects the amino acid and protein metabolism and the animals utilized amino acids for energy compensation, since the energy requirements could not be met from carbohydrates and fats due to breakdown of the metabolic cycles as a result of vitamin deficiency (Beare et al., 1953). Similar, conclusions can be drawn from the present study. Thus, all these suggests that pyridoxine deficiency results in imbalance of protein and amino acids metabolism (Fisher, 1960) and thus, necessiating dietary supplementation of pyridoxine at optimal levels.

Sure and Esterling in rats and Beare et al. (1953) in mice, reported poor ash content in the carcass of animals, when fed on a pyridoxine deficient diet. Even though no

significant variation in the ash content was observed between prawns fed on the pyridoxine deficient diet and those fed on diets containing pyridoxine concentration of less than 0.1 g. At very high dosage of pyridoxine, high ash deposition resulted probably due to the dietary stress as a result of hypervitaminosis. Also, in these high concentrations protein and lipids were low, which suggests that poor deposition of these nutrients resulted in the overall deposition of higher ash content.

Though significant variations in ash content of prawns was observed between treatments, there were no significant differences in the levels of inorganic elements - calcium, magnesium and phosphorus. Possibly, concentrations of other macro-inorganic nutrients may influence the differences in the ash values of these prawns.

Pyridoxine has been shown to influence the lipid content of the carcass of animals (Sure and Esterling, 1949; Beare et al. 1953; Mullar, 1964; Cowey and Sargent, 1972). It has also been reported to be involved in the essential fatty acid (EFA) conversion (Beaton et al. 1952; Witten and Holman, 1952; Sato, 1970) and promote the synthesis of fat from carbohydrate. However, certain researchers disclaim the relationship of vitamin B_6 and EFA metabolism (Williams and Scheier, 1961; Johnston et al. 1961).

In the present study, the quantity of lipids used in the diet was constant in all the treatments, so dietary effect of lipids on growth should be same. Yet, under deficiency of pyridoxine or when pyridoxine was added at a concentration of 0.1 g or more in the diet, lipid deposition in tissues was relatively poor in prawns. The present findings, indicate that pyridoxine may be interfering in the lipid metabolism. Thus, low lipid content could be due to utilization ot neutral lipids for energy production as studies on rat showed that neutral lipids are broken down rather than phospholipid and sterol fractions (Carter and Phizackerley, 1951).

The variation in lipid levels in the carcass has been speculated as a result of variation in the conversion of certain essential fatty acids by Sato (1970). Since essential fatty acids form important molecules for growth in prawns (New, 1976a) the analysis of the prawn carcass for the EFA profile could have further substantiated the role of vitamin B_6 on EFA conversion. Also, some studies in rats have shown that pyridoxine concentrations in the diets lead to changes in the total lipid and neutral lipid fractions but no specific changes in the phospholipid or sterol fractions of liver (Carter and Phizackerley, 1951). These observations, thus, may be applicable to prawns also. In addition to the changes in EFA, poor fat deposition

in animals fed on the diet deficient in pyridoxine may also be due to increased basal metabolic rate (BMR) or due to inefficient energy production as observed by Sure and Easterling (1949) and Beare et al.(1953).

Thus, the most preferable level of pyridoxine hydrochloride in the diet of juvenile P. indicus is about 0.02 g/100 g dry diet, since the best growth and food efficiency are recorded at these concentrations. When the vitamin is deleted or supplemented in the diet at high dosages ($\geqslant$0.05 g), growth retardation due to poor food intake, conversion and deposition of organic matter results.

CONCLUSIONS

The experimental study, clearly indicates that pyridoxine is indispensable in the diet of juvenile P. indicus and a concentration ranging from 0.01 g to 0.02 g/100 g dry diet seems optimum for supporting maximum growth. Pyridoxine levels in the diet in excess of the requirement, results in poor food intake accompanied by poor growth and deposition of energy nutrients in the body as a result of dietary stress in prawns. However, the requirements can be significantly influenced by

the type of species, size, age, molting stage, environmental conditions maintained and also on the dietary manipulations such as protein levels.

In general, the vitamin levels for fish and crustaceans diets are many times higher than those recommended for domestic land animals and levels required in purified fish diets are higher than those in commercial feeds (Heinen, 1984), because of the presence of vitamins in commercial feed stuffs (New, 1976a; Hastings and Cowey, 1977) and also because of the greater amount of leaching that occurs in crustacean diets (New, 1976a; Infanger et al., 1980; Heinen, 1984).

In the present study, detrimental effect was observed in treatment groups containing diets with more than 0.05 g/100 g dry diet of pyridoxine, resulting in poor growt , even though some prawns showed high protein content in the carcase. Detriment from excessive high levels of water soluble vitamins, especially pyridoxine, has also been reported by Provasoli and Shiraishi(1959) in Artemia, Conklin and Provasoli (1977) in Moina, Deshimaru and Kuroki(1979) in P. japonicus and it was suggested that crustaceans may not have an efficient mechanism for rid ing themselves of the excess vitamins (Heinen, 1984).

CHAPTER-VIII

NIACIN REQUIREMENT

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

Niacin was identified as the milkfactor which cured diseases like pellagra. Frapolli (1771) coined the term pellagra, 'roughened skin' and attributed the disease to some dietary deficiency. According to Hein (1964), Huber and Weidel (1867) were the first to isolate nicotinic acid as an oxidation product of nicotine. However, their work went unnoticed until Funk (1912) isolated it from rice polishings and suggested pellagra to be caused by niacin deficiency.

The biological role of niacin was first established in 1935 when nicotinamide was found to be a component of NAD^+ and $NADP^+$ (Warburg and Christian, 1935). Kuhn and Vetter (1935) isolated and stressed the importance of niacinamide for living tissues. Tunison et al. (1943) postulated that niacin to be a part of factor H for fish.

The vitamin exists in its amide form nicotinamide, under its physiological active state, serving as coenzyme for a variety of metabolic enzymes. These coenzymes serve as hydrogen acceptors from metabolites activated by certain anaerobic dehydrogenases passing H-molecule to flaveproteins in glycolysis, Kreb's cycle and other metabolic cycles.

Deficiency of niacin reduces the concentration of coenzymes in liver and muscle (Goldsmith, 1964). It has also been observed that niacin in the diet, increases the secretion of both free and total acids of the gastric juice (Goldsmith, 1964) in rats. Very high dosages of the vitamin decrease the concentration of cholesterol and other lipids in the serum (Goldsmith, 1964) and depress growth in rats (Poston, 1969b).

Most studies have shown slower development of niacin deficiency symptoms (Krashoff, 1946; Horwitt, 1958; Halver, 1972) due to many reasons. The symptoms are developed much slower in invertebrates than higher vertebrates; reason being that niacin is replenished through microbial population present in the intestinal region in many species (Ellinger, 1950; Mitchell, 1964), which produce the vitamin quantum just sufficient to meet the animal requirements (Halver, 1972). Besides, stores of niacin are more slowly exhausted under experimental conditions than some of the other vitamins, resulting in less defined and slowly developing syndromes in the species. Niacin demands are also met through the conversion of the amino acid-tryptophan present in the diet. Other factors such as environmental conditions and physiological state of the animals also have significant influence on the development of the symptoms due to the vitamin deficiency.

The first signs of niacin deficiency in fishes are, loss of appetite and poor food conversion (Halver, 1972). However, in the prawn M. rosenbergii, the conversion efficiency is not marked affected by feeding niacin deficient diet (Heinen, 1984). In fishes, continued deletion of the vitamin from diets, results in lesions of colon and muscle spasms (McLaren et al., 1947a; Halver, 1953 , 1957); but no such lesions were found in crustaceans (Heinen, 1984). Niacin requirement varies with the protein and tryptophan content in the diet (Holts, 1956). Apart from protein and amino acids, the type of carbohydrate (Mitchell, 1964), amount of dietary fat (Salmon, 1947a), micro-nutrients like steroids, trace elements like Cr^{++}, Zn^{++} and MoO_4 and a number of B vitamins significantly influence niacin requirements (Halver, 1980).

In aquatic systems, dietary losses are widely encountered due to leaching of the vitamin from diets. About 50% of the vitamin is lost from purified diets in 24 hrs due to leaching (Infanger et al., 1980). To compensate losses during diet preparation and as a result of leaching, higher dosages of the vitamin are incorporated in the diets. But in certain instances, higher dosages of water soluble vitamins in diets resulted in detrimental effects, as in Moina sps. (Conklin and Provasoli, 1977). Thus, considering the physiological role of niacin in the metabolism, the present study was undertaken to determine the vitamin level that can be incorporated in the diet of juvenile prawns P. indicus for proper growth, survival and utilization of the ingested food and protein.

MATERIAL AND METHODS

Niacin requirement in prawns was studied through laboratory experiments using isonitrogenous diets with vitamin-free casein as the major protein source. Diets containing graded levels of nicotinic acid (0.0 to 0.25 g/100 g dry diet) were prepared for the experiment. Composition of ingredients, formulation and preparation of diets, feeding level and schedule were similar to that described in Chapter I and III, excepting for the composition of vitamin mixture in which the amounts of nicotinic acid were varied (Table 29).

Experimental set up and rearing of animals were similar to those followed for earlier experiments. Table 28 shows the mean environmental conditions recorded during the experiment and also the initial length and weight of the animals taken for the study. All the parameters that were determined for protein requirement and other vitamin studies were also considered for the present experiment. Standard procedures as described in Chapter I were used for the collection and statistical analysis of the data.

TABLE 28: ENVIRONMENTAL PARAMETERS AND STOCKING SIZE OF JUVENILE PRAWNS.

Parameter	Mean values
Temperature (°C)	26.28 ± 0.7111
Salinity (ppt)	21.50 ± 3.2000
pH	7.55 ± 0.6200
Ammonia concentration in the water(NH_4-N mg/l/d)	0.0146 ± 0.0064
Initial length (mm)	15.40 ± 0.529
Initial weight (mg)	23.9 ± 0.0031

TABLE 29: DIETARY COMPOSITION OF EXPERIMENTAL DIETS WITH GRADED LEVELS OF NICOTINIC ACID.

Ingredient	g/100 g							
Nicotinic acid in the dry diet	0.0	0.025	0.05	0.075	0.10	0.15	0.2	0.25
α-Cellulose powder	0.25	0.20	0.174	0.15	0.125	0.075	0.025	0.0

RESULTS AND OBSERVATIONS

Survival:

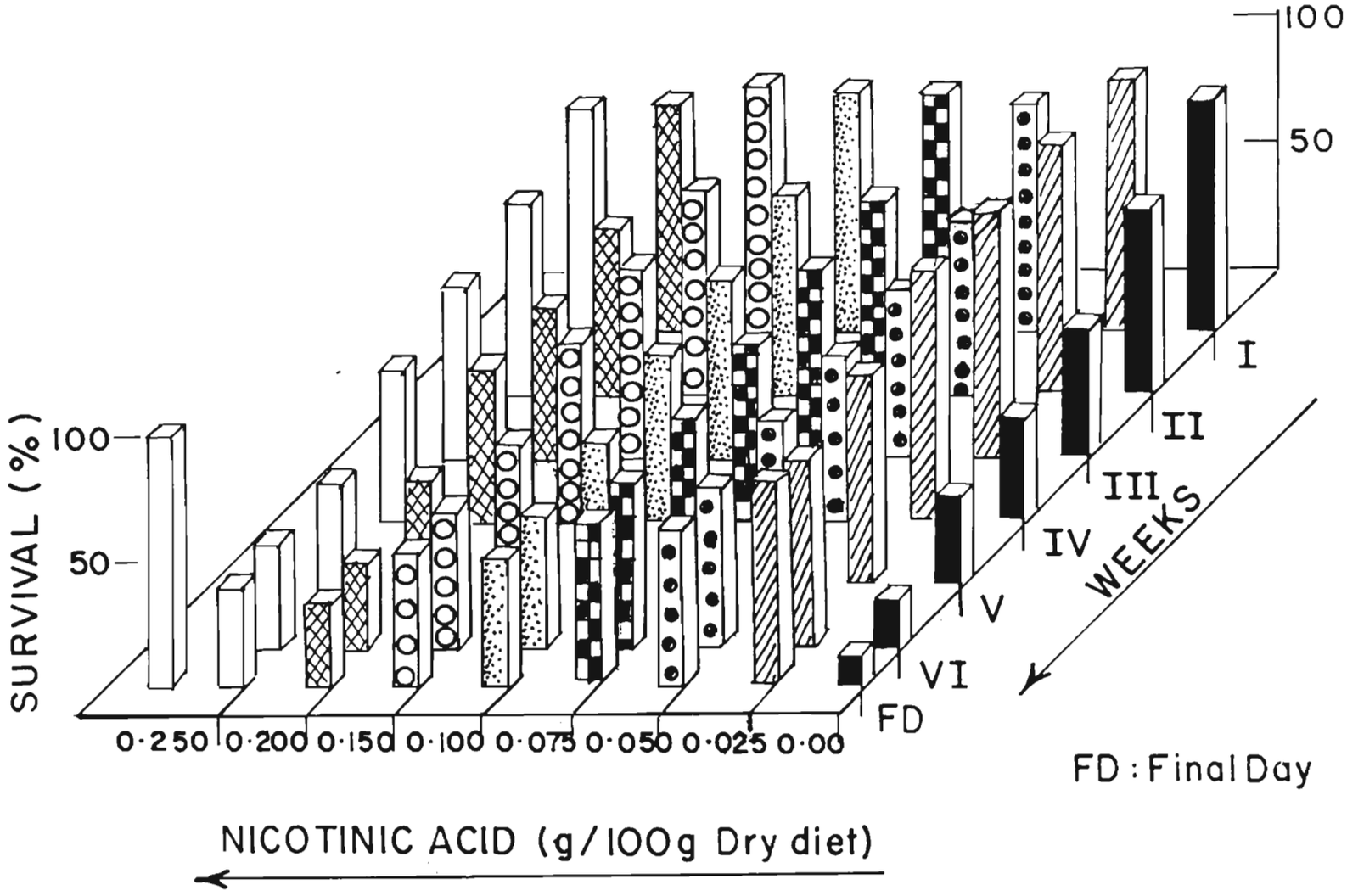
Survival rates recorded from the experiment are shown in Fig. 47. Analysis of variance of the data showed that the dietary concentrations of nicotinic acid significantly ($P < 0.01$) influence the survival rates. The prawn groups fed on the niacin deficient diet and those fed on diets containing higher concentrations (≥ 0.2 g) of niacin produced significantly ($P < 0.05$) lower survival rates than those fed on other diets. The maximum survival rate (68.8%) was observed at 0.025 g of niacin and the minimum (11.1%) in the niacin deficient diet. At very high concentrations of niacin (0.2 g and 0.25 g) survival rate was less than 40% (33.3% and 37.8%, respectively). In all other groups, survival rate ranged between 51.1 and 66.7%. The survival rate showed a gradual decline with increasing concentrations of the test vitamin, beyond 0.025 g in the diet.

Growth:

Data on mean percent gains in length, wet weight and dry weight are shown in Fig. 48. Analysis of variance of the data showed that the diets containing various concentrations of niacin significantly influence growth ($P < 0.05$ - percent gain in length and percent dry weight, and $P < 0.01$ - percent gain in wet weight). The prawns fed on the diet with 0.025 g of niacin showed significantly ($P < 0.05$) higher mean percent gain in length than those fed on other diets.

Fig. 47. Weekly percent survival of prawns fed diets with different levels of nicotinic acid.

FIG. 47

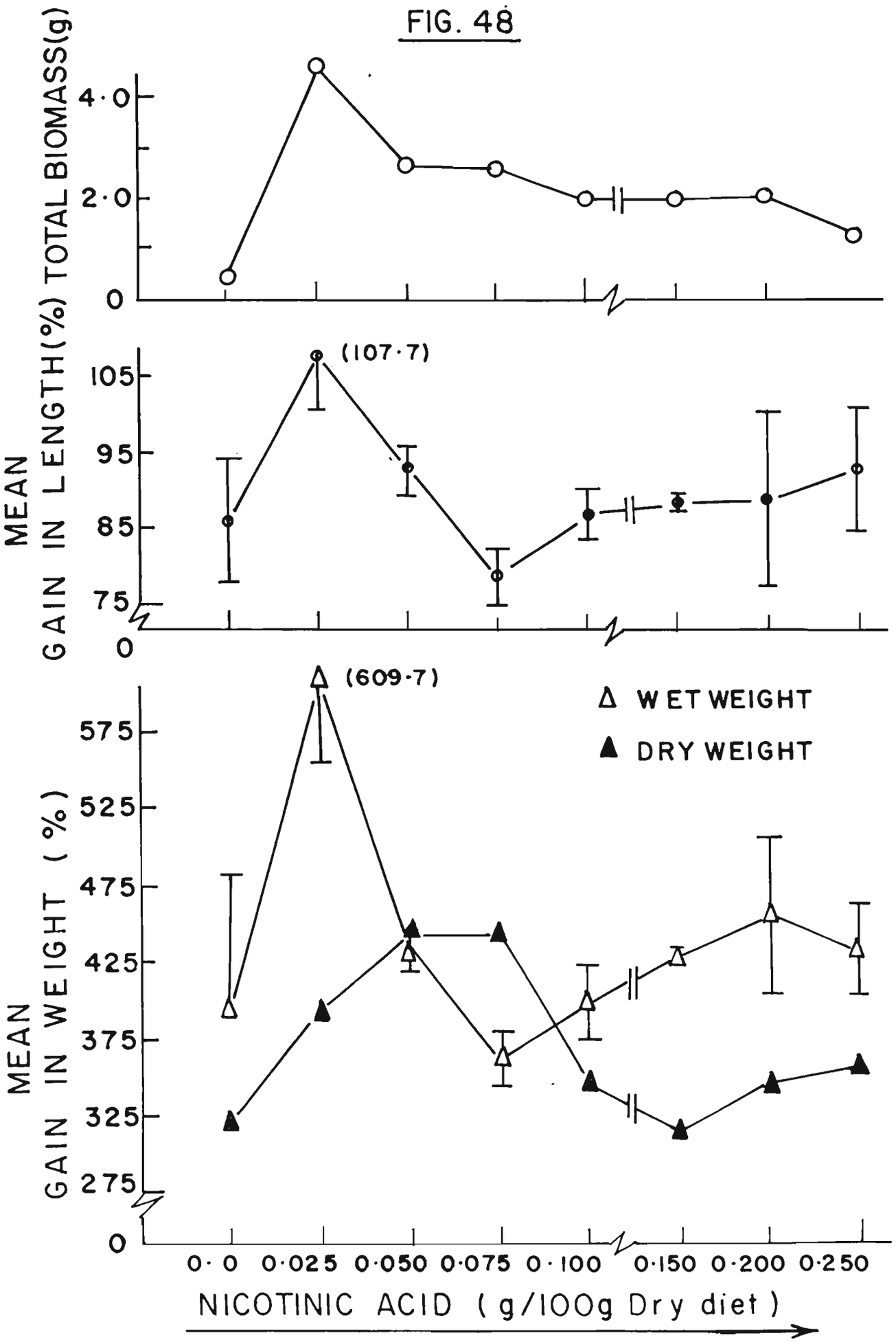

SURVIVAL (%)
100
50
0·250
0·200
0·150
0·100
0·075
0·050
0·025
0·00
NICOTINIC ACID (g/100g Dry diet)
100
50
I
II
III
IV
V
VI
FD
WEEKS
FD : Final Day

As in the case of mean percent gain in length, no specific trend could be observed (Fig. 48) for the mean percent wet weight gain in prawns. However, the diet containing 0.025 g of niacin produced significantly ($P < 0.05$) higher mean percent gain in wet weight than all other dietary levels of niacin. The highest mean percent gain in wet weight was obtained with 0.025 g of niacin (609.7%) and the lowest was recorded in prawns fed on the diet with 0.075 g of niacin(363.8%). The mean percent gain in wet weight of prawns from other treatments ranged between 396 and 457.1%. The niacin deficient diet and diets containing niacin concentrations higher than 0.15 g produced superior wet weight gains compared to many other diets. This growth was mainly due to the high cannibalism and devouring of the dead prawns by the surviving ones in these dietary treatment groups.

The data for mean percent gain in dry weight of prawns were considerably different from that for mean percent gain in wet weight (Fig. 48). For example, while the mean percent wet weight gain was significantly ($P < 0.05$) higher in prawns fed on the diet containing 0.025 g of niacin, the mean percent dry weight gain was significantly ($P < 0.05$) higher in prawns fed on diets containing 0.05 g and 0.075 g of niacin. However, no significant difference was observed between diets containing 0.05 g (442.7%) and 0.075 g(443%) of niacin. The prawns fed on the diet deficient in niacin (320.2%) and those fed on the diet containing 0.15 g niacin (315.3%), showed significantly ($P < 0.05$) lower dry weight gains than that recorded in prawns

Fig. 48. Percent gain in length and weight, and total biomass (g) of prawns fed diets with different levels of nicotinic acid.

FIG. 48


MEAN GAIN IN LENGTH(%) TOTAL BIOMASS(g)
4·0
2·0
0
(107·7)
105
95
85
75
0
(609·7)
Δ WET WEIGHT
▲ DRY WEIGHT
MEAN GAIN IN WEIGHT (%)
575
525
475
425
375
325
275
0
0·0 0·025 0·050 0·075 0·100 0·150 0·200 0·250
NICOTINIC ACID (g/100g Dry diet)

from most other treatments.

Specific Food Consumption:

The levels of niacin in the diets had highly significant ($P < 0.01$) effect on the specific food consumption in prawns (Fig. 49). The prawn groups fed on the niacin deficient diet showed the highest SFC of 18.8%, which was followed by those fed on diets containing niacin concentrations of 0.25 g.

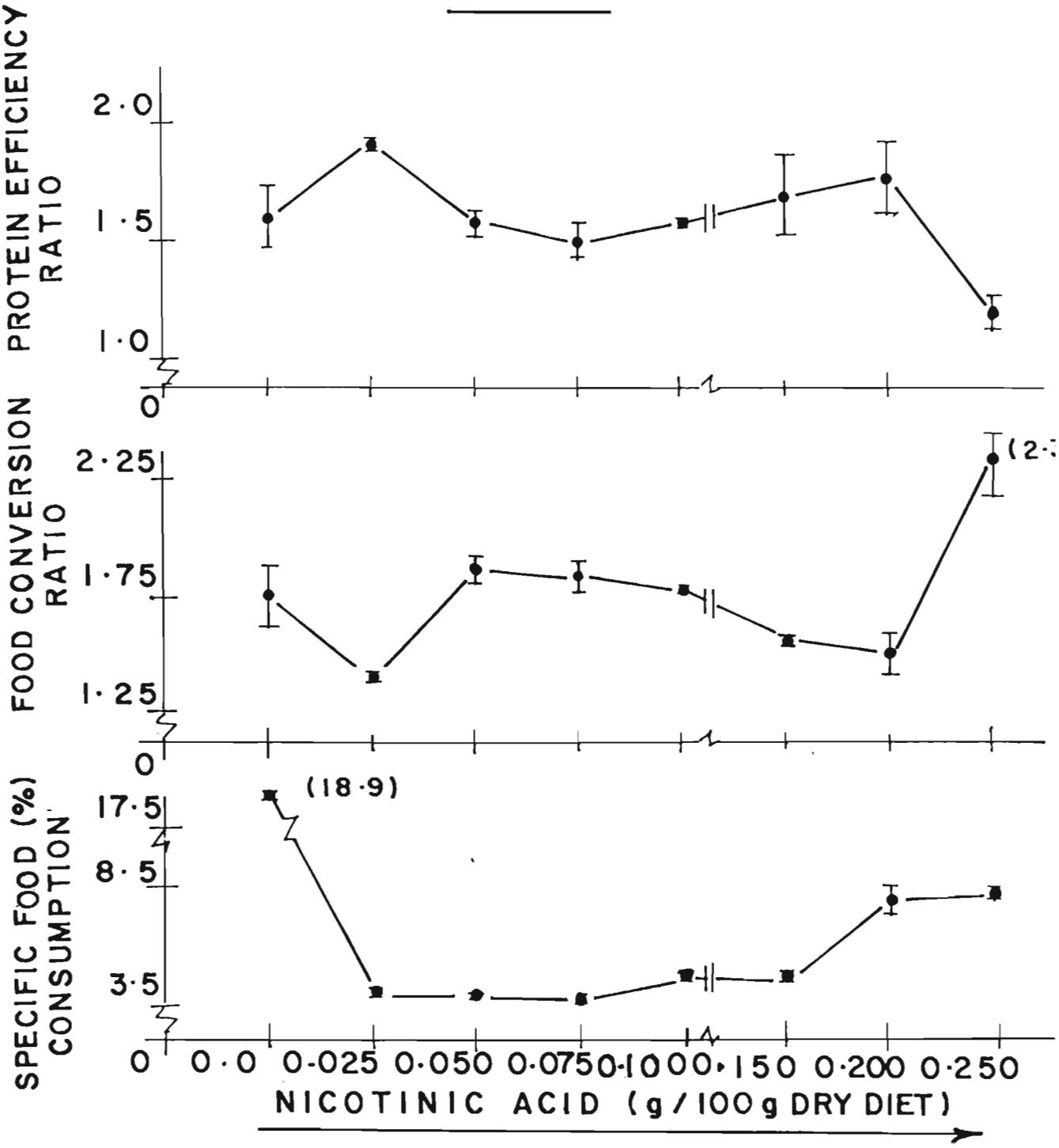
The SFC of prawns from the above three treatments was significantly ($P < 0.05$) higher than all other groups, indicating that deficiency of niacin, as well as niacin at high levels significantly affect food consumption. A steady decline in the SFC (Fig. 49) was observed with increasing concentrations of niacin upto 0.075 g in the diet and thereafter, it increased with a sharp rise at niacin concentrations of 0.2 g and 0.25 g.

Food Conversion Ratio (FCR):

The food conversion ratios for various dietary treatments are shown in Fig. 49. Statistical analysis of the data showed that the FCR was significantly ($P < 0.05$) influenced by the diets fed to the prawns. Diets containing 0.25 g of niacin gave significantly ($P < 0.05$) higher FCR (2.35) than diets containing other niacin concentrations. The lowest FCR was recorded in the treatment with 0.025 g of niacin (1.45). No significant difference in FCR could be observed between treatments in

Fig. 49. SFC, FCR and PER for diets with different levels of nicotinic acid.

FIG. 49


PROTEIN EFFICIENCY RATIO
2·0
1·5
1·0
0
FOOD CONVERSION RATIO
2·25
1·75
1·25
0
SPECIFIC FOOD (%) CONSUMPTION
(18·9)
17·5
8·5
3·5
0
0·0
0·025
0·050
0·075
0·100
0·150
0·200
0·250
NICOTINIC ACID (g / 100 g DRY DIET)

which niacin concentrations ranged from 0.05 to 0.2 g. The niacin deficient diet produced relatively higher FCR (1.74) than most diets with niacin.

Protein Efficiency Ratio (PER):

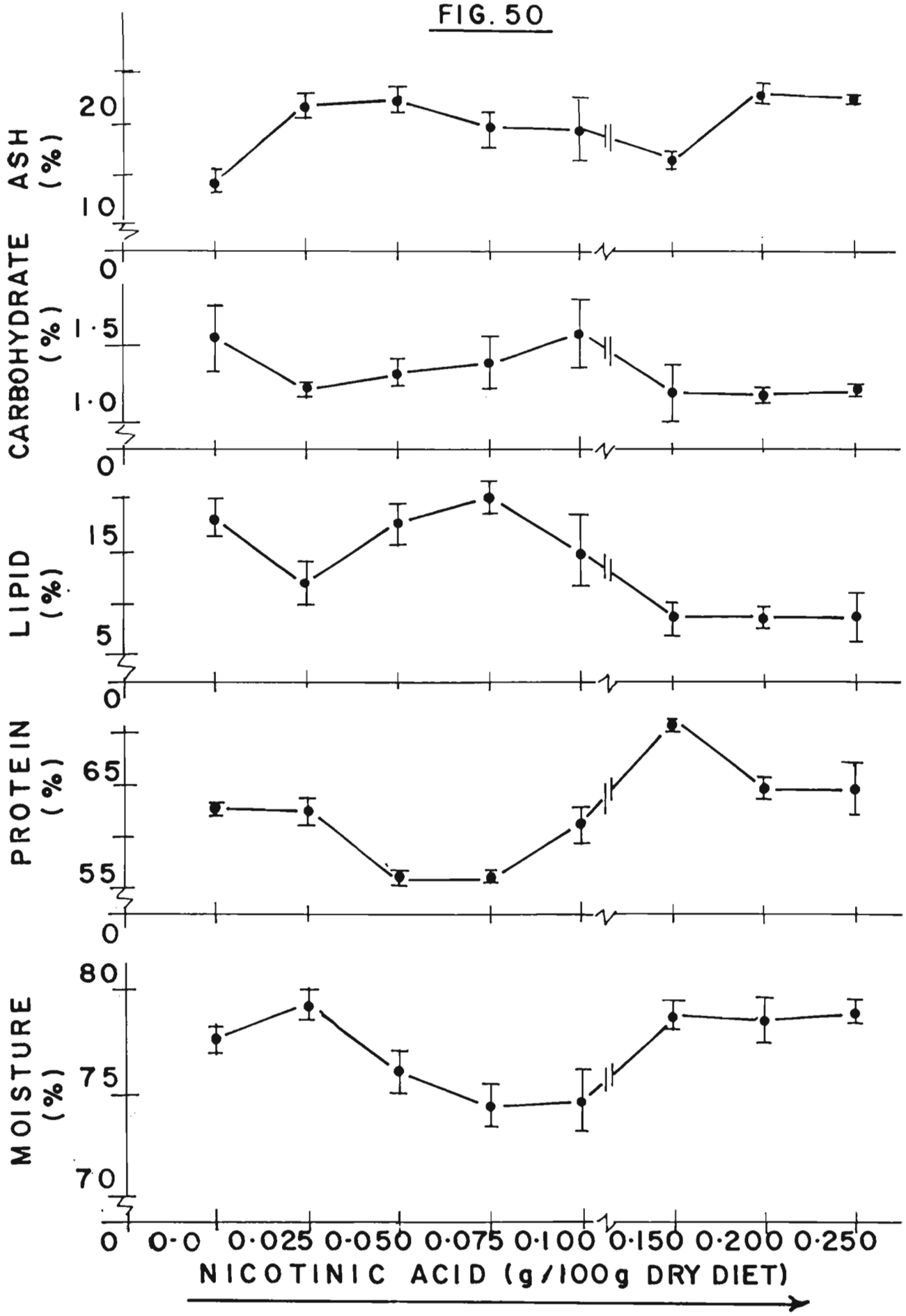
Protein efficiency ratios (Fig. 49) were also significantly ($P < 0.05$) affected by the niacin concentrations in the diet. The PERs for diets containing 0.025 g and 0.05 g niacin were signifi--cantly higher than that of other dietary levels of niacin. The highest PER was obtained with 0.025 g of niacin (1.91) and lowest with 0.25 g of niacin (1.18). Although, prawns fed on the niacin deficient diet recorded a PER of 1.59; this is not comparable due to the very low survival rates in this treatment.

Biochemical Composition:

The moisture, ash, protein, lipid and carbohydrate contents of prawns subjected to various dietary concentrations of niacin are shown in Fig. 50. Analysis of variance of the data showed that the dietary concentrations of niacin, significantly influence the moisture ($P < 0.05$), ash, protein and lipids ($P < 0.01$). Significantly ($P < 0.05$) lower moisture contents were observed in prawns fed on diets containing 0.075 g and 0.1 g niacin than prawns fed with other dietary concentrations of niacin. While, the prawns fed on 0.025 g of niacin had the highest moisture content (79.5%), those fed on the diet with 0.075 g of niacin had the lowest moisture content (74.5%), closely followed by

Fig. 50. Biochemical composition of prawns fed diets with different levels of nicotinic acid.

FIG. 50


ASH (%)
20
10
0
CARBOHYDRATE (%)
1·5
1·0
0
LIPID (%)
15
5
0
PROTEIN (%)
65
55
0
MOISTURE (%)
80
75
70
0
0·0
0·025
0·050
0·075
0·100
0·150
0·200
0·250
NICOTINIC ACID (g/100g DRY DIET)

prawns fed on the diet with 0.1 g of niacin (74.6%). In all other treatment groups, the moisture content in prawns varied between 76 and 79.1% and there were no significant differences between them. A gradual decline in the moisture content was observed up to 0.075 g and thereafter it increased with further increase in dietary niacin concentration. The moisture content in prawns fed on the niacin deficient diet did not significantly vary from that of prawns fed on diets with higher concentrations of niacin (0.15 g and above).

The prawns fed on the niacin deficient diet and those fed on the diet containing 0.15 g niacin had significantly ($P < 0.05$) lower ash contents than that of prawns from all other treatments. The maximum ash content was recorded with 0.2 g of niacin (22.9%) and the minimum with niacin deficient diet (14.3%). In prawns from other treatments the ash content varied between 16.1 and 22.4%. A comparison of the ash content in prawns fed on the niacin deficient diet with that of the prawns fed on the diet with 0.025 g, indicates that inclusion of niacin in the diet markedly raises the ash content in prawns.

No specific trend could be observed in the protein content in prawns with respect to the increasing concentrations of niacin in the diet. The maximum protein content was observed in prawns fed on the diet containing 0.15 g of niacin (71.5%) and the minimum in prawns fed with the diet containing 0.05 g (55.4%) niacin. In prawns from all other treatments, the protein

content ranged between 56 and 64.9%.

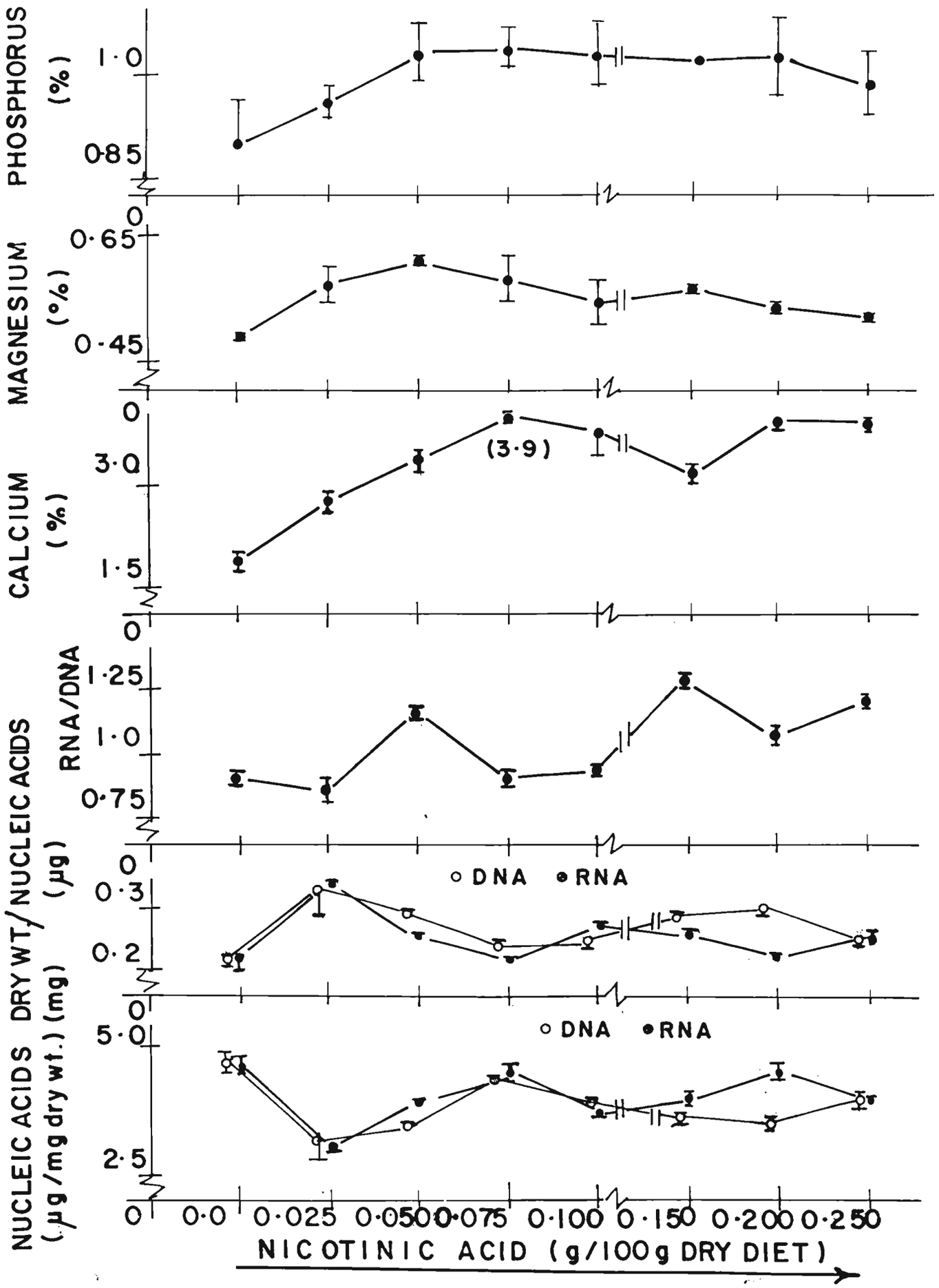
The prawns fed on diets containing higher niacin concentrations (> 0.15 g), had significantly ($P < 0.05$) lower lipid contents than those fed on diets containing lower concentrations of niacin (≤ 0.1 g). While the prawns fed on the diet with 0.075 g of niacin (20.5%) had the highest lipid content, those fed on the diet containing 0.15 g of niacin had the lowest lipid content (8.7%). The prawns fed on the niacin-free diet had relatively high lipid content (18.5%), but the data can not be compared with other treatment groups due to the high cannibalism exhibited by the prawns reared on this diet. The lipid content in prawns increased with the concentration of niacin in the diet up to 0.075 g and thereafter, it showed an abrupt decrease with further increase in niacin concentration.

The carbohydrate content in prawns did not differ significantly from treatment to treatment, indicating that the dietary niacin concentration has no significant influence on the carbohydrate accumulation. Yet prawns fed on the niacin deficient diet had relatively higher carbohydrate content (1.54%) than prawns from most other treatments with niacin in the diet.

The RNA content (Fig. 51) in prawns was significantly ($P < 0.05$) affected by the various dietary concentrations of niacin. The prawns fed on the diet deficient in niacin had significantly ($P < 0.05$) higher RNA content (4.62 µg/mg) than those from

Fig. 51. Biochemical composition of prawns fed diets with different levels of nicotinic acid.

FIG. 51


PHOSPHORUS (%)
1·0
0·85
0
MAGNESIUM (%)
0·65
0·45
CALCIUM (%)
0
3·0
1·5
(3·9)
0
RNA/DNA
1·25
1·0
0·75
0
NUCLEIC ACIDS (µg)
DNA RNA
0·3
0·2
0
NUCLEIC ACIDS DRY WT./DRY WT. (mg)
DNA RNA
5·0
2·5
0
0·0 0·025 0·050 0·075 0·100 0·150 0·200 0·250
NICOTINIC ACID (g/100g DRY DIET)

most other treatments. The prawns fed on the diet with 0.025 g niacin had the lowest (3.01 µg/mg) RNA content. In other groups of prawns, the RNA content ranged between 3.78 and 4.48 µg/mg. However, the dry weight/total RNA ratio (Fig. 51) was not significantly affected by the dietary concentrations of niacin.

The DNA content in prawns (Fig. 51) was also significantly ($P < 0.05$) influenced by the dietary concentrations of niacin. The prawns fed on the niacin deficient diet had significantly ($P < 0.05$) higher DNA content (4.65 ug/mg) than prawns from other treatments, whereas the DNA content ranged between 3.09 to 4.3 µg/mg. The dry weight/total DNA ratio (Fig. 51) was not significantly influenced by the diets. However, the prawns fed on the diet deficient in niacin showed lower ratio (0.22) compared to prawns fed with niacin in the diets. Among prawns fed on the diets with niacin, the highest ratio (0.33) was recorded with 0.025 g of niacin. The ratios in various other treatment groups ranged between 0.23 and 0.29.

The RNA/DNA ratio (Fig. 51) was however, significantly ($P < 0.05$) influenced by the dietary concentrations of niacin. The prawns fed on the diet with 0.025 g niacin had significantly ($P < 0.05$) lower ratio (0.86) when compared to prawn from most other treatments. The highest ratio was obtained in the case of prawns fed on the diet with 0.15 g of niacin (1.29), but this was not significantly different from the ratios (0.89 to 1.22) recorded in prawns from most other treatments.

The calcium, magnesium and phosphorus contents in prawns from various treatments are shown in Fig. 51. The calcium content in prawns was significantly ($P < 0.01$) influenced by the dietary levels of niacin. The prawns fed on the niacin deficient diet had significantly ($P < 0.01$) lower calcium levels (1.91) than prawns fed on diets containing various concentrations of niacin. However, in prawns from all other treatments, the calcium levels ranged between 2.78 and 3.99%. With the increase in concentrations of niacin in the diet, the calcium level showed an increase; but there was no significant increase in the calcium level beyond 0.075 g of niacin in the diet.

The magnesium content (Fig. 51) was not significantly influenced by the dietary concentrations of niacin. There was also no consistent trend in the magnesium content in prawns fed on the diets containing increasing concentrations of niacin. The highest (0.6%) and the lowest (0.49%) magnesium contents were recorded in the case of prawns fed on the diet with 0.075g of niacin and vitamin deficient diet, respectively.

Like magnesium, phosphorus content in prawns (Fig. 51) was also not significantly affected by the dietary concentrations of niacin. The phosphorus content in prawns ranged between 0.9% and 1.04%, the lowest being recorded in prawns fed on the niacin deficient diet and the highest in those fed on the diet with 0.075 g of niacin. Also, the phosphorus level increased with the concentration of niacin in the diets up to 0.075 g and thereafter showed a gradual decrease.

Ammonia Concentration in Water:

The Table 30 shows the mean ammonia concentration (mg/ in the aquaria. The treatment in which the prawns were fed the niacin deficient diet had significantly higher mean ammo concentration (0.03 mg/l/d) compared to other treatments. Tl ammonia concentration in water from other treatments did not differ markedly between themselves, as it ranged from 0.01 t 0.019 mg/l/d.

O B S E R V A T I O N S

During the 45 days of experimental study, observations were also made on molting, general activity and external morphology of prawns to ascertain if, there were any differen induced by the dietary treatments.

Molting:

Table 31 gives the mean total number of exuviae and carcass collected from different treatments. Relatively more numbers of exuviae were obtained from the treatments, where tl prawns were fed on diet with high dosages of niacin(≥0.2g). Tl number of exuviae collected from the replicates were not significantly different. The exuviae obtained from the treatments with lower concentrations of niacin were usually partly eaten by the cohabitors; whereas the exuviae collected from treatments with higher dosages (≥0.2g) of the viamin were

TABLE 30: AMMONIA CONCENTRATION IN SEAWATER HELD IN EXPERIMENTAL AQUARIA.

Concentration of nicotinic acid g/100 g dry diet	Mean ammonia concentration in seawater mg/l/d
0.0	0.030
0.025	0.019
0.05	0.011
0.075	0.011
0.10	0.011
0.15	0.01
0.20	0.012
0.25	0.013

usually complete. In prawn groups fed with 0.2 g and 0.25 g of niacin in the diet, the average number of exuviae collected were 42 and 45 nos, respectively. The number of exuviae collected from other treatments ranged from 24 to 37. From the niacin deficient dietary treatment, the highest number of exuviae (48 nos) were collected.

Post-molt deaths occurred usually as a result of cannibalism by prawns were freshly molted ones, due to the delay in the hardening of exoskeleton. During the first two weeks, cannibalistic tendencies were not evident in various treatments; but from the third week onwards cannibalism was observed in few treatments. Post-molt deaths were more in treatment groups fed on the diet deficient in niacin and those containing high concentrations of niacin in the diet (0.2 g and 0.25 g). In most of these cases, the healthy prawns were found to devour the freshly molted ones. Post-molt deaths were, however, relatively less in the treatments with intermediate concentrations of niacin in the diets.

Food Intake:

During the first two weeks of the experimental study, not much variation was observed in the left-over food between the various treatments. But from the third week onwards, prawns fed on the niacin deficient diet and those fed on diets containing more than 0.15 g niacin, showed poor food intake by leaving large amounts of food. They were also not attracted towards the food when introduced in the water; whereas, prawns fed on diets containing lower concentrations of niacin (< 0.15 g) showed almost uniform food intake, except on days when mass

TABLE 31: OBSERVATIONS IN PRAWNS FED WITH DIFFERENT EXPERIMENTAL DIETS

Concentration of niacin in the diet mg/100 g dry diet	Mean no.of moults recovered	Mean no. of post-molt deaths	Texture
0.000	48	29	SO
0.025	24	9	H
0.050	32	12	H
0.075	37	13	H
0.100	33	12	H
0.150	33	14	SO
0.200	42	27	SO
0.250	45	26	SO

H - hard, SO - soft

molting was observed. Also, these prawns were quickly attracted towards the food on introduction in the tanks.

Behaviour Toward Light:

When light from table lamp (1625 x 10^2 lux) was suddenly flashed into the experimental tanks, the responses of the animals varied considerably in different treatments. However, these responses were prominent only from the third week onwards. Prawns fed on diets with more than 0.1g of niacin responded with active, agitated and incoherent movements when the light was suddenly flashed into the experimental tanks. These incoherent movements subsided after few seconds in the case of prawns fed with 0.1 g and 0.15 g of niacin in the diet. In contrast to higher dosages, prawns fed with less than 0.1 g, but more than 0.025 g of niacin did not show much agitated movements in response to light and invariably, showed normal behaviour. The prawns fed with the niacin deficient diet and 0.025 g of niacin in the diet were passive in their responses towards the flash of light. These responses to light stimulus were also observed in the replicates.

External morphology:

The prawns fed on the niacin deficient diet had black-brownish spots in the abdominal region in all the replicates. The spots were also found in prawns fed with diets containing more than 0.15 g of niacin and were densely distributed along

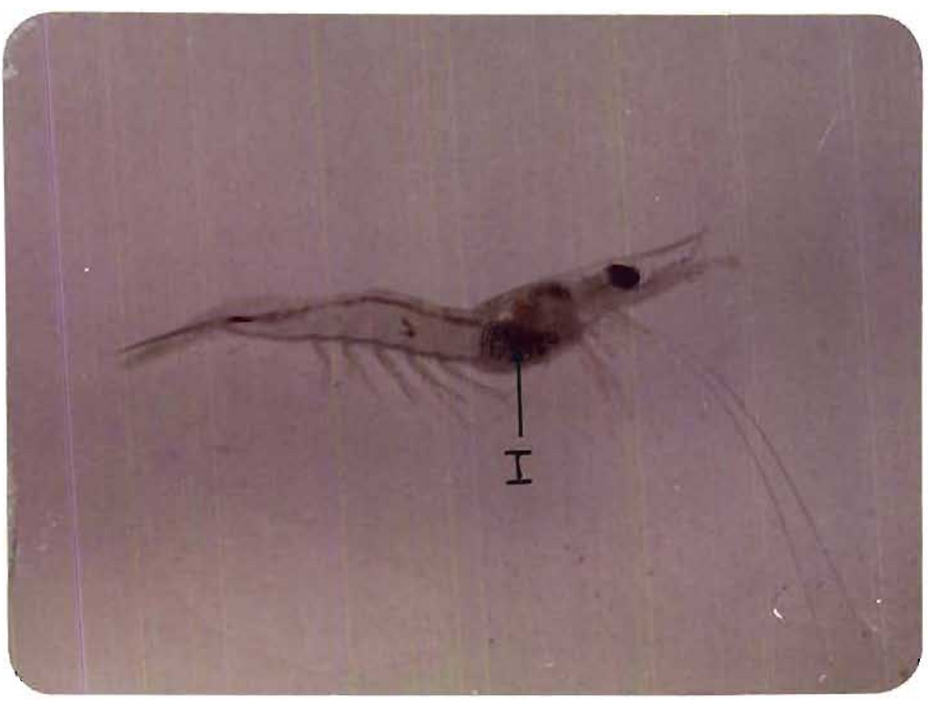
the margins of the abdomen as well as in the gill. However, these spots were not found in prawn groups fed on diets containing niacin concentrations ranging from 0.025 to 0.15 g.

Specific Pathological Observations:

In prawns fed with niacin deficient diet for more than 15 days, in certain specimens, spiral (shaped) black lesions (Plate VI), were observed in the gill region. Initially, these lesions were observed to develop on both the gills as light black bands, but in 6-7 days they became prominent black structures almost covering the gill region completely. With the development of these blackened lesions in the gill region, the prawns ceased to feed, became passive and died within 24 to 48 hrs, after these structures became prominent. Interestingly, these dead prawns bearing blackened lesions were not eaten by the cohabitors. These prawns had hard exoskeleton and were observed to be in the intermolt to pre-molt stages. However, further studies were hampered due to (improvised) fixation procedure. This incidence of blackened lesions was not seen as an out break of infectious disease because in the course of the experimental study only six prawns were spotted to develop these structures in all the three replicates of the niacin deficient treatment.

PLATE VI. Juvenile prawns showing blackening of gills due to nicotinic acid deficiency. I-infection.

PLATE VI



D I S C U S S I O N

Niacin forms an essential component of the coenzymes - nicotinamide adenine nucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), which function as hydrogen donors and acceptors in the biological oxidation processes for the release of energy from nutrients - carbohydrates, protein and lipids. Because of its functional role in biologicaltissues, it is required by all living cells (Halver, 1982). The present study also shows that niacin is an essential constituent in the diet of juvenile prawn, P. indicus for promoting growth, augmenting survival, optimum intake and conversion of food as well as protein.

From the present study, it is evident that when prawns are fed on a niacin deficient diet, heavy mortalities occur. Similarly, high concentration of niacin (0.1 g or more) in the diet, results in abrupt decrease in the survival rate. While niacin deficiency induced high mortality rate from the third week onwards, high concentration of vitamin in the diet induced high mortality from the fifth week onwards. This indicates that prawns are able to survive on tissue vitamin stores only for about two weeks and prolonged deficiency probably results in the breakdown of most of the energy yielding metabolic reactions, since NAD and NADP are important coenzymes taking part in these metabolic cycles. This high mortality could also be due to the heavy loss of nutrients during molting and poor turnover of

dietary energy, because of niacin deficiency. High mortalities due to niacin deficiency has also been reported by McLaren et al. (1947a) in fishes. On the other hand, high mortality rate in prawns fed with 0.1 g or more, reveals the detrimental effect as a result of hypervitaminosis. Similar observations of poor survival at high concentrations of niacin were reported in Moina sps (Conklin and Provasoli, 1977).

Although, the highest survival was recorded in prawns fed with 0.025 g of niacin, there was no significant difference in the survival rate of prawns fed on diets with 0.025, 0.05 and 0.075 g of niacin. The present findings are contradictory to that of Heinen (1984) who observed that inspite of 12 weeks of deficiency of the vitamin, the survival of juvenile M. rosenbergii was not affected and concluded that these prawns may not require niacin in the diet. The differences observed may be due to species specific variations in vitamin requirements.

According to Heinen (1984), deletion or supplementation of niacin in the diet did not have any significant influence on growth and he presumes that niacin may be dispensable in the diet of M. rosenbergii. However, the present study shows that niacin levels in the diets significantly influence the growth of juvenile P. indicus and that a dietary niacin concentration of 0.025 g produce maximum growth. However, growth seems to be inhibited at higher concentration (above 0.075 g) of niacin. Studies in finfish have also shown that excess of niacin in the diet inhibits growth (Poston, 1969b; Halver, 1982).

The ingestion of food by the prawns was also greatly affected both by niacin deficiency and high dosage of niacin in the diets. Initially, for the first two weeks, the prawns were active and fed normally. However, with the prolongation of experimental days, the prawns responded poorly towards the feed in these treatment groups. Data obtained from the experiment however, did not give a clear trend as to the effect of the niacin concentration on either FCR or PER. However, from the highest PER and lowest FCR at 0.025 g niacin in the diet, it can be assumed that the ingested food as well as proteins are more efficiently utilized for tissue growth and protein synthesis at this concentration of niacin. It is also clear from the results that increasing the dietary niacin concentration above 0.025 g in the diet does not proportionately improve FCR and PER but results in poor FCR and PER as a result of hyper vitaminosis.

In trouts and salmons, fed on niacin deficient diet, similar symptoms such as anorexia, reduced growth, poor feed conversion and muscular weakness and increased mortality were reported (McLaren et al., 1947a; Halver, 1982). These dietary symptoms in trouts and salmons were attributed to the inadequate supply of tryptophan in the diet as it is a precursor of niacin (Poston and Combs, 1980a) and it was presumed that salmonids must obtain preformed niacin to meat their requirements (Halver, 1982). Though it is not known whether tryptophan conversion to niacin is possible in P. indicus, yet the results clearly

demonstrate the essentiality of niacin in the diet of prawns.

The biochemical composition of the carcass of prawns also to some extent reveal the functional role of niacin. The moisture content of prawns was observed to be significantly affected by the dietary level of niacin. The significantly higher moisture contents observed in prawns fed with the niacin deficient diet and those fed on the diet with 0.1 g niacin, indicate that the metabolism of prawns may be influenced by the dietary stress, resulting in decreased rate of nutrient deposition. On the other hand, prawns fed with dietary concentration of niacin between 0.05 and 0.10 g recorded relatively low moisture contents, suggesting that the prawn's metabolism was not disturbed at these concentrations resulting in high growth and survival. The results also indicate that the ash, protein, lipid, calcium, magnesium and phosphorus contents of prawns are significantly influenced by the niacin concentration in the diets. Though the prawns fed on the niacin deficient diet, had significantly lower ash, calcium, magnesium and phosphorus and relatively high protein and lipid contents, these results do not truly reflect on the effect of niacin deficiency due to cannibalism and devouring of dead prawns by the cohabitors.

The relatively higher protein and low lipid contents in prawns fed with higher concentration of niacin ($\geqslant$ 0.1 g) indicates that lipid is increasingly utilised for metabolizable energy, sparing protein. In higher vertebrates it has been

shown that very high dosages of the vitamin, decrease the concentration of cholesterol and other lipids in the serum (Beaton et al., 1952). These findings in higher animals and that as reported in the present study clearly shows the interference of niacin at high dosages with that of lipid metabolism and may be it interferes with the carbohydrate metabolism also in prawns, as the levels of carbohydrates tend to decline with increasing concentrations of the vitamin in the diet.

The calcium, magnesium and phosphorus content in the carcass of prawns were also studied to ascertain, if these were affected by niacin levels in the diet. The calcium content was significantly low in the niacin deficient fed prawns compared to other treatments, which indicates that niacin deficiency results in poor calcium metabolism, probably during cuticle formation, making the prawns more prone to post-molt stresses leading to death. On the other hand, calcium levels in niacin fed prawns was high and showed almost an increasing trend with increasing concentrations of the niacin. Yet, prawns fed with 0.1 g or more concentration of niacin showed higher mortality rate, especially post-molt death, compared to prawns fed with 0.075 g or less of niacin in the diet. Thus, these results suggests that under niacin deficiency and niacin at high concentrations in the diet, the calcium metabolism is greatly affected in the prawns and this may be more pronounced during the molting cycle.

C O N C L U S I O N S

From the present study it is evident that juveniles of *P. indicus* have a dietary requirement of niacin and that the optimal requirement for maximum growth seems to be about 0.025 g/100 g dry diet. However, niacin concentrations as high as 0.075 g/100 g dry diet did not affect growth or survival which indicates that these prawns can also function efficiently even at these high levels without much dietary stress as a result of hypervitaminosis.

Conversely, when prawns were fed on diets without niacin or with very high dosage ($\geq$ 0.1 g) of niacin, the metabolism tends to be affected greatly. In the former case metabolic breakdown perhaps results, since from the third week onwards poor survival, growth, food intake and food conversion were observed. The niacin deficiency if prolonged, also results in the dietary deficiency syndromes such as blackening of gills, the intensity of which increased with the prolongation of the experimental period. These prawns ultimately died when the blackening spread around the gills.

CHAPTER-IX

PANTOTHENIC ACID REQUIREMENT

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

Pantothenic acid plays a stellar role in general metabolic pathways. It is a dipeptide derivative and its name (Panto-Greek 'Everywhere') implies its almost ubiquitous distribution. Williams et al. (1933) demonstrated the widespread distribution of the substance, its stimulative effect on the growth of microorganisms and named it pantothenic acid. Later, he and his associates found this factor in the crude concentrate of liver. Subsequent research revealed that this liver extract is required by numerous microorganisms and other animals for preventing certain deficiency symptoms (Woolley et al., 1939). Since the vitamin is unstable, it is used in its calcium salt form for nutritional studies in fish and allied aquatic species.

DeVaries et al. (1950) identified pantothenic acid as a part of the coenzyme A(CoA). Being a part of CoA it has many important metabolic functions. It has significant role in fatty acid oxidation and synthesis, synthesis of cholesterol and phospholipid, in PO_4 energy transfer (Jones et al., 1953; Lipmann et al., 1953) and in the acetylation of aromatic compounds (Hughes, 1953; Mitchell, 1964). Since pantothenic acid is a part of acetyl CoA, it has been shown to be required by all animal species studied so far, including the microorganisms (Chow, 1964; West et al., 1966).

Symptoms associated with pantothenic acid deficiency are mostly not specific and vary from species to species (Chow, 1964; Halver, 1957). Deficiency studies in rats and other higher vertebrates have shown retardation of growth, impairment of reproduction, achromatichia of the hair, imbalance of salt-water metabolism and reduction of CoA content in tissues leading to poor utilization of pyruvate (Chow, 1964). In higher animals, simultaneous deficiency of pantothenic acid and pyridoxine results in loss of conditional reflex performance (Chow, 1964), which reverses to normalcy on adequate supply of the vitamin. It has also been reported that deletion of both pantothenic acid and methionine from the diets of rats induced specific deficiency symptoms with lower levels of CoA, which returned to normalcy on adequate supply of the vitamin or methionine (Dinning et al.,1955).

Aquatic species, especially fishes like salmon and trout, reared at 10-19°C water temperature, fed with pantothenic acid deficient diets were found to exhaust the vitamin stores rapidly in 8-12 weeks. These fishes stop feeding and the gill filaments show proliferation of epithelial surface in addition to swelling and clubbing together of the filaments and lamellae (Philips et al., 1945), and the fishes show signs of sluggishness and prostration. Long periods of deletion of the vitamin results in necrosis, scarring and cellular atrophy of the tender gills (Halver, 1953 ; 1957a; Steffans, 1969). Rapid recovery was reported when the vitamin was supplemented in the diet. In M. rosenbergii ,Heinen (1984) has reported unusual post-molt

deaths in the case of pantothenic acid deficient treatment.

The quantitative requirement of this vitamin varies from species to species and is influenced by a number of factors. Low protein and high carbohydrate levels in the diet showed significant pantothenic acid deficiency symptoms, when compared to high protein and low carbohydrate diets in rats (Nelson et al., 1947).

The involvement of pantothenic acid in the exergonic reactions of the body, releasing free energy for various types of physiological work, is sufficient ground for expecting that the requirements for the vitamin will be in proportion to the amount of organic substrate so catabolised (Mitchell, 1964). However, no concrete conclusions have been drawn in this respect due to insufficient evidences. Experiments carried out till date to define the dosage requirements were based on the body weight. According to this, most workers administer the vitamin on the basis of minimum requirements to prevent dietary deficiency symptoms specific for the species (Chow, 1964).

Requirement for two species of crustaceans - Orconectes virilis and Cancer irroratus have been judged from the formation of acetylcholine by choline acetylase in the nerves (Fisher, 1960). It has been understood that for the formation of acetylcholine, CoA is essential, demonstrating the requirement of pantothenic acid in Crustacea (Fisher et al., 1955). In the lobster

(*Homarus gammarus*) nerves, acetylcholine is hydrolysed at a rate twenty times faster than in the case of frog's sciatic nerve (Marnay and Nachmanschn 1937). Likewise, Walop *et al.* (1950) reported rich concentration of choline esterase in *Carcinus maenas*.

Fritsch (1953) reported the effect of pantothenic acid on the longevity of *Daphina* sps. The life-span of *Daphina* was enhanced by about three times, when they were fed with *Chlamydomonas* sps. grown on a basal diet supplemented with pantothenic acid. However, from the studies on *M. rosenbergii*, Heinen (1984) reported that diets deficient in pantothenic acid promote higher growth rate compared to that of pantothenic acid supplemented control diets and concluded that possibly the vitamin was having inhibitory effect at higher dosages.

Thus, a proper understanding of the requirement of this vitamin is essential before its inclusion in dietary formulations of prawns. Since there is no information on the pantothenic acid requirement of *P. indicus*, the present study was undertaken.

M A T E R I A L A N D M E T H O D S

Pantothenic acid requirement in juveniles of the *P. indicus* was studied using graded levels of calcium pantothenate in isonitrogenous purified diets (Table 33). Preparation of experimental diets were same as described in earlier Chapter (I & II) and the diets were adjusted to 100% by using -cellulose.

TABLE 32: ENVIRONMENTAL PARAMETERS AND STOCKING SIZE OF JUVENILE PRAWNS

Parameter	Mean value
Temperature (°C)	28.9 ± 1.75
Salinity (‰)	21.6 ± 1.80
pH	7.94 ± 0.92
Ammonia concentration in the water (NH_4-N mg/l/d)	0.0083 ± 0.0014
Initial length (mm)	19.769 ± 0.671
Initial weight (mg)	43.9 ± 0.0022

TABLE 33: DIETARY COMPOSITION OF EXPERIMENTAL DIETS WITH GRADED LEVELS OF CALCIUM PANTOTHENATE

Ingredient	g/100 g							
Calcium pantothenate	0.0	0.025	0.05	0.075	0.10	0.15	0.20	0.25
α-cellulose powder	0.25	0.225	0.20	0.175	0.15	0.10	0.025	0.0

Experimental set up, monitoring of environmental conditions rearing of animals prior to the experiment and during experimental study were similar to that described in previous Chapters (I & III) Table 32 gives the mean environmental conditions and the initial length and weight of the animals used for the study.

Parameters considered for pantothenic acid requirement study were same as that used for protein requirement study (Chapter I). The methodology adopted for determination of various parameters and statistical analysis of the data were as that reported in Chapter I.

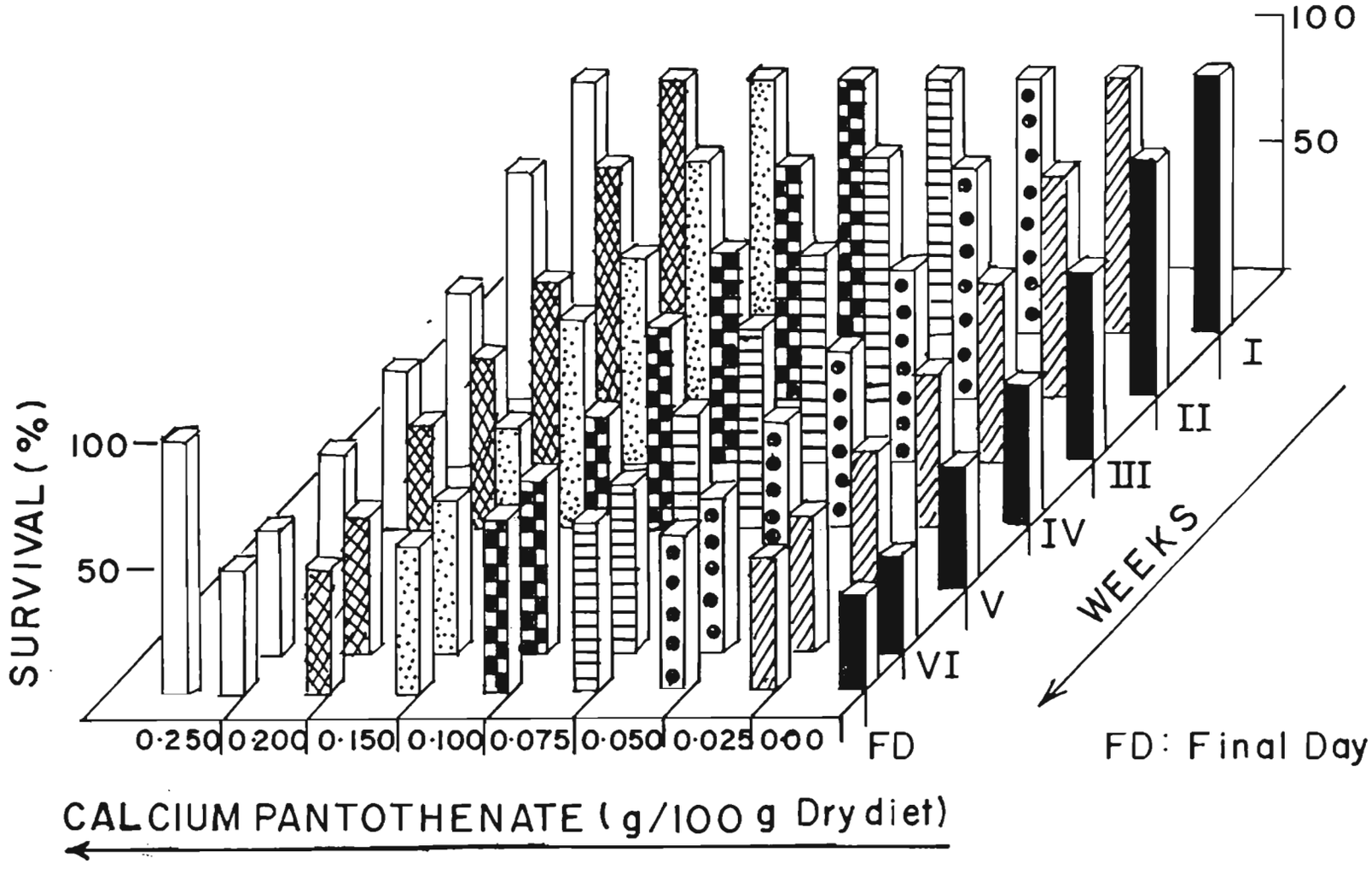
R E S U L T S A N D O B S E R V A T I O N S

Survival:

Survival rate of prawns was significantly ($P < 0.05$) influenced by the diets containing different concentrations of pantothenic acid (Fig. 52). The prawns fed the pantothenic acid deficient diet and those fed diets with 0.2 g or 0.25 g of pantothenic acid gave significantly ($P < 0.05$) lower survival rates than other treatments. The highest survival rate was recorded with 0.1 g of pantothenic acid (68.9%) and the lowest survival with pantothenic acid deficient diet (37.8%). In other treatment groups, the survival rate ranged between 48.9 and 66.7%. The survival rate showed an increasing trend upto 0.1 g of pantothenic acid in the diet and thereafter it showed a gradual decline. No significant variation in survival

Fig. 52. Weekly percent survival of prawns fed diets with different levels of calcium pantothenate.

FIG. 52


100
50
SURVIVAL (%)
100
50
I
II
III
IV
V
VI
WEEKS
0·250
0·200
0·150
0·100
0·075
0·050
0·025
0·00
FD
FD: Final Day
CALCIUM PANTOTHENATE (g/100 g Dry diet)

rate was observed in prawns fed diets containing pantothenic acid concentrations between 0.025 and 0.15 g.

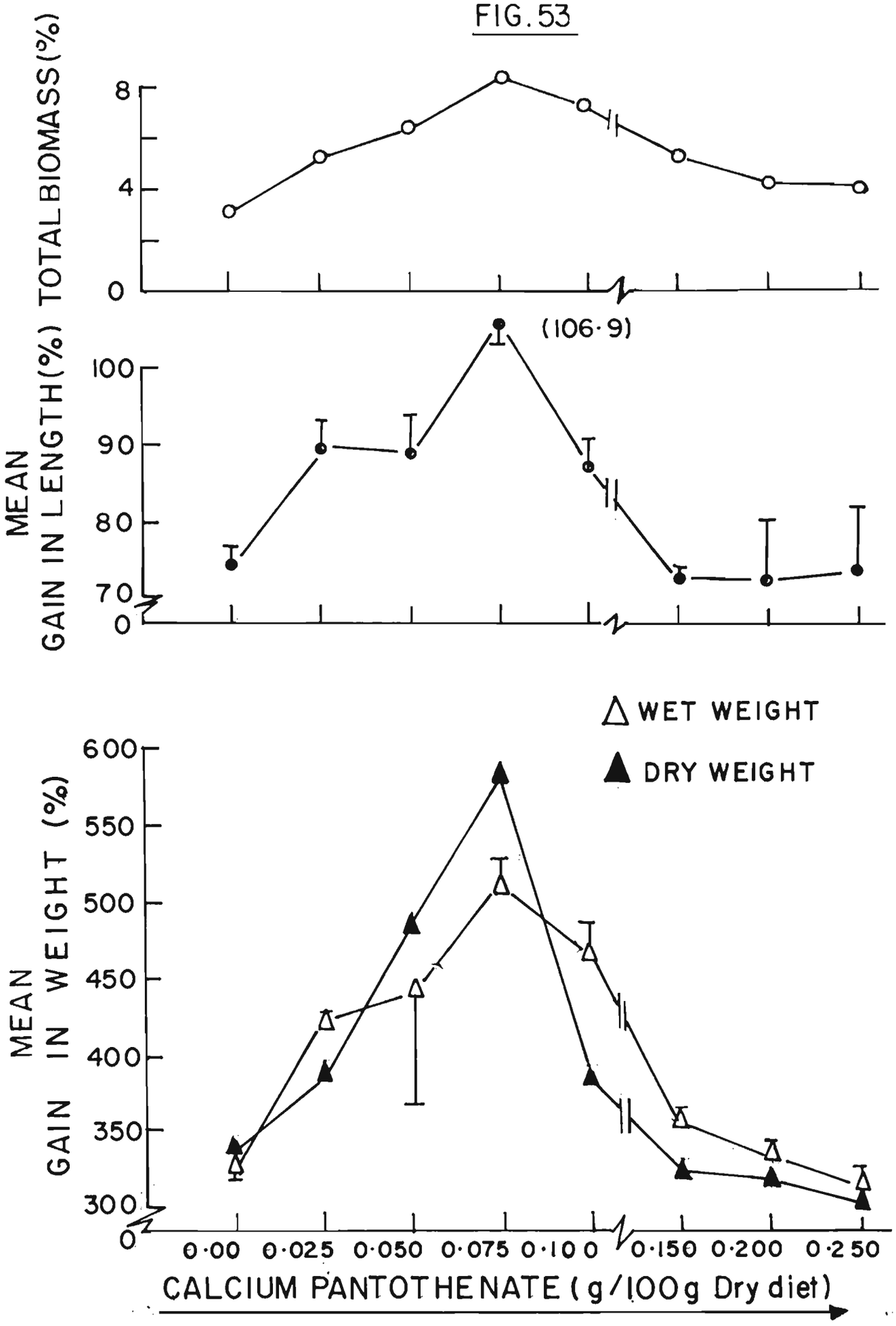
Growth:

Figure 53 shows the mean percent gain in length, wet weight and dry weight recorded in prawns fed on the diets containing different concentrations of pantothenic acid. Analysis of variance of the data showed that the concentration of pantothenic acid in diets have significant ($P < 0.05$) effect on growth. The prawns fed on the diet with 0.075 g of pantothenic acid showed significantly ($P < 0.05$) higher mean percent gain in length (107%), wet weight (511%) and dry weight (582%) compared to prawns from all other treatment groups. Similarly, prawns fed on diets with higher concentrations of pantothenic acid (0.15g and above) and those fed on the pantothenic acid deficient diet showed significantly ($P < 0.05$) lower percent mean gain in length, wet weight and dry weight compared to all other groups. These results indicate that growth of prawns is adversely affected when pantothenic acid is deficient or is in excess in the diet.

There were also no significant differences among the mean percent gain in length and wet weight of prawns fed on the diets containing 0.025 g, 0.05 g and 0.10 g. However, the mean percent dry weight gain was significantly ($P < 0.05$) higher in prawns from treatment with 0.05 g (485%) pantothenic acid when compared to all other treatments, except for the highest value observed at 0.075 g. The mean percent gain in length, wet weight and dry

Fig. 53. Percent gain in length and weight, and total biomass (g) of prawns fed diets with different levels of calcium pantothenate.

FIG. 53


MEAN GAIN IN LENGTH (%) TOTAL BIOMASS (%)
8
4
0
(106·9)
100
90
80
70
0
WET WEIGHT
DRY WEIGHT
MEAN GAIN IN WEIGHT (%)
600
550
500
450
400
350
300
0
0·00
0·025
0·050
0·075
0·100
0·150
0·200
0·250
CALCIUM PANTOTHENATE (g/100g Dry diet)

weight of prawns increased with the concentration of pantothenic acid upto 0.075 g and thereafter showed an abrupt decline with further increase in the pantothenic acid concentration in the diets.

Specific Food Consumption (SFC):

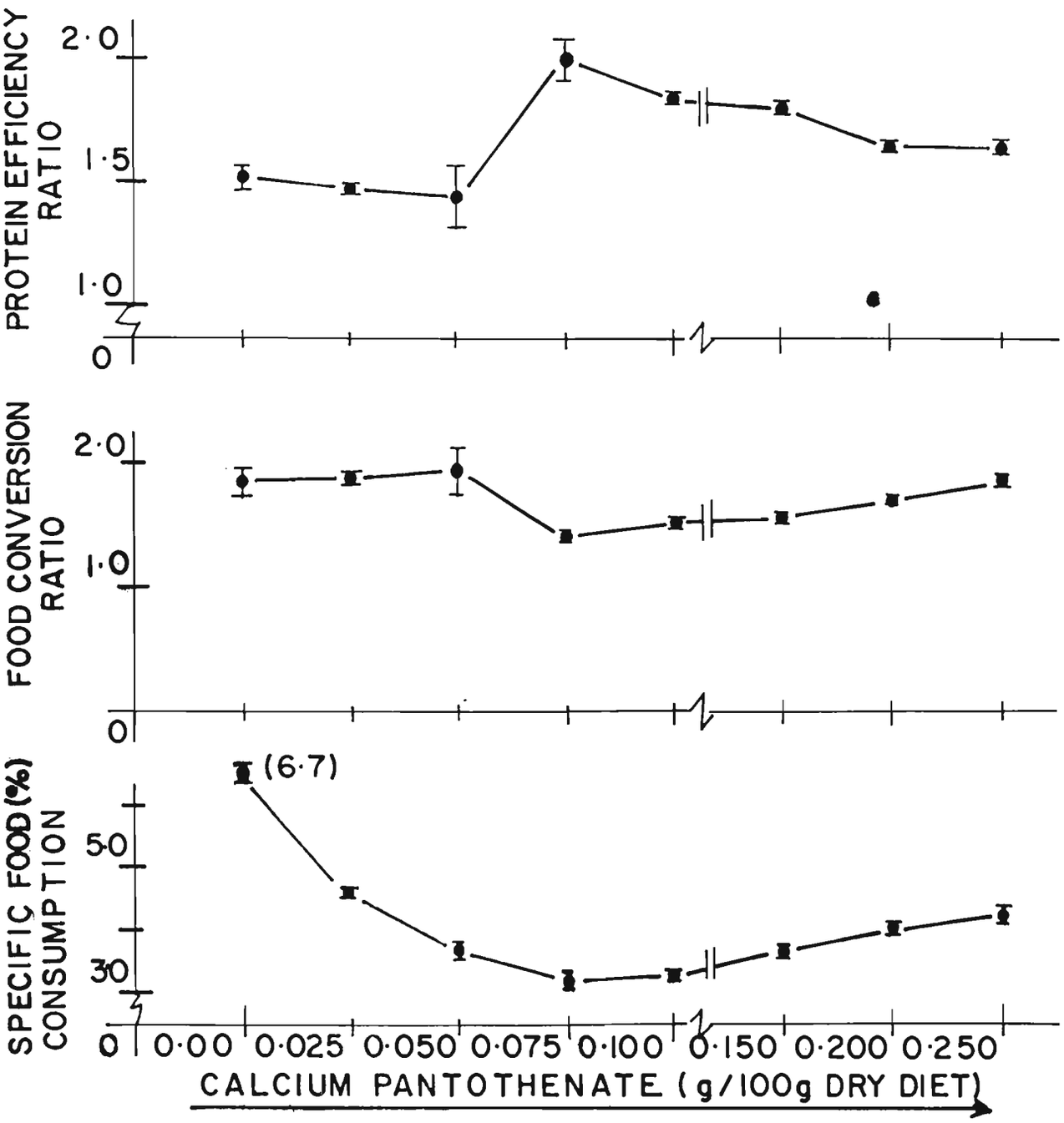
Specific food consumption in prawns was significantly ($P < 0.01$) influenced by the dietary levels of pantothenic acid. The SFC decreased as the pantothenic acid concentration in the diet increased up to 0.075 g and thereafter the SFC increased with further increase in pantothenic acid concentration in the diet (Fig. 54). The maximum SFC was observed in prawns fed on the pantothenic acid deficient diet (6.7%) and the minimum in prawns fed on the diet with 0.075 g of pantothenic acid (3.2%), closely followed by prawns fed on 0.1 g (3.3%) pantothenic acid in the diet. In all other treatment groups, the SFC ranged between 3.69% and 4.21%.

Food Conversion Ratio (FCR):

The diets with various concentrations of pantothenic acid also had significant ($P < 0.05$) influence on the food conversion ratio (Fig. 54). Although, no specific trend in FCR could be observed with increasing pantothenic acid concentration upto 0.075 g, there was a steady increase in the FCR with increasing concentrations of pantothenic acid in the diet beyond 0.075 g. The highest FCR was recorded for the diet containing 0.05 g of

Fig. 54. SFC, FCR and PER for diets with different levels of calcium pantothenate.

FIG. 54


PROTEIN EFFICIENCY RATIO
2·0
1·5
1·0
0
FOOD CONVERSION RATIO
2·0
1·0
0
SPECIFIC FOOD (%) CONSUMPTION
(6·7)
50
30
0
0·00
0·025
0·050
0·075
0·100
0·150
0·200
0·250
CALCIUM PANTOTHENATE (g/100g DRY DIET)

pantothenic acid (1.95) and the lowest for the diet with 0.075 g of pantothenic acid (1.39).

Protein Efficiency Ratio (PER):

Protein efficiency ratios were not significantly affected by the levels of pantothenic acid in the diets. However, minor variations were observed between treatment groups in the PER values (Fig. 54). The highest PER was obtained with 0.075 g of pantothenic acid (1.99) and the lowest was with 0.025 g(1.47), closely followed by 0.05 g (1.43) pantothenic acid in the diet. However, PER did not vary between the pantothenic acid deficient diet (1.51) and the diet with 0.25 g of pantothenic acid (1.52). In all other treatment groups, the PER values ranged between 1.65 and 1.82. However, no specific trend could be observed in the PER obtained from treatments with lower concentrations of pantothenic acid, but beyond 0.075 g of pantothenic acid in the diet, the PER values decreased gradually with increasing concentrations of vitamins.

Biochemical Composition:

The diets with different levels of pantothenic acid fed to the prawns had highly sifnificant ($P < 0.01$) effect on the moisture content in prawns (Fig. 55). However, only prawns fed with 0.075 g of pantothenic acid had significantly ($P < 0.05$) lower moisture content than prawns from all other treatments. There were no significant differences in the moisture content

of prawns among other treatments. The maximum moisture content was recorded in prawns fed on the diet with 0.10 g of pantothenic acid (78.9%) and the minimum in prawns fed on the diet with 0.075 of pantothenic acid (74.1%). However, no specific trend could be observed in the moisture content of prawns with increasing concentrations of pantothenic acid in the diet.

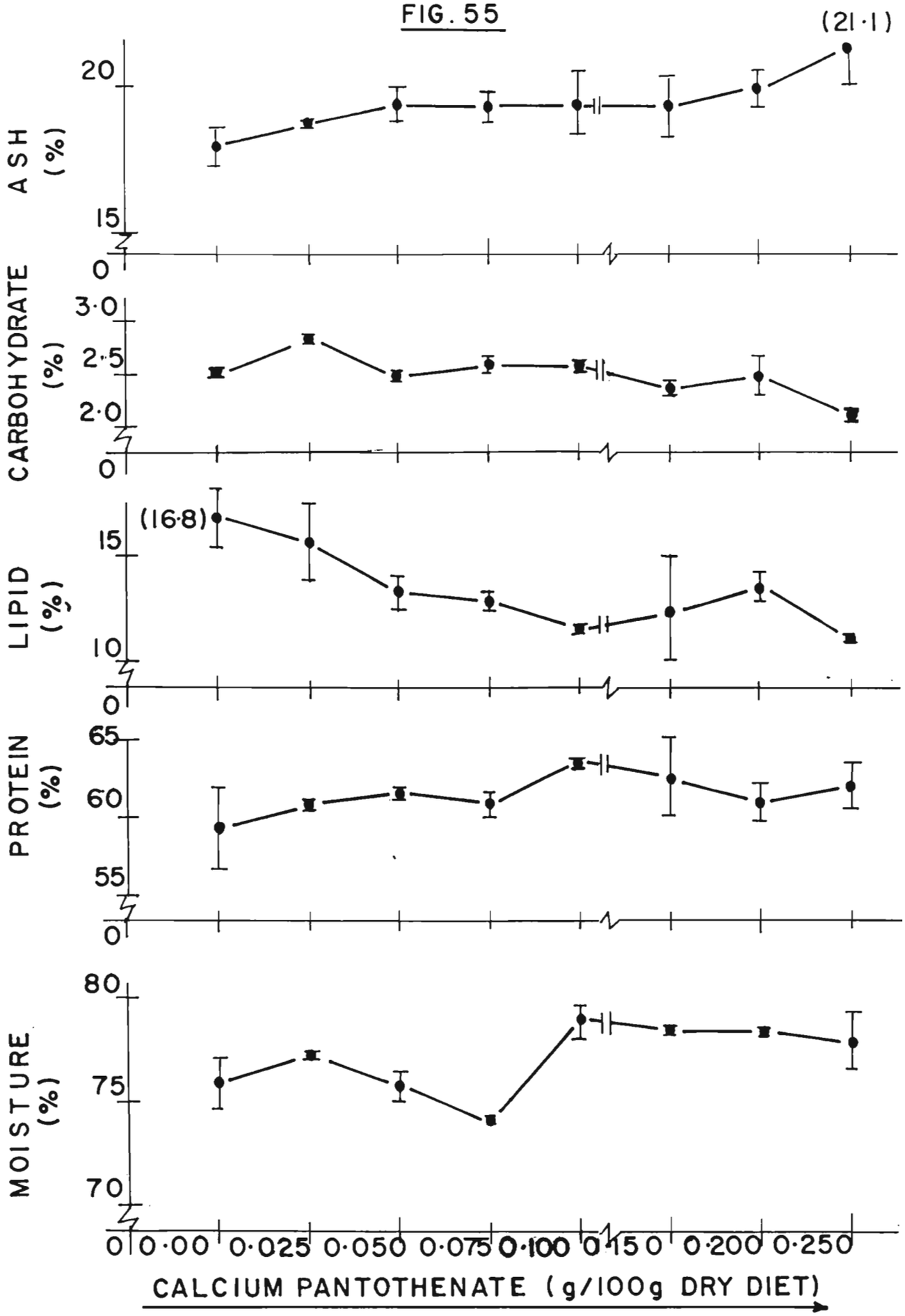
The ash content in prawns (Fig. 55) was not significantly influenced by the diets containing increasing concentrations of the test vitamin. The maximum ash content was recorded with 0.25 g of pantothenic acid (21.2%) and the minimum with the diet deficient in pantothenic acid (18.1%). In prawns from all other treatments, the ash content ranged between 18.8% and 19.9%.

The ash content in prawns (Fig. 55) was not significantly influenced by the diets containing increasing concentrations of the test vitamin. The maximum ash content was recorded with 0.25 g of pantothenic acid (21.2%) and the minimum with the diet deficient in pantothenic acid (18.1%). In prawns from all other treatments, the ash content ranged between 18.8% and 19.9%.

The protein content in prawns (Fig. 55) was significantly ($P < 0.05$) influenced by the dietary concentrations of pantothenic acid. The prawns fed on the diet deficient in pantothenic acid and those fed on the diet with 0.2 g of pantothenic acid only showed significant ($P < 0.05$) differences in the protein content with that of prawns fed on other diets. The prawns fed on the diet with 0.1 g of pantothenic acid had the highest protein (63.1%

Fig. 55. Biochemical composition of prawns fed diets with different levels of calcium pantothenate.

FIG. 55


(21·1)
ASH (%)
20
15
0
CARBOHYDRATE (%)
3·0
2·5
2·0
0
(16·8)
LIPID (%)
15
10
0
PROTEIN (%)
65
60
55
0
MOISTURE (%)
80
75
70
0 0·00 0·025 0·050 0·075 0·100 0·150 0·200 0·250
CALCIUM PANTOTHENATE (g/100g DRY DIET)

and the prawns fed on the diet deficient in pantothenic acid (59.2%) and 0.2 g pantothenic acid in the diets (60.7%) had relatively low protein contents. The protein content in prawns from other treatments insignificantly ranged between 60.6% and 62.4%. The protein content in prawns increased with the concentration of pantothenic acid in the diet upto 0.1 g and thereafter it showed gradual decline with further increase in the vitamin concentration.

The lipid content in prawns was also significantly ($P < 0.01$) influenced by the dietary concentration of pantothenic acid (Fig. 55). The prawns fed on the pantothenic acid deficient diet had significantly ($P < 0.05$) higher lipid content (16.8%), but those fed on diets with 0.1 g (11.6%) and 0.2 g (11.1%) of pantothenic acid had significantly ($P < 0.05$) lower lipid contents, compared to all other groups. In prawns from all other treatments, the lipid content ranged between 12.9 and 15.7%, but the observed differences were not significant.

The carbohydrate content (Fig. 55) in prawns was significantly ($P < 0.05$) influenced by the diets fed to them. However, significant differences ($P < 0.05$) in the carbohydrate contents were observed only between prawns fed on the diets containing 0.025 g and 0.25 g of pantothenic acid, with that of all other groups. The maximum carbohydrate content was recorded with 0.025 g of pantothenic acid (2.8%) and the minimum with 0.25 g of pantothenic acid (2.1%). In all other treatments groups,

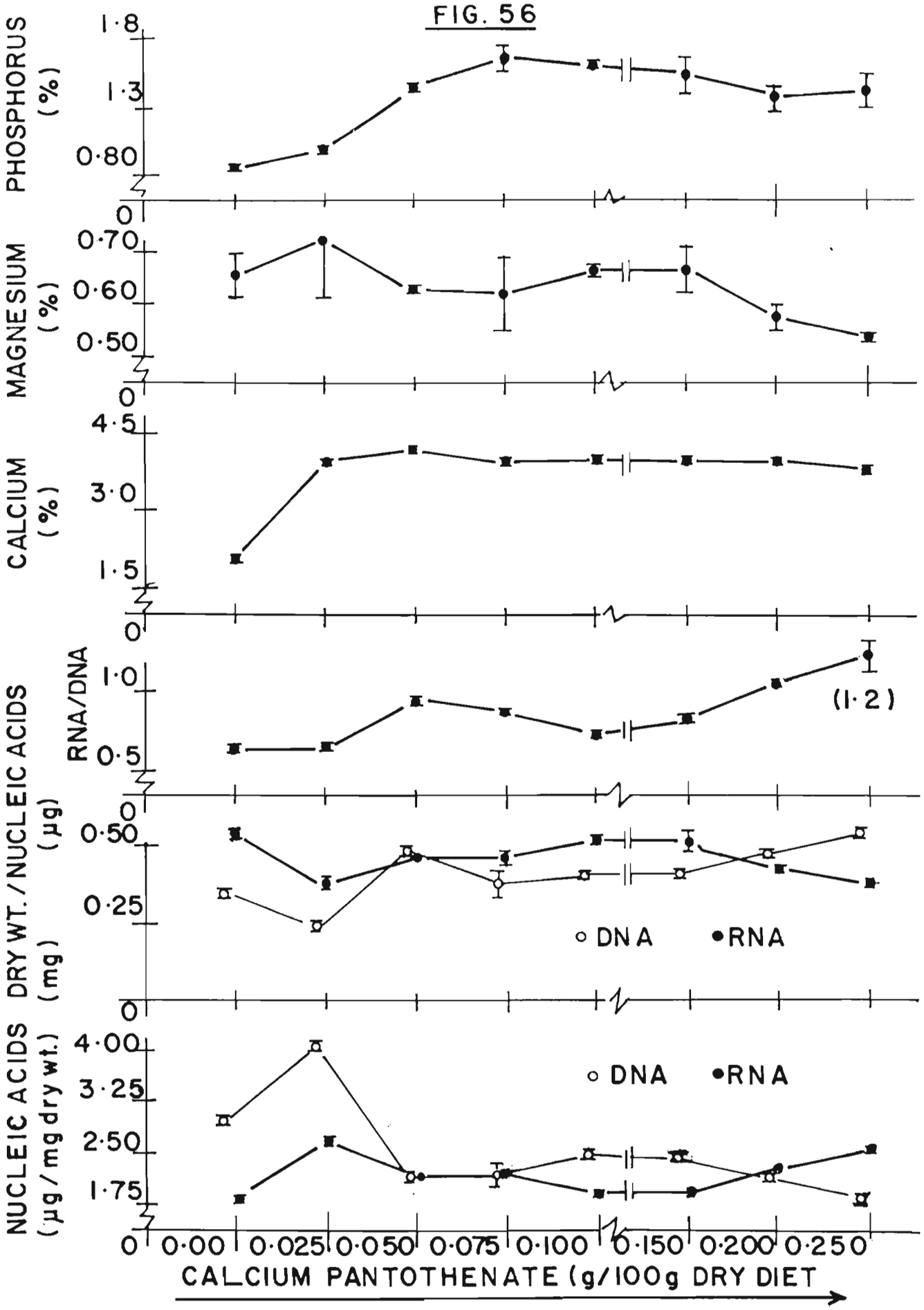
the carbohydrate content ranged between 2.4 and 2.6%. With increasing concentration of pantothenic acid in the diet beyond 0.025 g, the carbohydrate content in prawns showed a gradual, but steady decline.

RNA content (Fig. 56) in prawns was significantly ($P < 0.05$) influenced by the dietary concentrations of the vitamin. The prawns fed on diets containing 0.025 g and 0.25 g pantothenic acid had significantly ($P < 0.05$) higher RNA contents than that in prawns from other treatments. The maximum RNA content was recorded with 0.025 g (2.7 µg/mg) of pantothenic acid, closely followed by 0.25 g (2.6 µg/mg) of pantothenic acid. But the minimum RNA content was recorded in prawns fed on the pantothenic acid deficient diet (1.8 µg/mg). In other treatment groups, the RNA content in prawns ranged between 1.9 and 2.3 µg/mg, but the differences observed between them were not significant. Comparatively, no significant variation was observed in the dry weight/RNA ratio (Fig. 56) with respect to increasing dietary levels of the vitamin. The ratio ranged between 0.37 and 0.53, with the maximum in prawns fed the pantothenic acid deficient diet and the minimum in prawns fed on the diet with 0.025 g of pantothenic acid.

The DNA content in prawns (Fig. 56) was significantly ($P < 0.01$) affected by the dietary levels of pantothenic acid. Significant ($P < 0.05$) difference in the DNA content was observed between prawns fed on the diet with 0.025 g. of pantothenic acid, here the maximum DNA content was recorded (4 µg/mg), and those

Fig. 56. Biochemical composition of prawns fed diets with different levels of calcium pantothenate.

FIG. 56


PHOSPHORUS (%)
1·8
1·3
0·80
0
MAGNESIUM (%)
0·70
0·60
0·50
0
CALCIUM (%)
4·5
3·0
1·5
0
RNA/DNA
1·0
0·5
0
(1·2)
DRY WT./NUCLEIC ACIDS (µg)
0·50
0·25
0
○ DNA ● RNA
NUCLEIC ACIDS (µg/mg dry wt.) (mg)
4·00
3·25
2·50
1·75
0
○ DNA ● RNA
0·00 0·025 0·050 0·075 0·100 0·150 0·200 0·250
CALCIUM PANTOTHENATE (g/100g DRY DIET

fed on the diet with 0.25 g of pantothenic acid; where the minimum DNA content was recorded (1.8 µg/mg). The dry weight/DNA ratio in prawns (Fig. 56) was also significantly ($P < 0.05$) influenced by the dietary concentrations of the vitamin. However, differences in the ratio could be observed between prawns from treatments 0.025 g and 0.25 g of pantothenic acid diet with that from other treatments. The maximum ratio was observed in prawns fed the diet having 0.25 g pantothenic acid (0.54) and the minimum in those fed the diet with 0.025 g of panthothenic acid (0.25). In prawns from all other treatments, the ratio ranged between 0.34 and 0.47. Similar, to the DNA content, the dry weight/DNA ratio in prawns was relatively higher at higher concentrations of pantothenic acid in the diets.

The RNA/DNA ratio also was significantly ($P < 0.01$) affected by the dietary concentrations of pantothenic acid. (Fig. 56). There were no significant differences in the RNA/DNA ratio among prawn groups fed on diets containing 0.15 g or less of pantothenic acid. However, significant ($P < 0.05$) differences were observed between the RNA/DNA ratio in the above groups of prawns (< 0.15 g) and those fed diets with higher concentrations (≥ 0.2g) of pantothenic acid. Thehighest ratio (1.23) was recorded with 0.25 g of pantothenic acid, followed by 0.2 g(1.06) and the lowest ratio (0.63) with pantothenic acid deficient diets. In all other treatment groups, the ratio ranged between 0.73 and 0.95. The prawns

fed on the diets with higher concentrations of pantothenic acid ($\geq$ 0.2g) recorded relatively high RNA/DNA ratios.

The calcium levels in prawns shown in Fig. 56 were significantly ($P < 0.01$) influenced by the diets containing increasin concentrations of pantothenic acid. However, prawns fed the diet deficient in pantothenic acid had significantly ($P < 0.05$) lower calcium levels (2.03%) than the prawns fed on diets with various concentrations of pantothenic acid. From the figure, it is clear that the inclusion of pantothenic acid in the diet significantly enhances calcium content in prawns and that among the various dietary concentrations of pantothenic acid in the diet, ranging from 0.025 to 0.25 g fed to the prawns, there were no significant difference in the calcium content. The maximum calcium content was recorded with 0.05 g pantothenic acid (4.2%) in the diet. However, in all other treatments, the calcium content in prawns differed narrowly from the maximum value and ranged between 3.91% to 3.96%, with slightly lower value in prawns fed on diet with 0.25 g of pantothenic acid (3.8%).

The diets containing various concentrations of pantothenic acid also significantly ($P < 0.05$) affected the magnesium content of prawns (Fig. 56). The magnesium content in prawns fed on diets containing 0.025 g, 0.2 g and 0.25 g pantothenic acid was significantly ($P < 0.05$) different from that in prawns from other treatments while the maximum magnesium content was recorded with 0.025 g of pantothenic acid (0.73%), the minimum was observed with 0.025 g

of pantothenic acid (0.53%). The prawn groups fed on the pantothenic acid deficient diet and those fed on diets containing 0.1 g and 0.15 g pantothenic acid had almost the same magnesium content (0.65%). On the other hand, prawns fed with 0.05 g and 0.075 g pantothenic acid in diets recorded slightly lower magnesium content (0.62%). Though no specific trend could be observed with increasing concentrations of pantothenic acid in the diets, high concentrations seems to influence poor deposition of magnesium in prawns.

Analysis of variance of the data revealed the significant ($P < 0.05$) influence of dietary levels of pantothenic acid on phosphorus content in prawns (Fig. 56). The prawns fed on the diet deficient in pantothenic acid and those fed on the diet containing 0.025 g pantothenic acid had significantly ($P < 0.05$) lower phosphorus content than prawns fed on diets containing higher dosages of the test vitamin. It was also observed that with increasing concentrations of pantothenic acid in the diet, the phosphorus content increased up to 0.075 g and thereafter it declined with further rise in pantothenic acid concentration. While the prawns fed on the diet with 0.075 g of pantothenic acid (1.7%) had the maximum phosphorus, than those fed on the diet deficient in pantothenic acid (0.85%) had the minimum phosphorus content. Diets with 0.025 g pantothenic acid also produced relatively low phosphorus content (0.98%) in prawns. In all other treatments, the phosphorus content ranged between 1.39% and 1.54%.

Ammonia Excretion in Water:

Table 34 shows the mean ammonia concentration levels in the water. The pantothenic acid deficient dietary treatment had more ammonia concentration than the treatment with different concentrations of pantothenic acid. The maximum mean ammonia concentration/day observed in the treatment without pantothenic acid in the diet (0.011 mg/l/d) and the minimum in treatment with 0.1 g of pantothenic acid in the diet (0.006 mg/l/d). In all other treatments the ammonia concentration in the water ranged from 0.007 to 0.010 mg/l/d. The ammonia concentration in water was also significantly ($P < 0.05$) influenced by the dietary concentrations of pantothenic acid.

O B S E R V A T I O N S

Molting:

The mean number of exuviae collected per treatment, during the experimental period, is shown in Table 35. Usually, on most days, complete exuviae were obtained in the early morning, but on certain occasions, the exuviae were incomplete, as they were partly eaten by the cohabitors. Yet, the numbers of exuviae collected were relatively more in prawn groups fed diets containing pantothenic acid concentrations ranging from 0.025 g to 0.15 g. Relatively few number of exuviae were obtained from the treatment where prawns were fed the diet deficient in pantothenic acid as well as in treatments with higher concentrations (≥ 0.15 g) of pantothenic acid. On certain days, during the experimental study,

TABLE 34: AMMONIA CONCENTRATION IN SEAWATER HELD IN AQUARIA

Concentration of calcium pantothenate in the diet g/100 g dry diet	Mean ammonia concentration in water mg/l/d
0.0	0.011
0.025	0.009
0.05	0.009
0.075	0.007
0.10	0.006
0.15	0.008
0.20	0.008
0.25	0.008

TABLE 35: OBSERVATIONS IN PRAWNS FED WITH DIFFERENT EXPERIMENTAL DIETS

Concentration of Calcium pantothenate in the diet g/100 g dry diet	Mean nos. of molts recovered	Mean nos. of post-molt deaths	Texture of the body
0.0	12	7	SO
0.025	29	18	H
0.05	30	14	H
0.075	25	12	SO
0.10	32	13	SO
0.15	26	11	SO
0.20	20	13	SO
0.25	16	9	SO

H - Hard, SO - soft

the prawns fed the pantothenic acid deficient diet were observed to molt partially, along the abdominal region only. The occurrence of partial molting was more after three weeks of deprivation of pantothenic acid from the diet and invariably the prawns died before the complete molt was shed. However, no such case of partial-molting was observed in prawns fed diets with pantothenic acid. Also, in the case of the pantothenic acid deficient dietary treatment, the molted prawns were found to be passive even after 48 hrs of molting, and eventually died. No such passive activity was observed in the case of prawns from other treatment.

Post-molt deaths (Table 35) usually occurred in prawns fed the pantothenic acid deficient diet and those fed with high concentrations of pantothenic acid ($\geq$ 0.15 g) in the diets. Post-molt deaths were more from the fourth week onwards, especially in prawns fed the pantothenic acid diet and in those fed with 0.25 g of pantothenic acid in the diet. In prawns fed with other dietary levels of pantothenic acid, post-molt deaths were not common.

Food Intake:

No significant differences were observed in the food intake of prawns with respect to the treatment, during the first two weeks. However, variations in food intake, in different treatments, were noticed from the third week onwards. Food intake was considerably less in prawns fed on the pantothenic

acid deficient diet and those fed on the diet containing the highest concentration (0.25 g) of pantothenic acid. Large amounts of feed were left out in these treatments. Similarly, reduced feed intake was observed in prawns fed diets containing 0.15 g and 0.2 g of pantothenic acid, from the fourth week onwards. Prawns from these treatments showed poor attractability to feed, once introduced in the tanks and responded passively. However, in all other treatment groups, the prawns showed active response as soon as food was introduced, throughout the experimental period.

Behaviour Towards Light:

Table lamp light (1625×10^2 lux) when flashed into the experimental aquaria, the prawns showed variations in movements in response to the sudden flash of light. Initially, during the first two weeks there was no variation in the response of prawns from various treatments. However, from the third week onwards, prawns responded differently in the different treatments. The prawns/fed the pantothenic acid deficient diet passively responded to the light, and even disturbing the water column or hitting the sides of the tank did not evoke any significant active response in these prawns. Similar, behaviour was observed in prawns fed diets with 0.2 g or more of pantothenic acid from the fourth week onwards. However, in all other treatment groups, sudden flash of light stimulated active movement in prawns and this response was prominently observed in prawns fed diets

containing pantothenic acid concentration ranging from 0.075 g to 0.15 g. The movements were quick and lasted for few minutes before returning to normalcy. In prawns fed diets with lower concentrations of pantothenic acid (0.25 - 0.05 g), the movements were active and normal.

External Morphology:

Unlike in other vitamin studies, the prawns fed diets with various concentrations of pantothenic acid did not have any brown or black spots in the abdominal, gill or rostrum region, excepting in prawns fed on the pantothenic acid deficient diet which had brown spots in the anterior part of the rostrum. In three cases, each from two replicates of the treatment, where pantothenic acid deficient diet were fed to prawns, at the end of fourth week a dark black horse-shoe shaped structure was observed in both the gills. The intensity of the color of this structure enhanced day after day and within 5 days, the prawns died. During the development of these blackened regions, the prawns showed passive movements and responded poorly to the feed. However, prawns could not be preserved for histological studies due to poor fixation of the gills. No other distinct changes were observed in prawns from any of the other treatments.

DISCUSSION

Pantothenic acid is ubiquitous in distribution in all the living cells because of its participation in enzyme catalysed reactions, as it forms an intergal part of the acetyl coenzyme A (DeVaries et al., 1950). Its essentiality and requirement for proper growth and survival of a variety of organisms have been well established (Chow, 1964; West et al., 1966). The present study also shows that pantothenic acid is essential in the diet of juveniles of the prawn P. indicus. It is also evident that pantothenic acid deficiency in the diet leads to increased mortality rate of prawn. Halver (1982) also observed that deficiency of pantothenic acid results in decreased survival rates in a number of fishes. In a recent study in channel catfish fingerlings, it was observed that pantothenic acid deficiency results in complete wiping of the population in 10 weeks time (Wilson et al., 1983).

In the crustaceans, Daphnia pulex, pantothenic acid results in reduced longevity (Fisher, 1960). However, survival rate in juveniles of M. rosenbergii are not significantly affected by feeding the vitamin deficient diet (Heinen, 1984). But so far no studies have been made to demonstrate whether pantothenic acid is biosynthesized in M. rosenbergii or whether contributions from the microbes of the digestive tract mask the actual essentiality of the vitamin.

It is also evident from the present study that survival and growth of prawns are significantly influenced by the concentration of the vitamin in the diet. From the results, it is clear that growth and survival are significantly enhanced up to a dietary concentrations of 0.075 g and that concentrations above 0.1 g affect growth and survival, probably due to hypervitaminosis. Wilson *et al.* (1983) reported that with the increase in concentration of pantothenic acid in the diet, mortality rate reduced upto a dietary concentrations of 1 mg/100 g diet. However, they did not observe any variations in the mortality rate above this concentration of the vitamin. In the present study also, survival rate increased with the increasing concentrations of the vitamin in the diet up to 0.1 g, but further increase in dietary concentrations did not promote survival. However, the growth data clearly indicate that the prawns require a dietary concentration of about 0.075 g pantothenic acid/100 g dry diet for maximum growth, and further increase in pantothenic acid in the diet results in growth retardation, perhaps due to hypervitaminosis. On the contrary, Heinen (1984) reported relatively higher growth in prawns (*M. rosenbergii*) fed with pantothenic acid deficient diet, than their control counterparts having 0.06% pantothenic acid and he presumed that the poor growth is due to the detrimental effect of excess of vitamin dosage (Heinen, 1984).

The data for food consumption (SFC), food conversion ratio (FCR) and protein efficiency ratio (PER) also clearly

indicate that pantothenic acid is an essential vitamin and a concentration of 0.075 g/100 g dry diet is necessary to obtain optimum food intake and for best utilization of food and protein. Further, the data indicate that increasing the concentration of the vitamin in the diet above this concentration does not bring about any significant improvement in utilization of food and protein. The utilization of the ingested food as well as protein seems to be affected by the deletion of pantothenic acid from the diet. Poor food intake and conversion have also been reported in fishes fed with pantothenic acid deficient diets (Halver, 1982; Millikin, 1982). However, in juvenile M. rosenbergii, no significant variations in food intake and food conversion was observed in prawns fed on pantothenic acid deficient diet as compared to those fed on control diet with pantothenic acid (Heinen, 1984).

The vitamin deficient diet when fed to the prawns significantly influence their behaviour. These prawns showed passive response to various stimulus. In comparison, prawns fed with more than 0.15 g pantothenic acid in the diet showed hyperactivity. However, when fed with optimal levels (between 0.025 to 0.1 g) of pantothenic acid, prawns showed normal behaviour suggesting that underfeeding and overfeeding pantothenic acid in the diet significantly influence the metabolic activities of prawn. According to Halver (1957) deficiency of pantothenic acid causes scavring and atrophy of cellular layer which may result in the loss of conditional reflexes

(Gantt et al., 1959) leading to passive activity. The same may hold true for these prawns, when fed with pantothenic acid deficient diet.

It is also evident from the present study that long periods of deletion of pantothenic acid from the diet results in imbalances in the metabolism leading to poor growth followed by death of the prawns. Similar observations were reported in fishes where they were observed to become prostrate or sluggish, finally leading to death (Halver, 1957). Also in the present study it was observed that some of the prawns fed on the diet deficient in vitamin show partial molting along the abdominal region and pleopods only. Similar observations have been made in juveniles of M. rosenbergii (Heinen, 1984). However, partial molting was not prominent in first two weeks; probably the animals tend to subsist on body stores of pantothenic acid.

The biochemical composition of the prawns were also significantly influenced by the dietary concentrations of the vitamin. The lowest moisture content in prawns fed on 0.075 g vitamin in the diet, clearly suggests that at or near optimal concentrations of the vitamin, the prawns show maximum growth with high dry matter content. However, since there was no significant variation in the moisture content between prawns fed on 0.05 g and 0.075 g pantothenic acid, it is clear that for maximum accumulation of organic and inorganic component, 0.05 g of pantothenic acid in diet is good enough.

In higher vertebrates also moisture content has been found to be significantly influenced by pantothenic acid concentrations. These moisture content variations, however, should reflect on the deposition of other nutrients. Amongst the nutrients-ash, protein and carbohydrate were not significantly influenced by the different levels of pantothenic acid in the diet. In the case of pantothenic acid deficient diet fed prawns, the protein content was low and the ammonia concentration in water was high, suggesting active protein catabolism. According to Nelson and Evans (1945) pantothenic acid has sparing action on protein and thus, lower protein deposition in prawns are quite probable under pantothenic acid deficiency. Also, Halver (1982) reported that dietary insufficiency of pantothenic acid impairs the normal metabolism in mitochondria of the cells. So the imbalances as a result of dietary insufficiency of pantothenic acid could have been the probable cause of poor nutrient deposition in prawns.

Similarly, at high concentration ($\geqslant$ 0.15 g) of pantothenic acid, ash deposition was also high when compared to lower concentration of pantothenic acid. Possibly, hypervitaminosis results in the imbalance of salt-water metabolism, which was also observed in rats due to over-dosage of pantothenic acid feeding (Mitchell, 1964). However, the present results indicate that pantothenic acid has significant role in calcium metabolism. The relatively low values of calcium and phosphorus in prawns at high concentration ($\geqslant$ 0.15 g) of vitamin in the diet suggests that probably at these hyper-dosages, their utilization may be affected.

The role of pantothenic acid in the regulation of RNA-DNA concentration was quite clear. Under deficiency of the vitamin, the prawns had lower RNA and higher DNA contents with low level of protein synthesis resulting in poor growth. Conversely, prawns when fed with high dosage of vitamins($\geqslant$0.15 g) had higher RNA and low DNA contents, which suggests that higher protein synthesis to be taking place; yet the growth was poor. In these prawns, the synthesized proteins seems to be catabolized significantly, as high concentration of ammonia in water was recorded. However, when pantothenic acid is given at or near optimal levels (0.05 to 0.075 g), the concentration of RNA and DNA did not significantly vary and the growth was maximum suggesting that at these concentrations of the vitamin, the prawns are efficiently utilising the dietary proteins for tissue synthesis.

Similarly, the lipid metabolism is affected by the dietary concentrations of pantothenic acid. Under vitamin deficiency, the lowering of CoA concentration in tissues results in slow breakdown of nutrients as a result of imbalances in the metabolism (Mitchell, 1964). In the present study also, high lipid deposition in pantothenic acid deficient diet fed prawns could have been caused by poor utilization of lipids. Thus, all these parameters substantially support that prawns have an optimal requirement of pantothenic acid for maximum growth and survival.

CONCLUSION

From the present study in juvenile prawns, it is apparent that pantothenic acid is essential in the diet of prawns. However, Heinen (1984) showed that pantothenic acid is not essential in the diet of juvenile M. rosenbergii. Probably, there can be differences in the vitamin requirements between penaeids and non-penaeids (Heinen, 1984). It is also evident that juvenile P. indicus require a dietary pantothenic acid concentration of 0.05 g to 0.075 g/100 g dry diet for proper growth, survival and utilization of the food and protein.

Deficiency of pantothenic acid in the diet causes growth retardation, anorexia, poor food conversion, partial molting, passive activity and eventual death of prawns. All these may be caused by poor utilization of pyruvate in tissues as a result of reduction in CoA content as demonstrated in rats and other vertebrates (Mitchell, 1964).

On the other hand, prawns fed with high concentfations ($\geqslant$0.15 g) of pantothenic acid showed some symptoms as that of prawns fed with deficient diet which ultimately affected the growth and survival. Here the tissues may not be deprived of CoA, but the over-dosage of pantothenic acid may have a negative feed back effect on the formation of CoA, resulting in metabolic imbalances, since prawns do not have an efficient mechanism of ridding themselves of excess dietary water-soluble vitamins (Heinen, 1984), for which pantothenic acid may not be an exception.

SUMMARY

The present study was carried out to determine the requirement of protein and water-soluble vitamins in the diet of juveniles of the Indian white prawn *P. indicus*, using purified diets, and to evaluate the nutritive value of a few plant and animal protein sources for the same species. A total of ten statistically designed experiments were conducted in the laboratory under almost identical conditions and following similar methodologies.

Data on survival, growth, specific food consumption, food conversion ratio, protein efficiency ratio and biochemical composition of the prawns were collected from these experiments. Observations were also made on molting, food intake, response to photo-stimulus and changes in external morphology of the prawns. In few experiments, ammonia excretion by prawns was also recorded to elucidate the effects of experimental diets on excretion rates. Observations were also made on the histology of selected tissues of prawns from the experiments on ascorbic acid and choline requirements. Besides these, specific and non-specific symptoms observed in prawns, associated with deficiency or excess of the selected water soluble vitamins, were recorded. Analysis of variance was performed on the data to determine whether there were any significant influence of the test diets on the observed parameters. Least Significant

Difference Test was employed to determine the significant difference between treatments in the observed parameters.

The salient findings from these studies are given below:

1. Deficiency or sub-optimal levels of protein in the diet significantly affected the growth, survival, ingestion and utilization of food and protein and general maintenance of body functions in prawns. Prolonged period of protein deficiency induced cannibalistic tendencies in prawns and resulted in near complete wiping of prawn population.

2. The optimal protein requirements of these juvenile prawns was found to be within the range of 35-40%.

3. Supra-optimal levels of protein (beyond 40% protein) in the diet had deleterious effect on growth, survival, body composition and utilisation of food and protein. Excess of proteins in diets resulted in enhanced rate of protein catabolism resulting in increased ammonia excretion rates.

4. Among the natural protein ingredient sources used for compounding diets, proteins of animal origin significantly improved survival and growth in prawns. A mixture of animal protein sources proved to be a superior protein source when compared to only plant or mixture of plant-animal protein sources.

5. Amongst plant protein sources, soybean meal and ground nut oil-cake were found to be as good as many of the animal protein

sources in promoting survival, growth and feed efficiency. The results clearly showed that soybean meal and ground nut oil-cake can be successfully used as protein sources in compounded diets. Diets based on these protein sources, were readily accepted and the prawns showed high protein efficiency ratio and low food conversion ratio compared to all other plant protein sources tested.

6. The protein sources of crustacean origin, namely the prawn meal and crab meal were found to show better food conversion ratio and protein efficiency ratio amongst all animal protein sources tested. These prawns also had high levels of energy nutrients in the tissues.

7. The purified diet was used as reference diet and it produced better survival and growth. The protein efficiency ratio was also higher than most other protein sources tested, indicating that purified diets can be used effectively for nutritional requirement studies in prawns.

8. The deficiency of ascorbic acid, thiamine, niacin, pantothenic acid, inositol, riboflavin and choline in the diets severely affected the survival and growth of prawns. All these water-soluble vitamins were found to be indispensable for these prawns.

9. Prolonged deficiency of ascorbic acid in the diet resulted in general decline in the metabolic activity leading to poor food

intake aversion towards food and eventually death. The carcass composition clearly showed disturbances in the nutrient deposition. Histological examination of cellular layers of muscle and hepatopancreas showed lysis affected regions. The prominent externally visible changes observed in the prawns were blackening of gills and lesions in the abdominal region.

11. Inclusion of ascorbic acid at concentrations of 0.4 g and 0.8 g/100 g dry diet significantly improved growth, survival, food consumption, food conversion and protein efficiency as well as nutrient deposition. Histological studies showed normal cellular structures in the muscle and hepatopancreas.

12. Supra-optimal levels of ascorbic acid in the diet significantly affected growth, survival, food and protein conversion efficiencies, suggesting the deleterious effect of hypervitaminosis

13. Deletion of both choline and lecithin from the same diet, resulted in significant decrease in growth and survival and affected the food consumption and protein conversion. The prawns showed aversion towards food after two weeks which resulted in their passive activities and ultimately resulted in death. Histological examination of the muscle, nerve and hepatopancreas of these prawns showed normal cellular structures.

14. Supra-optimal levels of choline in diets resulted in lower growth rate and survival, suggesting that these prawns require optimal levels of choline in the diet for maximum growth and survival.

15. Deletion of both thiamine and carbohydrate from the same diet severely affected the growth and survival of prawns. The consumption and conversion of food, and protein efficiency ratio were significantly influenced by the diet. Supplementation of carbohydrate, but deletion of thiamine, resulted in better growth than the above diet. Deficiency symptoms associated with thiamine deletion in either treatments, were unstability, increased sensitivity to shock and aversion towards food.

16. The optimal thiamine requirement in prawns was observed to be around 0.01 g/100 g dry diet as high survival, growth and better food conversion and protein efficiency ratios were recorded at this concentration of thiamine.

17. Supra-optimal levels of thiamine did not produce much variation in survival, yet resulted in poor growth suggesting hypervitaminosis effect of thiamine on prawn's metabolism.

18. Pyridoxine deficiency did not significantly affect the survival; but the growth, efficiency of conversion of food and protein, and the body chemical composition were significantly affected by the deletion of the vitamin.

19. The pyridoxine requirement in prawns was in the range of 0.01 g to 0.02 g/100 g dry diet, for maximum survival and growth. Data for other parameters also support these findings.

20. Supra-optimal concentrations of pyridoxine in the diet produced significantly lowered growth rate and survival and

caused significant variations in other parameters studied.

21. Niacin deficiency in the diet resulted in extremely high mortality rates, suggesting that the vitamin is indispensable for the prawns. The prominent dietary deficiency syndromes observed were early setting of anorexia and aversion towards food. Prolongation of niacin deficiency caused blackening of gills in some prawns which resulted in ultimate death of the prawns.

22. Niacin when added in the diet at 0.025 g/100 g dry diet produced high survival and growth. These prawns showed better food conversion and protein efficiency ratios and nutrient deposition.

23. Supra-optimal dietary levels of nicotinic acid resulted retarded growth and lower survival rate. The other parameters studied showed similarly poor results.

24. Deletion of pantothenic acid from the diet significantly affected the growth and survival of prawns. These prawns showed deficiency syndromes such as anorexia and aversion towards food. The most significant syndrome observed was partial molting in the abdominal region in these prawns.

25. The optimal pantothenic acid requirement was found to be around 0.075 g/100 g dry diet. The high survival rate and growth, the food conversion ratio, protein efficiency ratio, biochemical composition and other observations support these findings.

26. The sub and supra-optimal dosages of pantothenic acid in the diet resulted in poor growth and survival in these prawns. The other parameters studied were also affected by low or high dosages of pantothenic acid.

27. Some of the significant deficiency and hypervitaminosis syndromes observed in prawns from various experiments are presented in Table 35.

Thus, the present findings clearly suggest the essential and optimal requirements for protein as well as water-soluble vitamins in the diet of juvenile P. indicus.

TABLE 36: SUMMARY OF DEFICIENCY/HYPERVITAMINOSIS SYNDROMES RECORDED IN JUVENILE *PENAEUS INDICUS* DURING THE PRESENT STUDY

Vitamin	Deficiency Syndromes	Hypervitaminosis Syndromes
Ascorbic acid	Poor food intake, conversion and protein efficiency, aversion towards food, high incidence of post-molt deaths, hyper activity and hyper sensitivity to photo-stimulus and shock, poor survival and growth, dystrophy of muscle and hepato-pancreas, blackening of gills.	Poor growth, poor food intake and conversion, aversion towards feed, delayed setting of hyper sensitivity to photo stimulus and shock.
Choline (with lecithin)	Poor growth and survival, poor food intake, aversion towards food, passive response to photostimulus and shock.	Poor survival and growth, high post-molt deaths, poor food intake and aversion towards food, hyperirritability, hyper sensitivity to photostimulus and shock.
Choline (without lecithin)	Poor growth and survival, poor food intake, aversion towards food, hypo-sensitivity to photo stimulus and shock and passive activity, dystrophy of muscle and hepatopancreas.	—
Thiamine	Poor growth and survival, poor food intake and aversion to food, hypersensitivity to photostimulus and shock.	Poor growth, delayed setting o food intake, aversion towards food, normal response to photo stimulus and shock.
Pyridoxine	Poor growth, poor food intake, aversion towards food, hypersensitivity to photo-stimulus and shock.	Poor survival and growth, poor food intake, conversion and aversion towards food, hyper sensitive to photostimulus and shock, hyperirritability.
Niacin	Very poor survival and growth, poor food intake and aversion towards food, hypo-sensitive to photostimulus and shock, highly inactive, develops black lesions on the body, and gills, muscle spasms when disturbed.	Poor survival and growth, high post-molt deaths, poor food intake and aversion towards food, hyperifratability, and very sensitive to photostimulu and shock.
Pantothenic acid	Very poor survival and growth, poor food intake and aversion towards food, hypo-sensitive to photostimulus and shock, sluggishness increases as deficiency prolonged, long periods of deficiency shows partial molting along the abdomen and other parts of the body, resulting in death.	Poor growth and survival, poor food intake and aversion towar food, passive activity, hypo-sensitive to photostimulus and shock, higher post-molt deaths
Riboflavin	Poor survival, poor food intake, aversion towards food, hyperirritability, incoordi-nated movements, sensitive to photosti-mulus and shock.	Not studied.
Inositol	Poor growth and survival, poor food intake, aversion towards food, sluggishness, hypo-sensitive to photostimulus and shock.	Not studied.

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