EFFECTS OF VARIOUS GROWTH PROMOTERS IN THE DIETS OF MACROBRACHIUM ROSENBERGII POST LARVAE

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THESIS

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DEPARTMENT OF AQUACULTURE

COLLEGE OF FISHERIES

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1994

DECLARATION

I hereby declare that this thesis entitled EFFECT OF VARIOUS GROWTH PROMOTERS IN THE DIETS OF *Macrobrachium rosenbergii* POST LARVAE is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma associateship fellowship or any other similar title of any other University or Society

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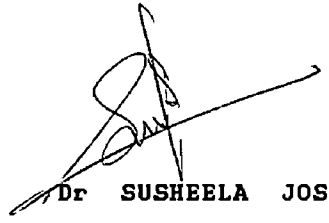

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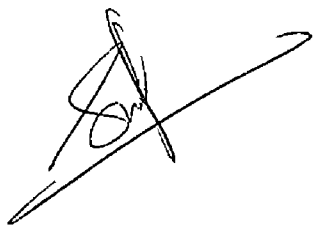

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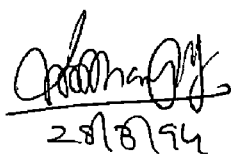

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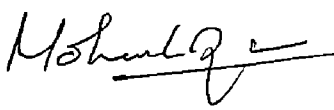

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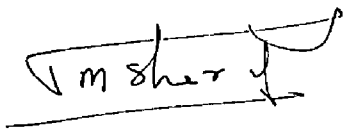

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INTRODUCTION

1 INTRODUCTION

The giant freshwaterprawn Macrobrachium rosenbergii (De mann) offers high farming potential due to its fast growth rate, high tolerance to wide range of temperature and salinity, acceptance to both plant and animal diets comparatively tame and less cannibalistic behaviour absence of major disease problems, compatibility with non predacious species of fish, short larval period and high internal and export value (Ling and Castello 1979) Though Macrobrachium culture has developed as a profitable venture in many parts of the world, the present production through farming of the species is only 5 - 6 % of the total world production through aquaculture Hence intensive farming techniques of the species have to be further developed

Inorder to develop intensive farming of Macrobrachium highly proteinaceous nutritive feeds are indispensable, since the success in the farming operation is mainly dependent upon the quality of feeds used The feed is normally the largest single item in the running of a shrimp farm and the suitability and cost effectiveness of the ration is of paramount importance for the commercial success of culture of any species (New, 1976)

Inorder to achieve maximum growth rate with minimum food conversion ratio, the cultured species must be

presented with the correct amount of feed with optimum quantity of protein, carbohydrate, fats minerals and vitamins But inorder to improve the growth above the physiological maximum, the species must either be genetically manipulated or given a suitable drug, with nil residue effect in the flesh, which will act pharmacologically, to improve the metabolic and/or digestive efficiency and promote protein deposition and hence growth (Matty, 1988) Improved nutrition use of growth promoters, controlled environment and selective breeding, are some of the techniques which have yielded substantial benefits in terms of increased growth rate

Use of additives in food of animals to promote faster growth, has been practised in meat producing industries for several decades But in the field of fisheries, application of growth promoting substances is relatively recent The major benefits of using growth promoting substances are, the time required for the cultured species to reach appropriate size can be reduced, the species can be grown to a larger size during the normal rearing period and food conversion efficiency can be improved, thus reducing the feed cost Growth promoters used in aquaculture are delivered in the feed and have nil residues in the flesh when marketed

Growth promoters in aquaculture feeds are applied quite widely in the culture practices of coldwater fishes channel catfishes and tilapias But the use of these

substances in prawn and shrimp feed is of very recent origin though many of the commercial shrimp feeds at present contain these promoters in minute quantity

In Macrobrachium rosenbergii culture, growth promoters have an important role since the rearing period of this species is more lengthy for attaining marketable size compared to penaeid shrimp species Thus a suitable growth promoter which accelerates the growth of the species and can reduce the rearing time is highly beneficial in reducing the feed cost and thus increase returns from Macrobrachium culture The growth promoters tried in the present study are those cleared by the U S Food and Drug Administration to be used in the feeds and hence used at the safe level in the aquaculture feeding studies

The objective of the present study is two fold The first one is to evaluate the efficiency of selected growth promoters viz oxytetracycline an antibiotic thyroxine a thyroid hormone and papain a proteolytic enzyme The second objective is to find out the optimum level of the selected growth promoter which gives maximum growth in the first experiment

REVIEW OF LITERATURE

2 REVIEW OF LITERATURE

Use of growth promoters in aquaculture is gaining importance as a means to increase production and thereby final economic returns Growth promoters are non-nutritive materials, which at very low levels of incorporation in the feed, increase the feed utilization (Viola and Arieli, 1987) Growth promoters are intended to improve the metabolic and/ or digestive efficiency and thereby promote protein deposition and growth in organisms Addition of these substances were found to improve the performance and feed efficiency by 10% to 20% (Akiyama et al 1991) Use of growth promoters in pig and poultry feed has been known since long But their application in aquaculture diets, especially in prawn diet is of recent interest, and comparatively few works have been reported Several classes of chemical compounds viz, antibiotics, vitamins, hormones arsenicals, tranquilisers, and enzymes have been reported to stimulate the growth of animals To be effective as a commercial growth promoter a drug must be cheap, capable of being delivered in the food and should have nil residue in the flesh when marketed (Matty 1988)

2 1 Antibiotics

Antibiotics may be defined as chemical

substances produced by micro organisms which in dilute solution have the capacity of inhibiting the growth of other microorganisms and even of destroying them

It was Moore et al (1946) who discovered that the addition of antibiotics in subtherapeutical levels in chicken feed increased growth rate and food conversion Later antibiotics were tried successfully in chicken and pig feeds by Stockstad and Jukes (1950) Mc Ginnis et al (1949) and Jukes and Williams (1953) Later these compounds were also tried in various meat producing animals with varying response

Antibiotics were first tried in the diets of fishes by Wagner (1954) in rainbow trout fingerlings but the result was not encouraging Later Snieszko (1957) & Malikova and Kotova (1961) tried antibiotics in feeds of salmonids but met with failure

Sukhoverkhov (1967) found that the use of terramycin at a level of 20 000 units given every three days increased the growth of Cyprinus carpio by 9 5% Mitra and Ghosh (1967) reported a growth enhancement in Indian major carps when fed on terramycin

The growth promoting properties of antibiotics were at first thought to be due to the presence of vitamin B12 in the preparation but it was later shown that the antibiotic gave greater response than pure vitamin (Mc Donald

et al 1973) Some other mechanisms of growth promotion by antibiotics have been discussed by Luckey (1963), Jukes and Williams (1953) & Francois (1962) Visek (1978) suggested that growth promoting concentrations of antibiotics modify the microflora or their products within the gastrointestinal lumen But Ahmed and Matty (1989) from their investigations of the bacteria in the intestine established that weight gain was neither due to an increase nor to a decrease of the bacteria but due to a protein sparing action of the antibiotic

The European Economic Community EEC (1985) has classified antibiotics into two groups therapeutic and infeed antibiotics Infeed antibiotics are permitted for inclusion at low levels in commercial diets of animals over a long period

Viola and Arieli (1987) tried a variety of antibiotics as growth promoters in tilapia and carp The various substances tried were Rumensein Avotan Payzone Virginiamycin and Zinc Bacterin In carp Virginiamycin gave the best performance while in tilapia Payzone at a rate of 15ppm showed better performance than the other substances Addition of Virginiamycin and Terramycin improved the growth of carp juveniles when they were fed on a high protein diet (40) while with low protein (25%) diet the antibiotics had little effect (Ahmed and Matty 1989) According to them these drugs have a sparing effect on the dietary proteins normally used for energy and may possibly be used as growth

enhancers Terramycin has been shown to have an energy sparing effect when fed to piglets (Francois 1962), while Virginiamycin has been shown to have a carbohydrate sparing effect when fed to swine (Vervaeke et al 1978)

Oxytetracycline at a level of 200 ppm was shown to enhance the growth of common carp (Rijkers et al 1980), while Chua and Teng (1980) found that Nitrovin was a more efficient growth promoter than 17 ∝ methyltestosterone in Epinephelus salmoides Incorporation of Nitrovin at a level of 25mg/Kg of diet improved the weight gain of carp fry by 11 8% (Parova et al 1982)

Among crustaceans, Corliss et al (1977) have reported weight increase in Penaeus aztecus by feeding with diets containing oxytetracycline In smaller shrimp, with mean weights of 143 4 mg, growth was rapid when fed with diets containing 100 mg and 1000mg of oxytetracycline/kg of feed But for larger shrimps growth inhibition was observed in all concentrations tested They also found that for smaller shrimps feed containing oxytetracycline was more efficiently converted than feed without oxytetracycline

On the otherhand Vaitheeswaran and Ali (1986) did not find any growth enhancement in the post larvae of Penaeus indicus when fed on diets containing oxytetracycline at a level of 10mg/100g of diet

Stuck et al (1992) studied the effect of antibiotics like oxytetracycline, pencillian/streptomycin, and chloramphenicol in Penaeus vannamei larvae and have reported that oxytetracycline administered at 25 or 50 ppm improved growth, while no growth was observed at 100 ppm level In the case of chloramphenicol even a low level of 2 5 ppm was found to be lethal to early protozoea Pencillin/Streptomycin at a level of 5 25 ppm was found to improve survival and growth but higher doses showed detrimental effect on organisms

Ciapara (1989)et al compared the effect of oxytetracycline and sulphamethazine on weight gain and survival of Penaeus monodon post larvae and adults when fed with pellets containing oxytetracycline and sulphamethazine at a rate of 250 mg/kg No significant difference between medicated and non medicated feeds were observed, but a significant difference in survival rate was found in medicated ones than the non medicated one

Various works using antibiotics have shown contradictory results Maynard and Loosli (1969) and Visek (1978), pointed out that the response in weight gain due to antibiotic supplementation in animal diets may be varied depending on the age of animal the antibiotic used and its dosage type of feed used and the nutrition of the recipient animal

2 2 Hormones

Natural hormones are specific chemical substances produced by living cells Hormones have the property of being effective even when present in extremely small amounts Some synthetic compounds such as diethylstilbestrol which does not occur in nature also has hormone-like properties

Growth promoting properties of certain hormones has been gaining importance in recent times, in aquaculture feeding trials with fishes and prawns Androgens, estrogens progestrogens pituitary growth hormone thyroxine and insulin are some of the hormones which play a significant metabolic role in th receipient organisms and may be capable of growth promotion alone or in combination with other hormone (Donaldson et al 1979)

Growth promoting efficiency of hormones in fishes has been reviewed by Donaldson et al (1979) and Matty and Lone (1985) Investigations on hormonal growth promoters for shrimps and prawns are very limited

2 2 1 Steroid hormones

Both natural and synthetic steroid hormones are used for enhancing growth in animals Androgens estrogens progestrogens and corticosteroids are the four different types of steroid hormones identified in fishes Of these

except corticosteroids, all others have anabolic action

2 2 1 1 Male sex hormones

Among androgens testosterone, and 17 ∝ methyl testostrone have been studied extensively

Testosterone was found to increase growth of juvenile coho salmon when fed at a rate of 1 and 10 mg/Kg of diet (Mc Bride and Fagerlund 1976) Matty and Lone (1979) reported growth enhacement in juvenile Cyprinus carpio when testosterone was fed at a level of 1 0 2 5, 5 0 and 10 mg/Kg of diet at a rate of 5% body weight Channa striatus showed an increased growth and food consumption when fed with testosterone (Nirmala and Pandian 1983)

17 ∝ methyltestosterone has been the most widely used synthentic androgen for enhancing growth in fishes and prawns In coho salmon (Onchorhyncus kisutch) 17 ∝ methyltestosterone administered at a level of 1 mg/Kg and 10 mg/Kg was found to enhance growth (Mc Bride and Fagerlund loc cit) The optimum dose for growth enhancement in Cyprinus carpio was found to be 2 5 -5 0 ppm of 17 ∝ a methyltestosterone (Lone and Matty 1980) This steroid increased growth and food conversion efficiency and also food consumption in fishes (Higgs et al 1982) In the case of eel Anguilla anguilla, diets containing 17 ∝ methyltestosterone at

a level of 1 ppm gave a better weight gain over the control (Degani 1985) When 17 α methyltestosterone was fed to Sarotherodon niloticus it showed a better weight gain (Ufodike and Madu 1986) Basavaraja et al (1988) have reported that the optimum dose of methyltestosterone for Indian major carps Cyprinus carpio and Tor khudree was 1 - 3 mg/Kg

Other androgens tried in fishes as growth promoters were stanozolal in Carassius auratus and Ictalurus punctatus (Bulkley and Swichart 1973) and 1-dehydrotestosterone acetate in Tilapia aurea (Guerrero 1975) In 1976 McBride and Fagerlund used Oxymethalone and 4-chlorotestosterone acetate in coho salmon, while 11-ketotestosterone was used by McBride and Fagerlund (1976) in coho salmon and Matty and Lone (1979) in C carpio Methonolone acetate (Matty 1975) dimethazine (Cheema and Matty 1977), 17 a ethyltestosterone (Liu et al 1978) norethandrolone (Matty and Cheema 1978) andrenoteststerone (Matty and Lone 1979) testosterone propionate (Schreck and Fowler 1982) and ethylstrenol (Lone and Matty 1983) were the other androgens used at different levels in different species of fishes

Androgens have been tried as growth promoters in prawns also Antiporda (1986) made preliminary studies on the effect of methyltestosterone on Macrobrachium rosenbergii The hormones was incorporated into a 30% crude

protein feed at levels of 2 5, 75 and 125 mg/Kg of feed for 60 days Growth in all treatments after 30 days post hormone treatement showed no significant difference

Vaitheeswaran and Ali (1986) tried testosterone in Penaeus indicus where they have observed that supplementation of diet with testosterone at a level of 2 5mg/100gm enhanced growth food conversion ratio and rate of survival

2 2 1 2 Female sex hormone

Female sex hormone estradiol was found to enhance growth in coho salmon when fed at a rate of 2 5 µg /Kg of diet (Yu et al 1979) Nirmala and Pandian (1983) reported an increased rate in feed consumption and feed conversion efficiency in Channa striatus, when low doses of this hormone was incorporated in feed Significant growth increment was also reported in yellow perch Perca flavescens (Malison et al 1988),and Anguilla anguilla (Degani 1986)

Diethylstilbestrol(DES) treatment was found to increase the growth of Labeo rohita and Cyprinus carpio (Nanjundappa and Varghese 1988 1989 Basavaraja et al 1989) Oral administration of 1 ppm sodium diethylstilbestrol significantly accelerated the growth of Anguilla japonica (Satoh and Nimura 1991)

In the case of progestrone, Ashby (1957) reported a substantial gain in length of brown trout, Salmo trutta , when treated daily with this hormone at a level of 50 100 µg /lit Contrary to this Mc Bride and Fagerlund (1976) showed that progestrone failed to induce growth in coho salmon O kisutch

Female sex hormones were tried in crustaceans also as growth enhancing agents Ethyloestrenol (orabolin) was tried in Penaeus indicus by Vaitheeswaran and Ali (1986) According to them, supplementation of the diet with ethylestrenol (orabolin) at 0 5 mg/100g did not enhance the growth of prawn, though it resulted in higher protein synthesis and retention of nitrogen

Contradictory to this, Shreeprakash et al (1987) have reported that the growth of Macrobrachium choprai could be accelerated by supplementing with orabolin They tried this hormone for larvae also and found that within 19 days post larval stage can be obtained whereas in normal case it takes more than 24 days

In the mud crab Scylla serrata an injection of diethylstilbestrol at a dose of 2 µg/g for 7 days was shown to increase feeding and moulting in bilaterally ablated ones, while, feeding with 14 18 µg/g diet at 3 days

interval notably accelerated growth (Wang et al 1989)

Ethylestrenol was found to be an efficient growth promoter for Penaeus indicus post larvae at a level of 8 mg /Kg (Raghunathan et al , 1992) They also reported that ethylestrenol treatment increased food conversion efficiency

2 2 2 Thyroid hormone

Two forms of thyroid hormone triiodothyronine (T3) and thyroxine (T4) have been found to produce increased growth rate in fishes

Thyroid hormone treatment enhances growth by increasing appetite and /or gross food conversion efficiency Though the exact mechanism by which thyroid hormone improves food conversion efficiency is not known, there is evidence for their involvement in protein, lipid and carbohydrate metabolism in fishes

Barrington et al (1961) studied the influence of thyroid powder and thyroxine in the rainbow trout Salmo gairdneri

Application of thyroid hormone in fish culture as growth promoter has been reviewed in detail by Donaldson et

al (1979) Higgs et al (1982),Mc Bride et al (1982), and Matty and Lone (1985)

Higgs et al (1976) reported that T4 administration at 0 5, 5 0 and 30 µg/gm levels enhanced growth in coho salmon Improved growth rate was observed in Oncorhynchus kisutch O tshawytcha Salmo salar, S gairdneri with T3 as a diet supplement (Mc Bride et al 1982) Atlantic salmon showed improved growth with 1 mg T3/Kg of feed (Refstie 1982) and Oreochromis mossambicus at a level of 20 mg T3 /Kg dry diet (Chaudhary et al 1989)

Improved egg viability hatchability larval survival growth and development were observed in Cyprinus carpio when they were treated with thyroid hormone (T4) at a level of 0 05 and 0 1 ppm (Lam and Sharma 1985) Treatment of post yolk sac larvae of milk fish C chanos with thyroxine (T4) at a level of 0 5 ppm markedly accelerated growth and development (Lam et al 1985) Thyroid hormone has significant effect in accelerating growth and early maturation in guppy Poecilia reticulata, when incorporated in the diet at a level of 5 10 and 20 mg/kg (Palave and Belsare 1992)

Studies on prawns using thyroid hormone as a growth promoter gave contradicting results According to Vaitheeswaran and Ali (1986), incorporation of thyroxine in the diets of Penaeus indicus did not give encouraging growth and

food conversion when administered at a level of 1 0 mg/100g while Pillai et al (1987) in their studies on Penaeus monodon showed that thyroxine in microquantites incorporated in the medium accelerated growth and ecdysis in P monodon They observed that the optimum dose was found to be 0 3 µg/l for post larvae and 5 0 µg/l for juvniles

2 2 3 Growth hormone

Porcine growth hormone administered at a level of 3 5 µg/g /week for 28 days significantly stimulated growth inSalmo salar at 11 5°C (Komourdjyan et al 1976) Injection with bovine growth hormone(bgh) at a rate of 3 5 µg/g /week for 14 days increased growth rate in sock eye salmon (Clarke et al 1977) and also in coho salmon (Market et al 1977) Adelman (1977 and 1978) studied the effect of bovine growth hormone on the growth and body composition of Cyprinus carpio Wilson et al (1988) reported that administration of recombinant bovine growth hormone resulted in significant increase in growth rate and food consumption in channel catfish Oral administration of 125 µg/g body weight recombinant bovine growth hormone resulted in significant increase in growth of coho salmon Adelman (1982) obeserved growth enhancement in Cyprinus carpio after injection of pituitary gland homogenate of adult carps Treatment of Salmo gairdneri with rain bow trout growth hormone gave a positive

growth increment (Agellon et al 1988)

Pituitary growth hormones (STH) was tried in crustaceans also Toullec and VanHormhoudt (1987) showed that human growth hormone like peptides is present in the prawn Palaeomon serratus Charmantier et al (1989)observed that injection of somatotropin (STH or Growth hormone) gave a more rapid growth than untreated animals over suceeding moults in American lobster Homarus americanus STH injection increased the growth rate of lobsters by 10 to 20 % The presence of human growth hormone like peptides in Penaeids and their possible involvement in the larval development in Penaeus indicus P vannamei and P stylirostris was shown by Toullec et al (1991) They also observed that human growth hormone supplementation in the diet of P vannamei larvae seems to have a positive effect on the size and the quality of animal estimated by their resistance to salinity stress

2 3 Exogenous digestive enzymes

Digestion is the process by which food in the digestive tract is split into simpler compounds that are capable of passing through the intestinal wall to be absorbed into the blood stream Proteins are hydrolysed into free aminoacids or short peptide chains, carbohydrates into simple sugars and fat into fatty acids and glycerols The ability of

organisms to digest a given food item mainly depends on the presence of appropriate enzymes (Smith 1980)

Supplemenation of the digestive enzymes in fish diets have proven to be advantageous in improving fish growth and food utilisation (Dabrowski 1979) These enzymes present in food or food organisms may support the digestive process in the fish (Jancarik 1964) They are of dietary origin (exogenous enzymes) and play an important role in the digestion and even growth of animals Dietary enzyme supplements seem to be especially important in juveniles which may lack some important enzymes A low production of digestive enzymes in fish is because of the simple morphological structure of digestive tract (Dabrowski 1979) Moreover the intestinal tract of a larval animal is more simply organised and shorter than that of adult (Stroband and Dabrowski 1979) Hence the advantageous effect of exogenous digestive enzymes on digestion and growth in young animals is obvious

Korneyev (1969) tried an enzyme preparation named Avomarin" which had proteolytic amylolytic and pectinolytic activity in carps It was found that this enzyme at a rate of 0 1% in the diet increased the growth by 20% Dabrowski and Glogowski (1977) studied the effects of adding bovine trypsin in the diets of common carp fry and found that the supplementation of enzyme increased the proteolytic

activity in the fish leading to a slightly higher growth rate than control

Addition of 0 2 (2 gm/Kg pellets) α amylase contributed to a significant increase in fish growth (Tomassian et al 1982) By using brewery enzyme addition 1 2 to 1 4 fold increase in the percentage daily biomass growth rate was obtained in Cyprinus carpio and Hypophthalmicthys molitrix (Boettcher 1985)

In invertebrates Yonge (1937) was the first to suggest that diet has a direct effect on digestive enzyme activities and since then it is known that, the dietary composition of crustaceans also seem to have an obvious influence on digestive enzyme activites (Maugle et al 1983 Lee et al 1984)

The relationship between digestive enzyme activities and growth in shrimp is not well defined Von Wormhoudt et al (1980) found a positive correlation between total digestive enzyme activites and growth in Palaemon serratus while Lee et al (1984) found no such correlation in Penaeus vannamei Maugle et al (1983)observed the growth of P japonicus to be positively correlated to hepatopancreatic enzyme activity Diet supplemented with 60 IU amylase/g dry weight at a 19 0% starch level gave a better growth over

control in juvenile P. japonicus They found that amylase supplement improved the digestibility ratio of dietary starch than the control

Chen and Lin (1990) have observed that addition of hepatopancreas powder of P. monodon or artemia nauplii acetone powder in the diet ofP. monodon post larvae promoted growth to a significant level Though the survival was not significant with enzyme supplementation, the authors have suggested the need to incorporate exogenous digestive enzymes in the diet of P. monodon post larvae to achieve optimum growth

Akiyama (1991) suggested the need to incorporate proteolytic and amylolytic enzymes in the shrimp feed to improve protein and carbohydrate digestion Papain and Bromelin at a level of 0 1% to 0 2% of the feed have been shown to improve the growth in prawn (Paul Raj 1993) since they are believed to help in the removal of decayed tissue and help to minimize inflammation The enzyme papain is a principal protease of papaya latex This enzyme has a broad specificity It hydrolyses small peptides and protein The optimum activity was found at pH 7 0 for egg albumin and casein and quite stable at elevated temperature (Yamamoto 1984)

2 4 Others

2 4 1 Moult hormones

The moulting hormone ecdysone which is found in insects and crustaceans has also been isolated from plants and supplementation of this hormones in media or diets was found to induce moulting thereby enhancing growth in crustacea

Kurata (1968) showed that injection of an insect moult inducing hormone the inokosterone obtained from roots of plants induced moulting in Penaeus japonicus An injection of 3 µg of inokosterone per gram live weight of shrimp gave a remarkable moult inducing effect but growth increase after the second moult was significantly less

Kanazawa et al (1972) studied the dietary effect of three different ecdysones isolated from plants on moulting and growth of prawns The different ecdysones used were inokosterone cyasterone, and ecdysterone All the three ecdysones induced moulting but growth rate of prawns supplemented with ecdysones was lower than in those fed on ecdysone free diet

2 4 2 Olaquindox

Olaquindox commercially known as Bayo-n-ox is a chemical growth promoter having chemical name 2 [N (2-hydroxy-ethyl)-carbmyl] 3-methyl quinoxalin-1 4 dioxidate Olaquindox appears to partition energy in the animals for protein synthesis (Akiyama et al 1991) Besides this chemical has been reported to possess antibacterial properties It was observed by Santiago in 1991 that 25mg Bayo n ox /Kg body weight produced significant growth improvement over control in Nile tilapia This growth promoter was found to be effective in accelerating growth in zoea, post larvae and juvenile of Penaeus orientalis by Jiamin et al (1989) They recommended a level of 100 - 300 ppm of this promoter in commercial feeds Akiyama (1991) recommended a dose 200 gm/MT of this drug

2 4 3 Dimethyl-β-Propiothein (DMPT)

A tertiary sulfonium compound DMPT significantly improved growth of gold fish (Nakajima et al 1989) DMPT was also found to be effective in accelerating growth in marine fishes like red sea bream, yellow tail and flounder (Nakajima et al 1990) Nakajima (1991) showed that, DMPT at a concentration of 0 1 mM solution highly stimulated growth and moult in stripped prawns Palaemon paucidens Other tertiary sulfonium compounds such as dimthyl acetothein (DMT) and

vitamin U also enhanced growth in *P paucidens*

2 4 4 Alfalfa

Alfalfa is a leguminous forage plant, which is a good source of vitamin K This plant also contains estrogen which has a benefical effect on the fattening of animals and is similar to that of giving synthetic hormones such as stilbestrol and hexosterol The product is also marketed as a homeomedicine

Rao *et al* (1983) concluded that supplementation of Alfalfa in the diet improves weight gain in *Penaeus indicus* juveniles Vaitheeswaran and Ali (1986) also showed that incorporation of alfalfa at the rate of 2 ml/ 100g enhanced growth rate in *P indicus*

2 4 5 Attractants

Dietary supplementation of feed attractants or stimulants plays a significant role in elevating feed efficiency Addition of feed attractants might elicit an increase in appetite and subsequently food intake and growth (Lindstedt 1971) and also improve survival and food conversion (Heinen 1980) Use of feeding stimulants has attracted considerable attention in development of rations for slow feeding crustacea, especially economically valuable species of

marine shrimp (Penaeus) and freshwater prawns (Macrobrachium) (Mayers 1987) Free amino acids and possibly small peptides serve as attractants for shrimps (Akiyama et al 1991) These products naturally occur in fish meal, shrimp meal squid meal crab meal and clam meal

Mackie (1973) observed that a synthetic mixture of substances identified in a squid extract when incorporated in the feed was highly attractive to the lobster Homarus gammarus The synthetic squid mixture included aminoacids betaine trimethylamine oxide trimethylamine, homarine hypoxanthin inosine, adenosine 5-monophosphate and lactic acid Carr (1978) showed that substances of less than ca 1000 molecular weight present in the extracts of crab and oyster stimulated feeding response in shrimp, Palaemonetes pugio Although betaine was present in considerable quantity in extracts this substance has only a modest stimulatory capacity Sick (1976) obtained poor results when 15 % betaine was added to a larval diet for M rosenbergii Deshimaru and Yone (1978) also found that addition of betaine at 1 5% level did not increase ingestion rate in P japonicus But Heinen (1980) recommends addition of betaine alone to feeds since it might be synergestic with dietary aminoacids Kanazawa et al (1970) have supported Heinen (loc cit) and reported that betaine and a similar compound morin stimulated the feeding behaviour in Penaeid prawns Adenosine 5 monophosphate (AMP)

has been shown to be a chemoattractant for the caridean shrimp Palaeomonetes pugio (Carr and Thompson 1983), M rosenbergii (Harpaz et al 1987) and P monodon (Hartati and Briggs 1993)

Squid protein and squid protein extracts have been shown to improve both growth rate and feed conversion in Penaeids Pascual (1980) observed a significant growth increment in P monodon juveniles, when fed with diets containing squid extract Purified diets incorporated with mussel extract and shrimp extract also improved growth of the species Cruz Rique (1987)observed that inclusion of squid protein even at a low level of inclusion of 1 5% enhanced growth in P japonicus juveniles as well as the juveniles of the P stylirostris and P vannamei while a higher rate of 6 and 16 % inclusion is required for P monodon But it was found that, it has no growth promoting effect in P indicus

Glutamic acid has been reported as a feeding stimulant for P japonicus (Takei 1969 Takei and Ai 1971) In P japonicus addition of glycine to the diet significantly stimulated the feed intake followed by the amino acid mixture taurine and serine in decreasing order while aspartic acid glutamic acid proline and betine were ineffective (Deshimaru and Yone 1978)

2 4 6 Monensin

This carboxylic ionophore is known to improve growth in sheep cattle poultry and pigs and combat disease (Anon 1990) Monensin was found to be effective in P vannamei when fed at a rate of 100 ppm Lower dose of 50 ppm and higher dose of 200 ppm showed a decrease in growth rate (Dielo Craetana and Pressman 1987)

2 4 7 Taurine

Shiau et al (1992) showed that taurine supplementation improves the growth of P monodon when the taurine content in the diet is low Better growth rate was obtained at high taurine level when salinity was high Taurine was found as a feed attractant for P monodon (Hartiati and Briggs 1993) for P japonicus (Deshimaru and Yone 1978) and M rosenbergii (Smith et al 1987)

2 4 8 Miscellaneous

A vault (1989) studied the use of dried flavomycin containing mycelium as a feed additive in penaeid shrimps In Japan addition of 40 to 67 ppm flavophospholipol in the feed increased the body weight of yellow tail by 21 5 to 66 7 while incorporation of flavophospholipol at 20 to 40 ppm in feed gave increased weight in P monodon and P

japonicus Flavomycin-40 was tried in M rosenbergii post larvae along with other commercially available growth promoters like Stefac-20 and Groviron by Reddy et al (1990) The growth promoters were incorporated at levels of 0 1 % and 0 2 % in a 40 % protein diet Among the three Groviron gave the best growth

Trimethylammonium hydrochloride (TMAH) gives a distinctive "faecal" odour to the feeds which may act as a feed attractant Many decapod crustaceans exhibited coprophagy which is taken advantage of by incorporation of TMAH in the feeds Chemotaxic response of prawn M rosenbergii to the TMAH treated feed was reported by Costa Pierce et al (1985) TMAH incorporation in the feed was found to increase the feed intake in M rosenbergii But TMAH was not found to increase feed ingestion or growth rate significantly in P monodon (Hartati and Briggs 1993)

MATERIALS AND METHODS

3 MATERIALS AND METHODS

The present study was conducted at the College of Fisheries Panangad during the period from 09 05 1993 to 18 09 1993 Two feeding experiments were carried out during the study The first experiment was envisaged to investigate the effect of various growth promoters such as antibiotic hormone and enzyme on the growth of Macrobrachium rosenbergii post larvae The growth promoter which gave the best performance was chosen for the second experiment to find out its optimum dietary level for producing maximum growth for the species

3 1 Experiment I Effect of different growth promoters on the growth of Macrobrachium rosenbergii post larvae

3 1 1 Experimental tanks The experiment was conducted in rectangular plastic containers of the size 60 x 40 x40 cm The tanks were filled to a height of 30 cm with filtered freshwater

In order to reduce cannibalism among the prawns uniform sized earthern tiles were provided as artificial substrata in each tank Gentle aeration was given in the tanks using diffusion stones from an air blower Air supply was uniform throughout the experimental period

3 1 2 Experimental prawns The post larvae of M rosenbergii used in the experiment were procured from Rosen fisheries Marathakkara Trichur They were transported to the College laboratory in oxygen filled polyethylene bags under minimum stress In the laboratory they were maintained in a 1 ton fiber reinforced plastic (FRP) tank containing freshwater, for one week for acclimation to the laboratory conditions During this period the prawns were fed on dried clam meat

For the study healthy prawns were selected from the stock and 10 prawns were assigned to each tank on a random basis They were acclimatised to tray feeding for another one week Before the start of the experiment the prawns were starved for a day and the prawns in each tank were weighed collectively The average initial weight of the post larvae ranged from 23 18mg to 25 93mg and the mean length ranged between 0 8 and 1 2 cm

3 1 3 Growth promoters used in the study The following growth promoters were used for the study

1 Antibiotic Oxytetracycline obtained as Oxytetracycline Hcl marketed under the brand name Terramycin by Pfizer (India) Ltd Oxytetracycline is categorised as infeed antibiotic by EEC (1985) and it is cleared by U S food and drug administration (USFDA) for use in animals and fishes In the present study the antibiotic oxytetracycline was incorporated into a basal diet at the rate of 10 mg/100

g of the diet

2 Hormone The hormone selected for the study was Thyroid hormone The hormone was obtained as thyroxine sodium in tablet form available under the name Eltroxin from Glaxo India Ltd The rate of incorporation of the thyroid hormone in the feed was 2 5 mg/ 100 g

3 Exogenous proteolytic enzyme, Papain a sulfhydryl protease obtained from Papaya latex was tried as the exogenous enzyme in the present experiment

For the study the enzyme preparation papain was obtained from Sisco Research Laboratories Bombay and was incorporated in to the diet at a rate of 200 mg/100g of basal diet

3 1 3 Experimental diets and their preparation Inorder to evaluate the efficiency of various growth promoting substances on the growth of M rosenbergii post larvae casein based purified diet was formulated to which the specified growth promoters were incorporated and fed to the prawns The purified diet was originally based on the formula recommended by Kanazawa et al (1982) for nutrition studies in Penaus japonicus Some modifications were made in the original composition based on recently published information regarding the nutrient requirement of M rosenbergii The ingredient

Table 1 Percentage composition of ingredients used in the basal diet

Ingredients	. weights
Casein	36 0
Egg albumin	4 0
Glucose	4 0
Sucrose	4 0
Potato starch	19 6
Glucosamine Hcl	0 8
Sodium citrate	0 3
Sodium succinate	0 3
Mineral mix	4 0
Vitamin mix	8 0
Cod liver oil	6 0
Maize oil	2 0
Cellulose	5 0
Cholestrol	1 0
C M C	5 0
	100 0

composition of the basal diet is given in Table 1

Casein the main protein source used in the diet is available in highly purified form (Kanazawa et al 1971 1976) and found to be assimilated satisfactorily by M rosenbergii (Hilton et al 1984 Briggs et al 1988) Since casein is lacking some of the essential aminoacids required for the prawns egg albumin was also added in the feed as protein source to ameliorate its deficiency

Polysaccharides were found to be more easily assimilable carbohydrate source than monosaccharides (Andrews and Sick 1972 Forster and Gabbott 1971, Pascual et al 1983 Alva and Pascual 1987) and hence potato starch was incorporated in the diet since it is available in relatively pure form and found as a good carbohydrate source for M rosenbergii (Gomez and Nakagawa 1990)

The lipid source used was a mixture of maize oil and cod liver oil which provides both w6 (linoleic) and w3 (linolenic) Poly Unsaturated Fatty Acids (PUFA) and were reported to be essential for prawns (Colvin 1976a, Kanazawa et al 1979 Reigh and Stickney 1989)

Crustaceans require cholestrol for their normal growth and survival (Castell et al 1975) and are incapable of de novo synthesis of cholestrol from simple sugars (Teshima and Kanazawa 1971 Kanazawa et al 1971) Recent

studies by Briggs et al 1988 and Sherif et al 1990) showed that cholestrol is requrred in the diets of M rosenbergii Based on the studies Cholestrol was added at 1 0 level in the purified diet used in the present study

Heinen (1988) found that trace mineral mix was essential for the normal growth of prawn M rosenbergii The mineral mix used in the present study was based on the nutritional studies of M rosenbergii by Stahl and Ahearn (1978) Table 2 shows the composition of the mineral mixture used in the study

The vitamin mixture contained both water soluble and fat soluble vitamins which are reported to be essential for crustaceans The mixture was made on the formula recommended by Kanazawa et al (1982) The composition of the vitamin mixture is given in Table 3

Glucosamine hydrochloride was added in the diet since it seems to help in the assimilation of dietary casein and enhance the growth in shrimps (Kanazawa et al 1971 and Vaitheeswaran & Ali 1986)

Carboxymethylcellulose was used as the binder to keep the pellets stable in water

Ingredients used were finely powdered and sieved

Table 2 Composition of mineral mixture used in the basal diet

Ingredients	% weights
Calcium biphosphate	1 00
Calcium lactate	37 24
Ferric citarte	2 97
Magnesium sulphate	13 20
Potassium hydrogen phosphate	25 40
Sodium chloride	18 64
Sodium biphosphate	1 04
Aluminium chloride	0 01
Potassium iodide	0 01
Cuperous chloride	0 01
Mangesium sulphate	0 08
Cobal+ choride	0 10
Zinc sulphate	0 30
	100 00

Table 3 Composition of vitamin mix used in the basal feed

Ingredients	weight (mg)
Thamine Hcl	5 0
Riboflavin	8 0
Paraminobenzoic acid	10 0
Biotin	0 4
Inositol	400 0
Niacin	40 0
Calcium pantothenite	60 0
Pyrodoxine Hcl	12 0
Menadione	4 0
β Carotene	9 6
α Tocopherol	20 0
Calciferol	100 0
Vitamin B12	0 1
Vitamin C	2730 0
Folic acid	0 9
Choline chloride	600 0
	4000 0
Cellulose	4000 0

through a 250 micron sieve Accurately weighed ingredients except vitamin mixture oil and growth promoter were first mixed thoroughly in a mortar To this, sufficient quantity of water was added and mixed thoroughly in order to get a dough like consistency This was then steam cooked for 10 minutes in an autoclave without pressure On cooling accurately weighed vitamin mixture and oils were added and mixed well Growth promoter was also added to the feed at this stage

After blending the mixture thoroughly the dough was then extruded through a hand pelletiser having a die of 3 mm diameter The extruded pellets were collected in an enamel tray and dried in an electric oven maintained at 40 $\pm$ 3°C for 24 hours to obtain dry pellets The pellets were then broken into small pieces packed in air tight containers and stored at 4°C in a refrigerator until used

3 1 4 Proximate composition of prepared diet

Analysis of proximate composition of the prepared diet was done using the following methods

For estimating moisture level Boyd s (1979) method was used The sample was heated to 105°C for 30 minutes and then dried at 65°C till a constant weight was obtained The crude protein content was estimated by microkjeldhals method (AOAC 1975) The nitrogen content was multiplied by a

Table 3 Composition of the diet used in the experiment 1

	Diet			
	T0	T1	T2	T3
Basal diet(g)	100	100	100	100
Oxytetracycline(mg)		10	--	-
Thyroid hormone(mg)			2 5	
Papain (mg)				200

factor of 6 25 to arrive at crude protein content Crude fat was extracted using petroleum ether (B P 40 - 60°C) in a soxhlet apparatus Method of Pearson (1976) was used for estimating the crude fiber The ash content was estimated by burning the sample at 550 ± 10 °C for 6 hours in a muffle furnace The carbohydrate content was found out by Hastings (1976) difference method as nitrogen free extract (NFE)

3 1 5 Feeding study for biological evaluation

The experiment was conducted in rectangular plastic tanks Each tank was stocked with ten numbers of uniform sized post larvae For each type of feed, 5 replicates were kept Hence for four treatments ie three test diets containing different growth promoters and one control without growth promoter a total of 20 tanks were used (Table 4) Treatments were allocated to each experimental units by random allocation method

The feed was given ad libitum every evening using feeding trays kept at the bottom of the tank close to the substratum provided Pellets were powdered into fine crumbles and sieved through a 650 micron mesh sieve before feeding to post larvae

The left over feed was collected and trays were cleaned thoroughly before the next feeding During the experimental period dead animals if any were immediately

collected and weighed The prawns were reared for 42 days with periodical replenishment of water in the tanks At the end of the feeding study the prawns were starved for one day and those in each tank counted and weighed collectively

3 1 6 Determination of body protein The body protein was estimated using digestion and nesselerisation method (Wooton 1964) Tissue was digested with conc sulphuric acid and digestion mixture till the solution became colourless The solution was then cooled and transferred to a 25 ml standard flask and neutralised with 10N NaOH solution making fine adjustments using 1N NaOH and the volume was made up using distilled water From this solution 1 ml was pipetted into a 50 ml standard flask containing about 40 ml distilled water 1 ml of nesselers reagent was added to this and made upto 50 ml and read O D at 400 nm A standard curve was plotted with standards made with standard $(NH)_4$ cl From the standard curve the amount of nitrogen present in the test solution could be found out Using a factor of 6 25, the percentage nitrogen in the sample was converted into percentage crude protein

3 1 7 Monitoring of water quality parameters

Observations of water quality parameters like water temperature pH and dissolved oxygen were made at weekly intervals inorder to find out the changes in these

parameters

Temperature was measured using mercury bulb thermometer with an accuracy of 0 1°C pH was measured using universal pH indicator solution manufactured by Glaxo (India) Ltd Dissolved oxygen was measured by standard Winkler s method (Strickland and Parsons 1972)

3 1 8 Evaluation criteria

Parameters like net weight gain Specific growth rate (SGR) , Percentage survival Food conversion ratio(FCR) Protein efficiency ratio(PER) and Productive protein value(PPV) were determined inorder to study the influence of various growth promoters on the growth of M rosenbergii post larvae

3 1 8 1 Net Weight Gain This gives the increase in the weight of prawns during experimental period when fed with various growth promoters Net weight gain was calculated using the formula

Net weight gain Final weight - Initial weight

3 1 8 2 Percentage growth The percentage growth of the animal was calculated by the following method

$$\text{growth} \quad \frac{\text{Final measurement} \quad \text{Initial measurement}}{\text{Initial measurement}} \times 100$$

3 1 8 3 Specific growth rate Specific growth rate (SGR) was calculated as

$$\text{SGR (.)} \quad \frac{\log_e W2 \quad \log_e W1}{T2 - T1}$$

where,

W1 - weight at time T1

W2 - weight at time T2

The calculated values give the average percentage increase in body weight per day over 42 days

3 1 8 4 Survival rate The survival rate of the prawns is expressed in terms of percentage This was calculated as follows

$$\text{Survival \%} - \frac{\text{Initial number} \quad \text{number of dead prawns}}{\text{Initial number}} \times 100$$

3 1 8 5 Food conversion ratio This refers to the ability with which an animal can convert the feed consumed into edible and other products (Devendra 1989) This gives an idea about the amount of feed required to produce a unit increase

Table 5 Composition of diets used in experiment II

	Diet			
	T1 0	T1 1	T1 2	T1 3
Basal feed (gm)	100 0	100 0	100 0	100 0
Oxytetracycline (mg)	5 0	10 0	20 0	40 0

in the weight of prawn Food conversion ratio was calculated using the formula

$$FCR = \frac{\text{Average weight of food consumed in dry weight}}{\text{Average live weight gain}}$$

3 1 8 6 Protein efficiency ratio Protein efficiency ratio is defined as the weight gain per unit intake of protein (Paulraj 1982) It was calculated using the following formula

$$PER \quad \frac{\text{Gain in body weight}}{\text{Protein intake}}$$

3 1 8 8 Productive protein value Productive protein value was calculated using the following formula

$$PPV\ (\%) = \frac{\text{Final body protein} \quad \text{Initial body protein}}{\text{Protein consumed}} \times 100$$

This gives the measurement of body protein deposition in the prawns with unit amount of protein consumed

3 2 Experiment II Study to determine the optimum level of antibiotic Oxytetracycline

3 2 1 Rearing facilities The experimental tanks used for the feeding study were the same as described in the case of the first experiment

3 2 2 Experimental animals Post larvae of M. rosenbergii obtained from the Rosen hatchery were brought to the College and acclimatised to the laboratory conditions as described earlier A basal diet as described in the experiment I was used in the experiment II to incorporate the selected growth promoter oxytetracycline which gave the best performance in experiment I Growth promoter antibiotic Oxytetracycline was incorporated at four levels 5 10,20 and 40 mg/100 g diet (Table 5) The preparation of the diet was as described in experiment I

The proximate composition of the diet prepared was determined as in the case of experiment I

3 2 3 Feeding study Five replicates were used for each treatment Thus for four levels of the antibiotic tested 20 tanks were used

Each tank was stocked with 10 numbers of healthy

post larvae after taking their initial weights as described earlier

Respective feeds were given ad libitum to prawns in each treatment Feeds were placed in the feeding trays kept at the bottom of the tank close to the hide-out provided Powdered pellets which were sieved through a 650 micron sieve were given to the post larvae

The left over feed was collected and trays were cleaned before next feeding The experiment was done for 6 weeks with periodical replenishment of water in the tanks

3 2 4 Recording the water quality parameters Water quality parameters like temperature pH and dissolved oxygen were monitored at weekly intervals as described earlier

3 2 5 Evaluation criteria The biological evaluation of the feeds containing the antibiotic was done by measuring the following parameters Net weight gain Percentage growth Survival rate FCR and PER as described earlier

3 3 STATISTICAL ANALYSIS

Analysis of variance was carried out for the collected data Pair wise comparison was performed by multiple t test technique (Snedecor and Cochran 1968)

RESULTS

4 RESULT

The results of the experiments, carried out to evaluate the effect of the antibiotic hormone and enzymes as growth promoters on the growth of Macrobrachium rosenbergii post larvae are detailed below For convenience, treatments are denoted as T0 for control, T1 for the antibiotic oxytetracycline T2 for the hormone thyroxine and T3 for the enzyme papain

4 1 Proximate composition of the formulated feed

Data on the proximate composition of the formulated feed analysed is presented in the Table 6

The feed contained 7 16% moisture 36 19% protein 7 1225. fat 4 38% fibre, 14 023 ash and 31 1281% NFE

4 2 Evaluation of various growth promoters

4 2 1 Water quality maintenance

1 Temperature Table 7 depicts the fluctations in water temperature recorded from the experimental tanks at weekly intervals during the study period Water temperature ranged

Table 6 Proximate composition of the basal diet used in the experiment

	T0	T1	T2	T3
Moisture	6 65	6 89	7 77	7 32
Protein	36 76	35 43	36 06	36 50
Lipid	6 89	7 48	7 34	6 78
Carbohydrate	31 33	31 79	30 39	31 00
Ash	13 94	13 99	14 08	14 08
Fibre	4 426	4 412	4 360	4 322

from 26 46 °C to 30 44 °C

2 pH Table 8 gives the details of variations in pH observed in the rearing tank during the study period Slightly alkaline pH values were observed during the rearing period, ranging from 7 2 to 8 3 pH values were found to be uniform in all treatments during the study period although slight variations were noticed

3 Dissolved oxygen Levels of dissolved oxygen recorded at weekly intervals are given in the Table 9 Dissolved oxygen values ranged from 6 67 to 8 32 ppm in the experimental tanks during the rearing period Dissolved oxygen levels were found to remain almost constant during the study period, since, mild aeration was provided in the experimental tanks

4 2 2 Efficiency of various growth promoters

4 2 2 1 Growth The data regarding weight gain of prawns fed with feeds containing different growth promoters are given in detail in Table 10

The initial average weights of prawn post larvae used as test animals were found to be 25 23 mg for T0 (control) 23 176 mg for T1 (diet containing antibiotic) 25 93 mg for T2 (diet containing thyroid hormone)and 25 23 mg forT3

Table 7 Range of water temperature in the experimental tanks during the study period to evaluate the efficiency of various growth promoters

	WEEKS					
TEMPERATURE	1	2	3	4	5	6
MEAN	27 27	27 19	28 63	29 50	28 82	28 42
± SE	0 29	0 31	0 54	0 51	0 56	0 01
RANGE	(26 98- 27 53)	(26 46- 27 68)	(27 96-29 09)	(28 87 30 44)	(27 09 29 13)	(28 03-28 72)

Table 8 Fluctuations in pH values observed in experimental tanks during the study to evaluate the efficiency of various growth promoters

	WEEKS					
pH	1	2	3	4	5	6
MEAN	7 52	7 94	8 06	8 08	7 88	7 86
± SE	0 24	0 31	0 21	0 41	0 22	0 19
RANGE	(7 2-7 8)	(7 6-8 3)	(7 8-8 3)	(7 9-8 2)	(7 8-8 2)	(7 6-8 2)

Table 9 Variations in Dissolved oxygen content in the experimental tanks during the study period to evaluate the efficiency various growth promoters.

	WEEKS					
DISSOLVED OXYGEN	1	2	3	4	5	6
MEAN	7 01	6 77	7 28	7.28	8 02	7 83
± SE	0 56	0 68	0 42	0 48	0 73	0 93
RANGE	(6 67 7 35)	(6 8 7 05)	(7 03-7 80)	(7 16 7 62)	(7 60-8 32)	(7 35-8 32)

(diet containing the enzyme papain) The mean final weights were found to be 51 954 mg for T0 66 842 mg for T1 60 994 mg for T2 and 57 402mg for T3 The net weight gain of the prawns fed on various growth promoters were found to be significantly different Maximum weight gain was observed for prawns fed on diets with antibiotic oxytetracyline The average weight increase being 43 6660 mg This was followed by prawns fed with thyroid hormone where the weight gain was 35 064 mg The exogenous proteolytic enzyme Papain gave average weight increase of 32 172 mg The growth rate recorded for control was 26 7240 mg The average percentage weight increase of the post larvae from their initial size was 106 434% 188 716 136 468% and 127 648% for the different treatments T0 T1, T2 and T3 Graphical representation of the growth observed in the experiment is given in Fig 1

The average daily increment in weight was 0 6363 mg for the control, 1 0397 mg for T1 0 8349 mg for T2 and 0 766 mg for T3 The maximum daily weight increment and maximum average weight gain (43 6660) was observed in the prawns fed on diet containing the antibiotic oxytetracyline

Analysis of variance (Table 11) showed that the growth of prawns was significantly different in various treatments

4 2 2 2 Specific growth rate Table 10 gives the data on SGR

Table 10 Growth and Specific Growth rate of M. rosenbergii post larvae fed on feeds containing various growth promoters.

TREATMENT	REPLICATION	AV INITIAL WEIGHT (mg)	AV FINAL WEIGHT (mg)	WEIGHT GAIN (mg)	AV LIVE WEIGHT GAIN (mg)	/ WEIGHT GAIN	AV % WEIGHT GAIN + SE	SPECIFIC GROWTH RATE	MEAN + SE
	1	22 40	47 45	25 05		111 83		1 79	
	2	23 47	49 35	25 88		110 27		1 77	
					26 72		106 43		1 73
T0	3	25 43	52 42	26 99	+ 1 46	106 13	± 5 71	1 72	+ 0 07
	4	29 80	58 75	28.95		97 15		1 62	
	5	25 05	51 80	26 75		106 79		1 73	
	1	22 22	60 46	38 24		172 10		2 38	
	2	22 09	68 32	46 23		209 28		2 68	
					43 67		188 72		2 52
T1	3	25 10	69 43	44 33	+ 3 70	176 61	+17 83	2 42	+ 0 15
	4	23 01	70 60	47 59		206 82		2 67	
	5	23 46	65 40	41 94		178 77		2 44	
	1	23 10	59 03	35 93		155 54		2 23	
	2	30 37	67 70	37 33		122 92		1 91	
					35 06		136 47		2 05
T2	3	23 45	59 36	35 91	± 2 27	153 13	+16 66	2 23	+ 0 17
	4	25 91	57 25	31 34		120 96		1 89	
	5	26 82	61 63	34 81		129 79		1 98	
	1	25 94	58 25	32 31		124 56		1 93	
	2	25 98	56 70	30 72		118 24		1 86	
					32 17		127 65		6
T3	3	24 19	58 85	34 66	+ 2 18	143 28	+ 9 95	2 12	+ 0 10
	4	25 82	59 65	33 83		131 02		1 99	
	5	24 22	53 56	29 34		121 14		1 89	

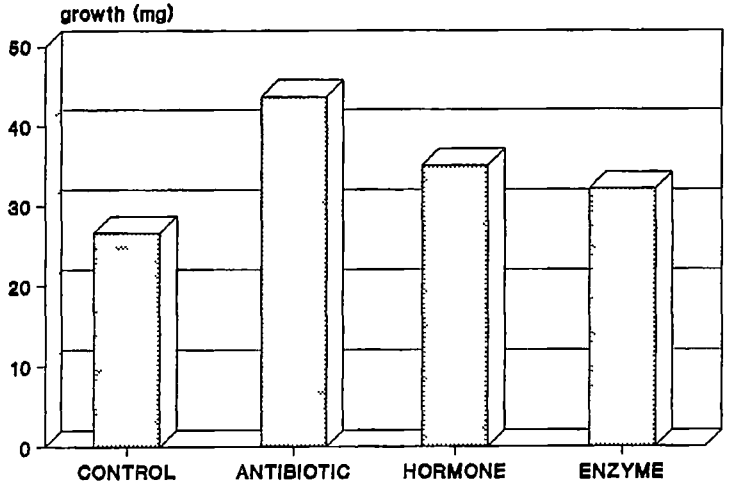

Fig 1 Growth of M. rosenbergii post larvae fed on various growth promoters

Table 11 Analysis of variance of the data on growth of *M rosenbergii* post larvae fed on various growth promoters

SOURCE	D F	S S	M S	F
Diet	3	750 9336	250 3112	38 88*
Error	16	103 0137	6 4384	
TOTAL	19	853 9472		

Comparison of treatment means based on critical difference

Critical difference 3 40

Diet	T1	T2	T3	T0
Treatment means	43 666	35 064	32 172	26 724

Underscored means are not significantly different

* significantly different at 5 % level

of prawns from different treatments The prawns which received treatment T1 gave the highest SGR (2 5180) while the control prawns gave the lowest SGR (1 7260) The SGR value for treatment T2 was 2 0480 and for treatment T3 was 1 9580 Graphical representation of SGR is given in Fig 2 Analysis of the data shows significant difference between treatments (Table 12)

4 2 2 3 Survival The percentage survival values of the prawn post larvae in the various treatments are given in Table 13 The overall survival was 69 5 % The highest survival was obtained in T1 (72%) and the lowest of 66% in T2 Graphical representation of the data is also given in Fig 3

4 2 2 4 Food conversion ratio The lowest average FCR was observed in T1 (2 93) where oxytetracycline was incorporated in the diet and the highest FCR was in T0 (4 36) which was the control with no growth promoters Table 14 gives the food conversion ratio obtained in different treatments Analysis of variance of treatments shows significant difference between various treatments(Table 15) Graphical representation of the FCR is given in Fig 4

4 2 2 5 Protein efficiency ratio Average protein efficiency ratio is highest in T1 (0 9769) while PER is lowest in the T0 (0 6566) The PER values for T2 and T3 are

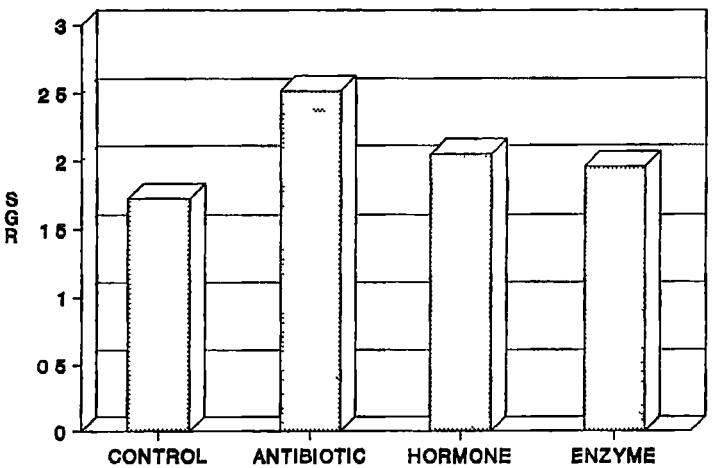

Fig 2 Specific growth rate of M. rosenbergii post larvae fed on various growth promoters

Table 12 Analysis of variance of the data on the specific growth rate of *M rosenbergii* post larvae fed on various growth promoters

SOURCE	D F	S S	M S	F
Diet	3	1 6592	0 5531	34 23*
Error	16	0 2586	0 0162	
TOTAL	19	1 9178		

Comparison of treatment means based on critical difference

Critical difference 0 17

Diet	T1	T2	T3	T0
Treatment means	2 518	2 048	1 958	1 726

Underscored means are not significantly different

* significantly different at 5 % level

Table 13 Percentage Survival values observed in the experimental tanks during the study to evaluate the efficiency of various growth promoters

TREATMENT	REPLICATION	SURVIVAL (%)	MEAN +SE
T0	1	60	70 +14 14
	2	80	
	3	90	
	4	60	
	5	60	
T1	1	70	72 +10 95
	2	90	
	3	70	
	4	70	
	5	60	
T2	1	70	66 +5 477
	2	60	
	3	70	
	4	60	
	5	70	
T3	1	60	70 +10 0
	2	80	
	3	80	
	4	60	
	5	70	

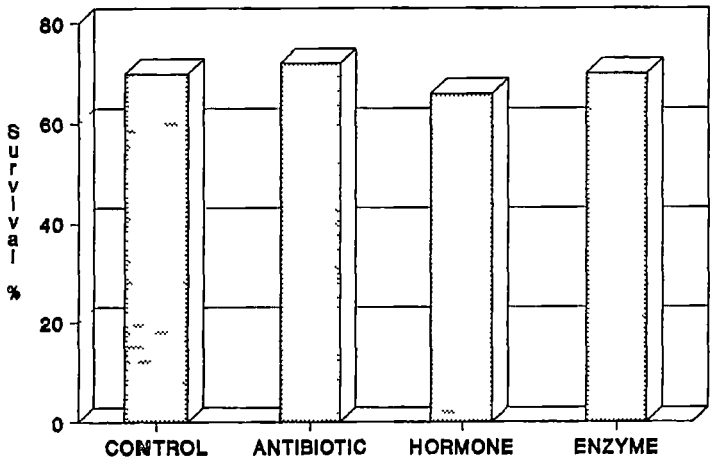

Fig 3 Survival rate of M rosenbergii post larvae fed on various growth promoters

Table 14 Food conversion ratio of M rosenbergii post larvae fed on feeds containing various growth promoters

TREATMENT	REPLICATION	AV INITIAL WEIGHT (mg)	AV FINAL WEIGHT (mg)	AV.LIVE WEIGHT GAIN (mg)	AV WEIGHT OF FEED CONSUMED (mg)	FOOD CONVERSION RATIO	MEAN +SE
T0	1	22 40	47 45	25 05	109 87	4 39	4 36 +0 2306
	2	23 47	49 35	25 88	112 54	4 35	
	3	25 43	52 42	26 99	120 47	4 46	
	4	29 80	58 75	28 95	115 58	3 99	
	5	25 05	51 80	26 75	123 51	4 62	
T1	1	22 22	60 46	38 24	120 91	3 16	2 93 +0 2192
	2	22 09	68 32	46 23	124 75	2 70	
	3	25 10	69 43	44 33	131 89	2 96	
	4	23 01	70 60	47.59	129 58	2 72	
	5	23 46	65 40	41 94	131 28	3 13	
T2	1	23 10	59 03	35 93	117 76	3 28	3 35 +0 1424
	2	30 37	67 70	37 33	124 61	3 34	
	3	23 45	59 36	35 91	116 64	3 25	
	4	25 91	57.25	31 34	112 75	3 60	
	5	26 82	61 63	34 81	114 41	3 29	
T3	1	25 94	58 25	32 31	113 15	3 50	3 70 +0 2626
	2	25 98	56 70	30 72	123 07	4 01	
	3	24 19	58 85	34 66	116 38	3 36	
	4	25 82	59 65	33 83	127.03	3 76	
	5	24 22	53 56	29 34	112 99	3 85	

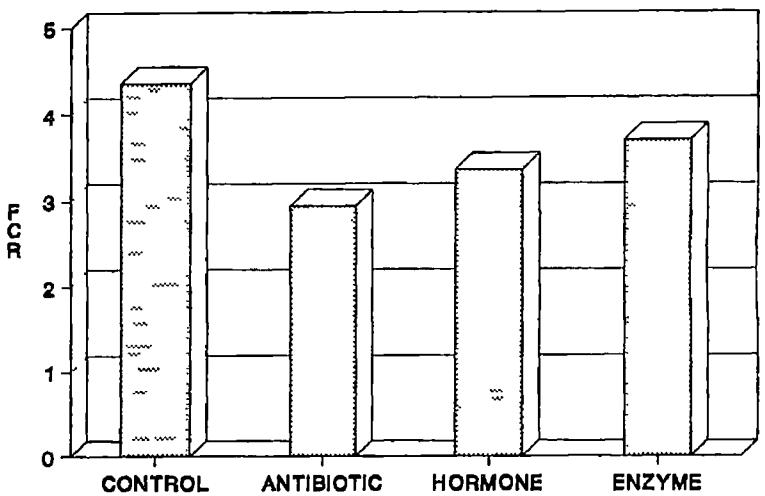

Fig 4 Food conversion ratio of M. rosenbergii post larvae fed on various growth promoters

Table 15 Analysis of variance of the data on the Food conversion ratio of M rosenbergii post larvae fed on various growth promoters

SOURCE	D F	S S	M S	F
Diets	3	5 4470	1 8146	37 89*
Error	16	0 7664	0 0479	
TOTAL	19	6 2111		

Comparison of treatment means based on critical difference

Critical difference 0 29

Diet	TO	T3	T2	T1
Treatment means	4 362	3 696	3 352	2 938

* Significantly different at 5% level

0 8586 and 0 7576 respectively Table 16 gives the details of PER values for different treatments F ratio at 5% level shows significant difference between treatments (Table 17) Graphical representation of the data is given in the Fig 5

4 2 2 6 Productive protein value Productive protein value gives an indication to the protein deposition in the prawn The average initial body protein of the prawn post larvae was estimated as 13 6. on wet weight basis At the end of the experiment on growth studies there was a significant increase in the body protein with the amount of protein deposited in the muscle tissue differing significantly in various treatments The average value of the gain in body protein for different feeds were 3 6609 in T0 6 0247 in treatment T1 4 8318 in T2 and 4 4202 in T3 The PPV was highest in treatment T1 with a mean value of 13 4764 while in treatment T0 T2 and T3 the mean values were found to be 8 995 11 7710 and 10 4246. respectively Data on the initial and final body protein and productive protein values are given in Table 18 and graphical representation of PPV is given in Fig 6 Analysis of variance at 5% level shows a significant difference between treatments (Table 19)

Table 16 Protein efficiency ratio of M rosenbergii post larvae fed on feeds containing various growth promoters.

TREATMENT	REPLICATION	AV. INITIAL WEIGHT (mg)	AV. FINAL WEIGHT (mg)	AV. LIVE WEIGHT GAIN (ng)	AV WEIGHT OF PROTEIN CONSUMED (ng)	PROTEIN EFFICIENCY RATIO	MEAN + SE
T0	1	22 40	47.45	25 05	38 4545	0 6514	0 6566 +0 0361
	2	23 47	49.35	25 88	39.3850	0 6571	
	3	25 43	52 42	26 99	42 1645	0 6401	
	4	29 80	58.75	28 95	40 4530	0 7156	
	5	25 05	51 80	26 75	43 2285	0 6188	
T1	1	22.22	60.46	38 24	42 3185	0 9036	0 9770 +0 0736
	2	22 09	68 32	46 23	43 6625	1 0588	
	3	25 10	69.43	44.33	46 1615	0 9603	
	4	23.01	70 60	47.59	45 3530	1 0490	
	5	23 46	65 40	41 94	45 9480	0 9128	
T2	1	23 10	59.03	35 93	41 2160	0 8717	0 8536 +0 0341
	2	30 37	67 70	37 33	43 6135	0 8559	
	3	23 45	59 36	35 91	40 8240	0 8769	
	4	25 91	57 25	31 34	39.4625	0 7942	
	5	26 82	61 63	34 81	40 0435	0 8693	
T3	1	25 94	58 25	32 31	44 8102	0 7210	0 7576 +0 0554
	2	25 98	56 70	30 72	43 0745	0 7132	
	3	24.19	58 85	34 66	40 7330	0 8509	
	4	25 82	59.65	33 83	44 4605	0 7609	
	5	24 22	53 56	29 34	39 5465	0 7419	

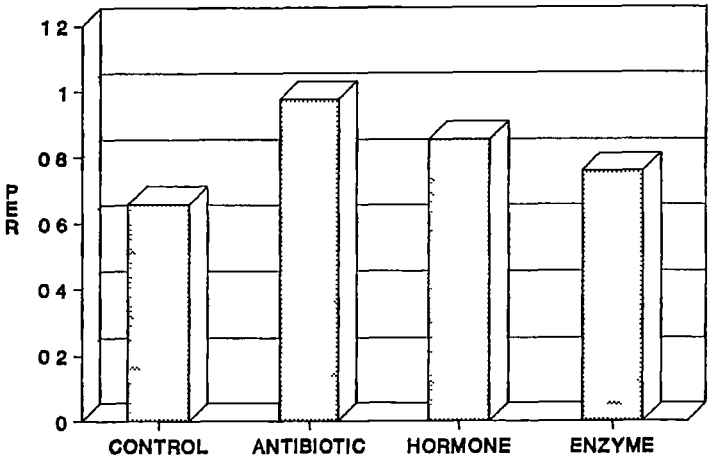

Fig 5 Protein efficiency ratio of M rosenbergii post larvae fed on various growth promoters

Table 17 Analysis of the data on protein efficiency ratio of *M rosenbergii* post larvae fed on various growth promoters

SOURCE	D F	S S	M S	F
Diets	3	0 2801	0 0934	34 07*
Error	16	0 0438	0 0027	
TOTAL	19	0 3239		

Comparison of treatment means based on critical difference

Critical difference 0 07

Diets	T1	T2	T3	T4
Treatment means	0 9769	0 8536	0 7578	0 6566

* significantly different 5 % level

Table 18 Productive protein values of M rosenbergii post larvae fed on feeds containing various growth promoters.

TREATMENT	REPLICATION	INITAL BODY PROTEIN	FINAL BODY PROTEIN	GAIN IN PROTEIN	AVERAGE PROTEIN GAIN	PROTEIN CONSUMED	PRODUCTIVE PROTEIN VALUE	MEAN + SE
T0	1	3 0464	6 4769	3 4305	3 6609 +0 2019	38 4545	8 9209	8 995 +0 500
	2	3 1919	6 7373	3 5453		39 850	9 0017	
	3	3 4585	7 1564	3 6979		42 1645	8 7702	
	4	4 0528	8 0223	3 9695		40 4530	9 8126	
	5	3 4068	7 0681	3 6613		43 2285	8 4696	
T1	1	3.0219	8 2975	5 2756	6 0247 +0 5118	42 3185	12 4660	13 4763 ±1 0196
	2	3 0042	9 3824	6 3781		43 6625	14 6077	
	3	3 3146	9 5327	6 1181		46 1615	13 2407	
	4	3.1294	9 6969	6 5676		45.3530	14 4811	
	5	3 1906	8 9735	5 7830		45 9480	12 5860	
T2	1	3.1416	8 0783	4 9367	4 8318 +0 30026	41 2160	11 9776	11 7710 +0 4536
	2	4.1303	9 2647	5 1344		43 6135	11 7725	
	3	3 1892	8 1323	4 9431		40.8240	12 1083	
	4	3 5238	7 8604	4 3367		39 4625	10 9894	
	5	3 6475	8 4556	4 8081		40 0435	12 0072	
T3	1	3 5278	7 9744	4 4466	4 4202 ±0 3116	44 8102	9 9232	10 4246 +0 7619
	2	3 5333	7 7611	4 2278		43 0745	9 8200	
	3	3 2898	8 0589	4 7691		40 7330	11 7100	
	4	3.5115	8 1661	4 6546		44 4605	10 4700	
	5	3 2939	7 3270	4 0031		39 5465	10 2000	

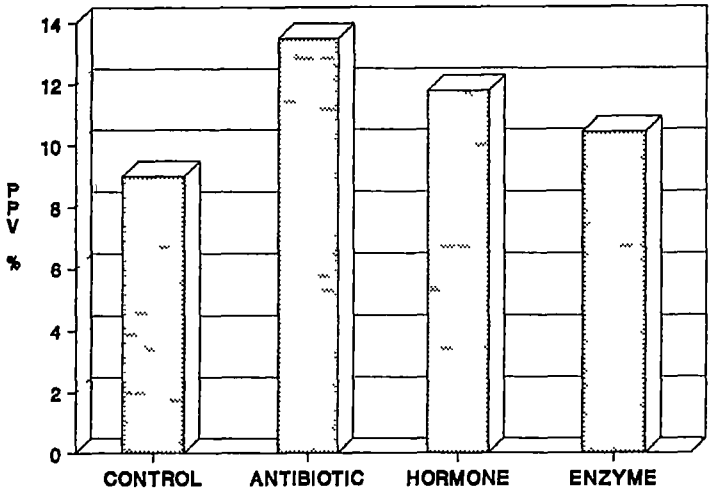

Fig 6 Productive Protein Value of M.rosenbergii post larvae fed on various growth promoters

Table 19 Analysis of variance of the data on productive protein value of M rosenbergii post larvae fed on various growth promoters

SOURCE	D F	S S	M S	F
Diet	3	54 8320	18 2773	35 22*
Error	16	8 3042	0 5190	
TOTAL	19	63 1362		

Comparison of treatment means based on critical difference

Critical difference 0 97

Diets	T1	T2	T3	T0
Treatment means	13 4763	11 7710	10 4246	8 995

* significantly different at 5 level

4 3 Optimum level of selected growth promoter oxytetracycline for M. rosenbergii post larvae

The analysis of the results obtained from the first experiment based on the efficiency of various growth promoters, showed that the antibiotic oxytetracycline was the best among the three types of growth promoters in Macrobrachium rosenbergii postlarvae

4 3 1 Proximate composition of diet The proximate composition of the basal diet was the same as that of the first experiment The diet was prepared based on the formula recommended by Kanazawa et al. (1982) for P. japonicus with few modifications on the basis of recent information regarding M. rosenbergii

Four different diets having varying amounts of oxytetracycline was prepared incorporating at levels of 5 mg/100g 10 mg/100g 20 mg/100g and 40mg/100g of the basal diet

Proximate analysis of feed showed the average protein content as 36 % fat 7% fibre 4% ash 14% and the rest as NFE

4 3 2 Water quality maintenance

1 Temperature The water temperature was found to vary from

25 03 °C to 28 36 °C in the experimental tanks during the period of study (Table 20)

2 pH pH values were found to be uniform in all experimental tanks in the same range as observed in the first experiment Slightly alkaline pH was observed in the tanks the range being 6 5 to 8 2 Table 21 shows mean pH and the range in each treatment

3 Dissolved oxygen As in the previous experiment dissolved oxygen values showed minimum fluctuations in various treatments Aeration provided in the experimental tanks kept the dissolved oxygen at a constant level The dissolved oxygen values ranged between 6 15 to 8 2 ppm during the study Table 21 gives mean dissolved oxygen values in various treatments and their ranges

4 3 3 Growth response of M rosenbergii to different levels of Oxytetracycline

Treatments with different antibiotic levels 5 mg/100g 10 mg/100g 20 mg/100g and 40 mg/100g have been denoted as T1 0 T1 1 T1 2 and T1 3 respectively

4 3 3 1 Growth The initial average weights of prawns used for the study were 22 642 mg 22 586 mg 23 486 mg and 22 142 mg respectively for the treatments T1 0 T1 1 T1 2 and T1 3

Table 20 Temperatures recorded from rearing tanks during experiment II to find out the optimum level of Oxytetracycline

	WEEKS					
TEMPERATURE	1	2	3	4	5	6
MEAN	25 64	26 80	27 13	28.12	28 14	27 95
+ SE	0 39	0 19	0 14	0 15	0 13	0 18
RANGE	(25 03-26 02)	(26 52-27 01)	(26 92-27 30)	(27 96 28 36)	(28 01 28 35)	(27 68-28 11)

Table 21 Fluctations in pH values recorded in different experimental tanks during the study to find out the optimum level of Oxytetracycline

	WEEKS					
pH	1	2	3	4	5	6
MEAN	6 86	7 36	8 04	8 06	7 84	7 72
+ SE	0 11	0 21	0 11	0 17	0 15	0 16
RANGE	(6 5-7 3)	(7 1 7 6)	(7 9 8 2)	(7 8-8 2)	(7 6-8 0)	(7 6-8 0)

Table.22 Dissolved oxygen values recorded in experimenal tanks during experiment to find out the optimum level of the antibiotic Oxytetracycline

	WEEKS					
DISSOLVED OXYGEN	1	2	3	4	5	6
MEAN	6 97	6 99	7 31	7 02	7 15	7 94
± SE	0 15	0 16	0 34	0 11	0 15	0 23
RANGE	(6 82-7 17)	(6 15- 7 82)	(6 91-7 14)	(6 91 7 14)	(6 91-7 32)	(7 6-8 2)

The final average weights obtained were 59 6825mg for T1 0 62 554 mg for T1 1 63 328 for T1 2 and 61 362 mg for T1 3 Maximum growth increment of 39 974 mg was obtained for treatment T1 1 where oxytetracycline has been incorporated at a rate of 10 mg/100g of diet This was followed by T1 2 with average weight increment of 39 8420 mg The average weight gain by the remaining two levels of antibiotic were for T1 0 - 37 0445 mg and for T1 3 39 22 mg Analysis of variance of the data has given a F ratio of 5 7332 (Table 24) which shows a significant difference between treatment (P< 0 05) Fig 7 gives a diagrammatic representation of the growth observed in the experimental tanks

Average daily weight gain of prawns were 0 8820 mg 0 9518 mg 0 9486 mg and 0 9338 mg for treatments T1 0 T1 1 T1 2 and T1 3 respectively

Table 23 gives the details of the initial and final weights growth increment and their mean values during the experiment II

4 3 3 2 Specific growth rate The SGR values obtained in the experiment ranged from 2 32 to 2 43 The highest SGR value was observed in treatment T1 1 (2 43) and lowest was in treatment T1 2 (2 38) Table 23 gives the data of SGR values in different treatments with graphical representation of the SGR

Table 23 Growth and Specific Growth Rate of M rosenbergii fed on feeds containing various levels of Oxytetracycline

TREAT MENT	REPLI CATION	AVE INITIAL WEIGHT (mg)	AVE FINAL WEIGHT (mg)	GROWTH (mg)	MEAN GROWTH (mg)	% GROWTH	MEAN % GROWTH	SPECIFIC GROWTH RATE	MEAN + SE
	1	23 31	60.60	37 29		159 97		2 27	
	2	21 56	57 93	36 37		168 69		2 35	
					37 04		165 21		2 32
T1.0	3	22 32	60 43	38.11	±1 21	170 74	+20 31	2 37	+0 19
	4	26 10	61 41	35 31		135 29		2 04	
	5	19 92	58 04	38.12		191 37		2 55	
	1	21 01	60 57	39 59		188 43		2 52	
	2	22 19	63 20	41 01		184 81		2 49	
					39 97		178 57		2 43
T1 1	3	21 93	60 07	38 14	+1 55	173 92	+17 83	2 40	+0 16
	4	27 16	67 89	40 73		149 96		2 18	
	5	20 64	61 04	40 40		195 74		2 58	
	1	23 47	62 77	39 30		167 45		2 34	
	2	26 26	66 94	40 68		154 91		2 23	
					39 84		171 98		2 38
T1 2	3	21 72	61 33	39 61	+0 94	182 3	+21 22	2 47	+0 18
	4	26 94	67 86	40 92		151 89		2 20	
	5	19 04	57 74	38 70		203 26		2 64	
	1	25 43	64 11	38 68		152 10		2 20	
	2	21 84	62 45	40 61		185 94		2 50	
					39 22		178 14		2 43
T1 3	3	21 42	58 91	37 49	+1 67	175 02	+16 90	2 4	+0 15
	4	20 88	62 20	41 32		197 89		2 60	
	5	21 14	59 14	38 00		179 75		2 45	

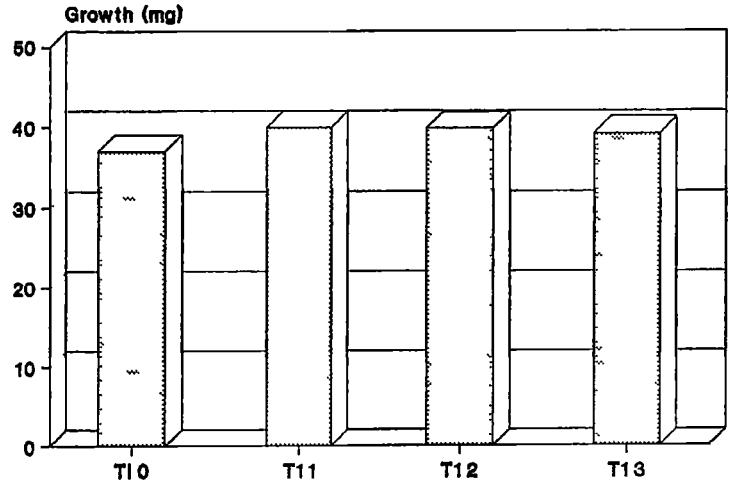

Fig 7 Growth of M rosenbergii post larvae fed on various levels of oxytetracycline

Table 24 Analysis of variance of the data on growth of *M rosenbergii* post larvae fed on various levels of Oxytetracycline

SOURCE	D F	S S	M S	F
Diets	3	27 7500	9 2500	5 73*
Error	16	25 8164	1 6135	
TOTAL	19	53 5664		

Comparison of treatment means based on critical difference

Critical difference 1 70

Diets	T1 1	T1 2	T1 3	T1 0
Treatment mean	39 97016	39 8432	39 2198	37 0376

Underscored means are not significantly different

* Significantly different at 5 . level

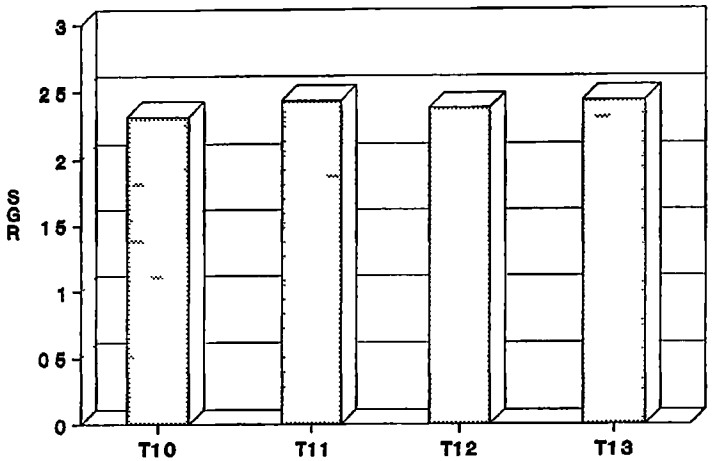

Fig 8 Specific growth rate of M. rosenbergii post larvae fed on various levels of oxytetracycline

Table 25 Analysis of variance of the data on specific growth rate of M rosenbergii post larvare fed on various levels of O ytetracycline

SOURCE	D F	S S	M S	F
Diets	3	0 0468	0 0156	0 55
Error	16	0 4543	0 0284	
TOTAL	19	0 5011		

values (Fig 8) Anova for the SGR values are given in the Table 25

4 3 3 3 Survival Average survival was 79% Maximum survival was observed in treatment T1 1 and T1 2 with 86. each Treatment T1 0 gave a survival of 74 and T1 3 gave 70 Table 26 and Fig 9 give the survival rates in various treatments

4 3 3 4 Food conversion ratio Mean FCR values in various treatments were 3 31 for T1 0 2 89 for T1 1 3 08 for T1 2 and 3 39 for T1 3 Lowest FCR was found in treatment T1 1 and highest in T1 3 Table 27 gives the data on feed consumed weight increment and FCR of various treatments Analysis of variance (Table 28) of the data shows that there is significant difference between the treatments ($P< 0.05$) Fig 10 shows the graphical representation of the FCR in various treatments

4 3 3 5 Protein efficiency ratio The average PER values for various treatments were T1 0 - 0 8686 T1 1 0 9913 T1 2 - 0 9310 and T1 3 0 8271 The maximum PER value was observed in treatment T1 1 where oxytetracyline has been incorporated at a rate of 100 mg /kg of diet The data on PER values in various treatments are given in Table 29 There is significant difference between various treatments as observed

Table 26 Percentage Survival values observed in the experimental tanks during the study to find out the optimum level of Oxytetracycline.

TREATMENT	REPLICATION	SURVIVAL (%)	MEAN +SE
	1	80	
	2	60	
T1 0	3	60	74 ± 13 42
	4	90	
	5	80	
	1	90	
	2	80	
T1.1	3	90	86 ± 5 48
	4	80	
	5	90	
	1	100	
	2	90	
T1 2	3	70	86 + 11 40
	4	80	
	5	90	
	1	70	
	2	50	
T1 3	3	80	70 + 12 25
	4	70	
	5	80	

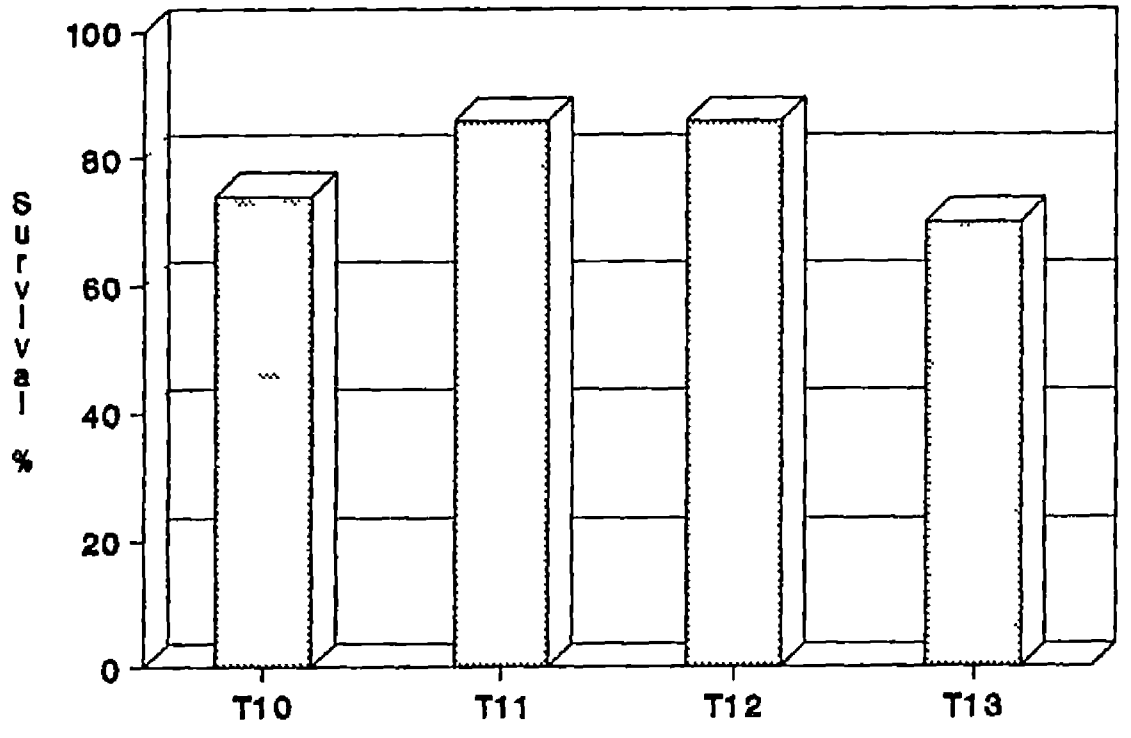

Fig 9 Survival rate of M rosenbergii post larvae fed on various levels of oxytetracycline

Table 27 Food conversion ratio of *M. rosenbergii* post larvae fed on feeds various levels of Oxytetracycline

TREATMENT	REPLICATION	AVE INITIAL WEIGHT (mg)	AVE FINAL WEIGHT (mg)	GROWTH (mg)	FOOD CONSUMED (mg)	FOOD CONVERSION RATIO	MEAN + SE
	1	23.31	60 60	37 29	113 39	3.04	
	2	21 56	57 93	36 37	130 47	3 59	
T1 0	3	22 32	60 43	38 11	116 94	3.07	3 31 + 0 3053
	4	26 10	61.405	35.31	130 29	3.69	
	5	19 92	58 037	38 12	120 83	3 17	
	1	21 02	60 573	39 563	108 48	2 74	
	2	22 19	63 203	41 01	113 05	2 76	
T1 1	3	21 93	60 072	38.14	114 55	3 00	2 89 + 0 2059
	4	27.16	67 894	40 73	112 45	2 76	
	5	20 64	61 039	40 40	129 55	3 21	
	1	23 47	62.771	39 30	122 57	3 12	
	2	26 26	66 942	40 68	123 33	3 03	
T1 2	3	21.72	61 330	39 61	113 26	2 86	3 08 ± 0 2103
	4	26 94	67 859	40 92	121.61	2 97	
	5	19 04	57 744	38 70	132 26	3 42	
	1	25 43	64 112	38 68	131 79	3 05	
	2	21 84	62 452	40 61	133 19	3 28	
	3	21 42	58 91	37 49	141 47	3 77	3 39
T1 3	4	20 88	62.199	41 32	136.57	3 31	+ 0 2757
	5	21 14	59 141	38 00	135 02	3 55	

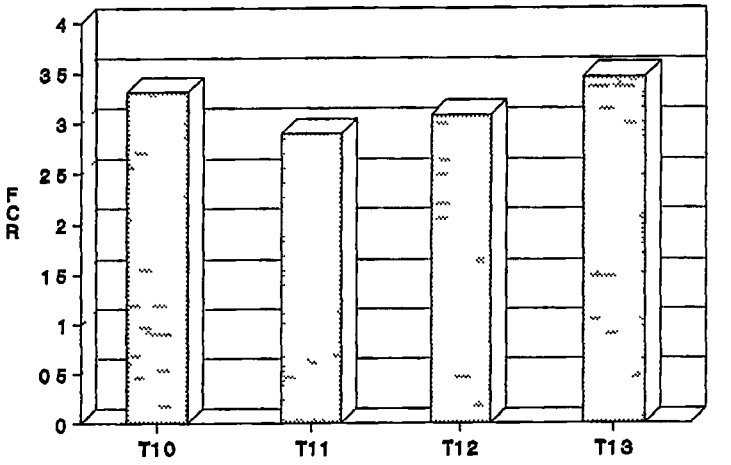

Fig 10 Food conversion ratio of M. rosenbergii post larvae fed on various levels of oxytetracycline

Table 28 Analysis of variance of the data on food conversion ratio of *M rosenbergii* post larvae fed on various levels of Oxytetracycline

SOURC	D F	S S	M S	F
Diets	3	0 9478	0 3159	5 71 *
Error	16	0 8858	0 0554	
TOTAL	19	1 8336		

Comparison of the treatment means basde on critical difference

Critical difference 0 32

Diets	T1 3	T1 0	T1 2	T1 1
Treatment means	3 4638	3 31146	3 0798	2 89382

Underscored means are not significantly different

* Significantly different at 5 % level

in ANOVA (Table 30) Fig 11 shows the variations in the PER values of the various diets

Table 29 Protein efficiency ratio of *M rosenbergii* post larvae fed on feeds containing various levels of Oxytetracycline

TREATMENT	REPLICATION	AVE INITIAL WEIGHT (mg)	AVE FINAL WEIGHT (mg)	GROWTH (mg)	PROTEIN CONSUMED (mg)	PROTEIN EFFICIENCY RATIO	MEAN + SE
T1 0	1	23 31	60 60	37 29	36 69	0 9395	0 8686 + 0 0776
	2	21 56	57 93	36 37	45 66	0 7965	
	3	22 32	60 43	38.11	40 93	0 9311	
	4	26 10	61 41	35 31	45 60	0 7743	
	5	19 92	58 04	38 12	42.29	0 9014	
T1 1	1	21 02	60 57	39 563	37 97	1 0427	0 9912 + 0 0675
	2	22 19	63 20	41 01	39 57	1 0364	
	3	21 93	60 07	38 14	40 09	0 9514	
	4	27 16	67 89	40 73	39 36	1 0348	
	5	20 64	61 04	40 40	45 34	0 8910	
T1.2	1	23 47	62 77	39 30	42.90	0 9161	0 9310 ± 0 0612
	2	26 26	66 94	40 68	43 17	0 9423	
	3	21 72	61 33	39.61	39 64	0 9992	
	4	26 94	67.86	40.92	42 56	0 9615	
	5	19 04	57 74	38 72	46 29	0 8360	
T1 3	1	25 43	64 11	38 68	46 13	0 8385	0 8271 + 0 0471
	2	21 84	62 45	40 61	46 62	0 8711	
	3	21 42	58 91	37 49	49 51	0 7572	
	4	20 88	62 20	41 32	47 80	0 8644	
	5	21 14	59 14	38 00	47 26	0 8041	

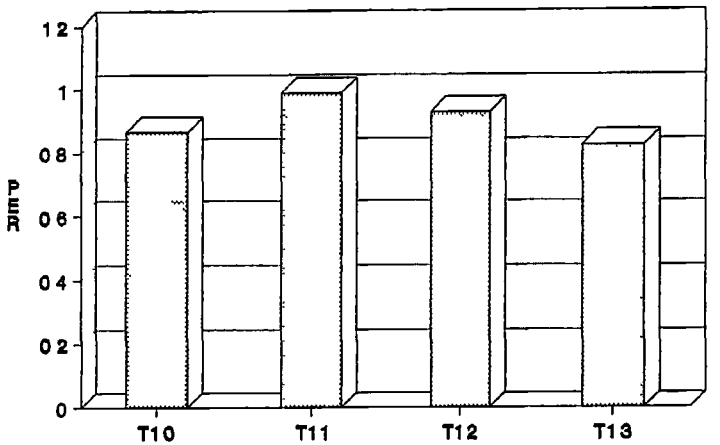

Fig 11 Protein efficiency ratio of M rosenbergii post larvae fed on various levels of oxytetracycline

Table 30 Analysis of variance of the data on protein efficiency ratio of M rosenbergii post larvae fed on various levels of oxytetracycline

SOURCE	D F	S S	M S	F
Diets	3	0 0776	0 0259	6 26 *
Error	16	0 0661	0 0041	
TOTAL	19	0 1437		

Comparison of treatment means based on Critical difference

Critical difference			0 09	
Diets	T1 1	T1 2	T1 0	T1 3
Treatment means	0 9913	0 9310	0 8686	0 8271

Underscored means are not significantly different

* Significantly different at 5 % level

DISCUSSION

5 DISCUSSION

The results obtained in the present study are discussed below in detail in light of the previous works, on the use of growth promoters in the diet of various cultured species Efficiency of diets water quality and survival are also discussed

5 1 Proximate composition of the formulated feed

Pelleted purified diet based on casein as a major protein source was used in the present study Such a diet was formulated originally for Penaeus japonicus by Kanazawa et al (1982) with a protein content of 50 . But in the present study the protein content of the diet was adjusted to 36 Balaz and Ross (1976) reported that protein content of more than 35 % is required to maintain adequate growth in juvenile M rosenbergii while Sick and Millikin (1983) estimated the protein requirement of early juvenile M rosenbergii to be around 40 % and larger prawns to be 25 30 % Employing a casein based semi purified diet Gomez et al (1988) found that a protein level of 13 - 25 % is enough to bring maximum growth in M rosenbergii In studies using purified crab protein D Abramo and Reed (1988) reported that 33 35 was the optimum dietary protein level while 30 % was found to be enough by Freuchtenichet et al (1988)

In commercial feeds of M rosenbergii lipid levels of 6 9 %, have been reported in Thailand (ASEAN/UNDP/FAO 1988), 5 -8 % in French Guiana (IFREMER 1989) and 2 4 % in Taiwan (Hsieh et al 1989) Using a 2 1 cod liver oil / corn oil mixture Sheen and D Abramo (1989) found that a 6 % inclusion rate was optimal Shrimps and prawns appear to utilize complex carbohydrate more effectively than simple ones (New 1976) Fair et al (1980) reported that dietary fibre levels upto 30 % do not appear to suppress growth in M rosenbergii The proximate composition of the feed prepared in this study conforms to this general picture

5 2 Water quality parameters

5 2 1 Temperature Temperature is an important factor which affects growth in ectotherms In the present study water temperature ranged between 26 4 °C to 30 44 °C which is within the optimal range New (1990) reported that M rosenbergii adults tolerate wide temperature range of 18 34 °C while for larvae the optimum range is 26 31 °C Temperature below 14 °C or above 35 °C are reported to be lethal for post larvae optimum being 29 - 31 °C Akiyama et al (1982) observed that rearing temperatures of 18 22 °C markedly stunt the growth in the species

5 2 2 Dissolved Oxygen Avault (1987) recommended the dissolved oxygen level should preferably be 70 % saturation for M rosenbergii adults though low levels as low as 1ppm are tolerated The optimum level of dissolved oxygen in pond condition for Macrobrachium culture ponds is 6 -8 ppm (Vasquez et al 1989) In the present study the dissolved oxygen content in the experimental tanks was well within the optimum range for Macrobrachium It was found to range between 6 67 - 8 32ppm

5 2 3 pH pH of water is another factor which has been reported to affect the growth of prawns During the present study, the pH values were almost uniform in all experimental tanks The pH values vary between 7 2 and 8 3 The optimum range pH reported for adults was 7 0 8 0 while for larval rearing the desirable pH was 7 0 8 5 (New 1990) Striuss et al (1989) reported that high unionized ammonia nitrogen and high pH have a synergistic toxic effect on prawns pH range of 7 0 8 5 is reported to be optimal for prawn culture

5 3 Evaluation of growth promoters

5 3 1 Growth rate Growth rate of prawns in various treatments indicated that the prawns fed on growth promoter oxytetracycline gave maximum growth The net weight gain in this treatment was 163 % more than the control which does not contain any kind of growth promoters The results

indicate that antibiotic oxytetracycline seems to be an efficient growth promoter for M. rosenbergii post larvae Corliss et al (1977) reported that addition of oxytetracycline in the diets of P. aztecus improved growth rate Oxytetracycline at levels of 10 -30 mg /Kg of feed has been reported to be a growth stimulant for animals in general (Maynard & Loosli 1969) But Vaitheeswaran and Ali (1986) reported that oxytetracycline has no growth promoting effect in P. indicus A similar observation has also been reported by Ciapara et al (1989) for P. monodon with oxytetracycline But Stuck et al (1992) reported that oxytetracycline at 25 or 50 ppm improved survival and growth in P. vannamei There are many reports of growth promotion by antibiotics in fishes (Ahmed and Matty 1989 Viola and Arieli 1987 Rijikers 1980 Chua and Teng 1980)

Growth promoting effect of antibiotics may partly be the result of their therapeutic effects It has been suggested that they reduce or eliminate the activity of microorganisms causing subclinical infections reduce bacteria which produce toxins retarding growth and increase the absorptive capacity of the intestine (Visek 1978) The use of antibiotics leads to a reduced requirement for vitamin B12 and an increased conversion of food nitrogen into body nitrogen

Thyroid hormone and proteolytic enzyme papain

which were the two growth promoters tried, also showed increased growth than control The percentage increase of growth over the control being 131 % for the former and 120 % for the latter

Vaitheeswaran and Ali (1986) observed that thyroid hormone has no growth promoting role in P indicus although this hormone has been considered as potential growth promoter for fishes (Donaldson et al 1979 Higgs et al 1982 Mc Bride et al 1982 Matty and Lone 1985)

Recent work by Pillai et al (1987) showed a positive role of thyroxine on moulting and growth of P monodon where post larvae showed an enhanced growth rate when thyroxine was incorporated in the medium at a level of 3 ug /lit In the present experiment thyroid hormone was incorporated at a level of 2 5 mg/100 g of the diet

According of Akiyama (1991) papain is a potential feed additive in the prawn feed which improves the growth by 10 20 % when incorporated into feeds at a level of 0 1 to 0 2% This view has been supported by Paulraj also n 1993 In the present experiment papain which was incorporated into the prawn diet at a level of 0 2 % has clearly accelerated the growth of prawns than the control diet but the growth was less than those treated with diets based on antibiotic and hormone growth promoters

5 3 2 Specific Growth Rate Specific growth rate can be considered as an index of growth in the evaluation of diets The results of the present study indicate an increase of average SGR over the control in the different growth promoters used The highest SGR was obtained for diets in which antibiotic oxytetracycline was incorporated at 10 mg/100g of diet which indicates its better utilization and efficient conversion Corliss et al (1977) found that oxytetracycline stimulated the growth and better SGR in P aztecus Ahmed and Matty (1989) showed that oxytetracycline improves the SGR in carps when incorporated in high protein diet (40 %) Improved SGR of the experimental animals were obtained for the other two growth promoters used, thyroid hormone treatment showed better SGR than the enzyme papain

5 3 3 Survival Overall survival of of 69 5 % was observed in experiment I Maximum survival was observed in treatment T1 where antibiotic was incorporated in the feed Incorporation of antibiotic in the feed has been reported to increase survival in fishes and prawns by several workers (Corliss et al 1977, Stuck et al 1992) Chan and Lawrenece (1974) reported the effectiveness of oxytetracycline Oleanodomycin combinations in reducing bacterial populations in larval shrimp cultures They suggested that this antibiotic combination could be used to treat Vibrio and other bacterial infections in mysis and post larval stage of shrimp The antibacterial property of the antibiotic may be one of the

reasons for improved survival In the present study lowest survival of 66% has been observed in T2 where thyroid hormone was used as growth promoter

5 3 4 Food Conversion Ratio The efficiency with which an animal can convert food for the growth process is reflected in the ratios of food consumed to the live weight it has gained Thus low food conversion ratios indicate high efficiency in food utilization In the present study the lowest food conversion ratio of 2 93 was obtained for diets containing antibiotic,whereas the food conversion values for control diet was 4 36

Sandifer and Joseph (1976) reported a food conversion ratio of 2 2 and 4 0 at the end of 3 weeks and 6 weeks respectively when M rosenbergii juveniles were fed on Purina Marine Ration a commercial pellet feed Reduced FCR values of 1 9 and 2 2 were observed by them in the juveniles during 3 weeks and 6 weeks study period with the same feed whose quality was augmented with shrimp head oil Fair and Fortner (1981) reported a FCR of 1 7 and 1 3 for M rosenbergii post larvae of initial weight of 0 150 g when fed on intact and pulverised pellets of Purina Marine Ration Balaz and Ross (1976) reported mean food conversion ratios of 0 90 to 1 24 after 24 weeks, in prawns having an initial mean weight of 0 1 g with feeds based on locally available

materials Post larvae of M rosenbergii of initial weight 90 mg gave a FCR of 1 71 in a 153 day feeding experiment with commercial pellets (Smith and Sandifer,1980) while Roberts and Bauer (1978) reported FCR values of 1 85 - 2 5 in Macrobrachium grow outs in South Carolina

Addition of antibiotic has been reported to reduce feed intake in shrimp by Corliss et al 1977 They have observed that the feed containing oxytetracycline was more efficiently converted than feed without oxytetracycline In the present study also incorporation of oxytetracycline was found to reduce the food consumption and hence reduce food conversion ratio Corliss et al (1977) reported a FCR of 4 2 for P aztecus of size 143 4 mg when fed on diets with 100 1000 mg of oxytetracycline while the control gave FCR of 12 0 Vaitheeswaran and Ali (1986) reported a FCR of 4 01 & 2 75 for feeds incorporated with thyroid and oxytetracycline respectively while casein based control diet gave a FCR of 2 84 James et al (1990) reported a FCR of 4 97 for M rosenbergii post larvae of 3 85 mg in weight when fed on a casein based diet

In the present study the FCR value shown by control diet is higher than the values reported by various authors Sandifer and Joseph (1976) reported FCR of 4 0 which is nearer to the FCR shown by the control in the present

study FCR values are affected by a variety of factors like age of animal species and environmental factors (New 1976 Condrey 1982 Goswami and Goswami 1982 De Silva 1989)

Results of the present study indicate that addition of the growth promoters in the feed reduced FCR values Although all the growth promoting substances tried gave a significant reduction in FCR values than their control the antibiotic gave the best performance

5 3 5 Protein efficiency ratio Colvin (1976 b) reported a protein efficiency ratio of 0 49 0 95 in P. indicus when feed containing different seed oils were fed to them Sedgwick (1979) obtained PER values in the range of 0 076 0 902 in P. merguiensis when the animals were fed on feeds made of freeze dried Mytilus edulis wheat starch cod liver oil etc James et al (1990) obtained PER values of 0 36 for casein based control diet and 0 51 for Spirulina based test diet

In the present study, highest PER was obtained in feed containing oxytetracycline This indicates that addition of antibiotic may have increased the utilization of protein in the feed for growth This is reflected in the increased growth rate and lower FCR values Control diet which did not contain any growth promoters gave the lowest PER value of 0 6566 which is indicative of lower utilization of the protein present in

the feed Incorporation of other two growth promoters in feed also improved the protein efficiency ratio than the control though not as high as oxytetracycline

5 3 6 Productive Protein Value PPV is considered as an appropriate and simple measure of utilization of dietary protein by organisms Steffens (1981) used productive protein values for comparing the protein utilization in Rainbow trout and carp Degani et al (1989) also used this index to study the protein utilization by Clarius gariepinus In the present study, productive protein value is maximum being 4 3818 in prawns fed on diets containing antibiotic oxytetracycline This indicates more efficient utilization of protein present in the diet when antibiotic was incorporated to the feed Results with the other two growth promoters also indicate better utilization of protein for growth than the control James et al (1990) reported a productive protein value of 6 67 for Spirulina based diet and 9 42 for casein based diet for M rosenbergii post larvae of size 3 85 mg In the present study also the control gave a PPV of 8 99 which is comparable to the value obtained by James et al (1990) But the productive protein value reported by Steffens (1981) and Degani et al (1989) in fishes is high compared to those reported in prawns

In general protein utilisation depends

essentially on the fish species and size environmental factors protein quality and level of dietary protein It is also seen to be influenced by utilizable dietary energy kind of energy source and amount of feed (Steffens 1981) The dependence of protein utilization on fish size and other factors was also shown by De Silva et al (1989)

The results of the present study clearly indicate a better utilization of dietary protein for growth in the diet incorporated with antibiotic The sparing effect on the protein normally used for energy by the antibiotic as reported by Ahmed and Matty (1989) may be the reason for increased growth observed in the experiment

5 4 Optimum level of oxytetracycline

Four levels of oxytetracycline were tested in the second experiment The levels were fixed based on the level tested in the first experiment In the first experiment the level tested was 10 mg/100g of feed Hence in the second experiment one lower level (5 mg/100 g) and two higher levels (20 mg/100g and 40 mg/100g) were tested

The results of the experiment to evaluate the optimum level of oxytetracycline showed that 10mg of oxytetracycline /100g of feed gave the best growth while the

feed containing 5 mg/100 g gave the lowest growth Analysis of variance of the data on growth and comparison of treatment means, based on critical difference indicate that the diets containing oxytetracycline at 10 mg 20 mg and 40 mg/100g of feed have no significant difference between the levels tested This result is in confirmation with the result obtained with P aztecus by Corliss et al (1977) where 100 1000 mg/Kg feed gave a similar growth enhancement

The effect of antibiotics on growth seems to be dependent on the age size, species etc of the animals tested (Maynard and Loosli 1969 Visek 1978) This may be the reason for difference in action of the antibiotics on the growth of prawns as reported by Stuck et al (1992) Ciapara et al (1989) and Vaitheeswaran et al (1986)

Specific growth rate is considered as an efficient index indicating the efficiency of the feeds on the growth of the animals In the present study also specific growth rate shows a similar trend where maximum SGR was obtained in T1 1 where antibiotic was incorporated at 10 mg/100g The analysis of variance of data shows that there is no significant difference in the specific growth rate at various levels of antibiotics Antibiotic incorporation in the diet was found to increase growth in carp (Ahmed and Matty 1989) in prawns P aztecus,P monodon (Corlisset al 1977

Ciapara et al 1989)

There is no significant difference in survival between various treatments containing various levels of oxytetracycline as observed in Anova Lowest and highest antibiotic levels gave a lower survival rate than the remaining two levels (10 and 20 mg/100g) Increased survival rate at higher levels may be due to modifications in the bacterial flora by the action of antibiotics but the lower survival rate shown by the highest level may be because of the reason that 40 mg/100g is the maximum tolerable limit to the post larvae of this size

Incorporation of oxytetracycline is found to reduce the food conversion ratio in prawns Corliss et al (1977) reported that feed containing oxytetracycline was more efficiently converted by P aztecus than the feed without oxytetracycline He found that the 10 mg/100g of feed reduced the FCR significantly than the control In the present experiment also the feed incorporated with oxytetracycline at the rate of 10 mg/100g of feed reduced the FCR values significantly than the other treatments Vaitheeswaran et al (1986) also reported a reduction in FCR values when oxytetracycline was incorporated in the diets of P indicus at a level of 10 mg/100g of diet

Ahmed and Matty (1989) observed an

increase in the food conversion efficiency in common carp when antibiotics were incorporated in the diet and the results of the study indicated that as the antibiotic dose increased the FCE also increased with maximum value being observed in 10 mg/100 g of a low protein diet

Protein efficiency ratio was highest in the diet with 10 mg of oxytetracycline per 100 gram of feed High PER values indicate a better utilization of protein present in the feed PER values at 10 mg/100g and 20 mg/100g show no significant difference The results indicate that at 10 mg/100g, more protein in the feed may have been used for growth Ahmed and Matty (1989) suggested a protein sparing action of the antibiotics where proteins normally used for energy is being used for growth

SUMMARY

6 SUMMARY

The present investigation has been carried out to evaluate the efficiency of various growth promoters viz oxytetracycline thyroxine and papain on the growth of M rosenbergii post larvae The optimum level of the selected growth promoter which gave the best performance in terms of growth rate and survival has also been studied

1 The various growth promoters used in the study were antibiotic oxytetracycline hormone thyroxine and enzyme papain

2 The growth promoters were incorporated in a casein based purified basal diet, with an average protein content of 36 fat content of 6 89% and moisture content of 7% The NFE value of the feed was found to be 31%

3 Four test diets prepared for the experiment, were control diet T0 without incorporartion of any growth promoters diet T1 with oxytetracycline diet T2 with thyroid hormone and diet T3 with enzyme papain The growth of prawns fed on various test diets in a 42 day experiment showed that prawns fed on oxytetracycline(T1) gave the best growth than those fed on diets containing thyroid hormone papain enzyme or control diet The average growth increment for diet T1 T2 and T3 were 43 67mg 35 06mg and 32 172 mg respectively

4 Incorporation of growth promoters in the feed has increased the specific growth rate of the animals Thus the specific growth rate of the animals fed on diet T1 containing oxytetracycline was 2 5180 while it was only 1 7260 for the control diet T0

5 The food is found to be more efficiently converted by prawns when oxytetracycline was incorporated in the feed This is reflected in the lower food conversion value of 2 93 obtained for prawns when fed on diet T1 containing oxytetracycline The FCR values were significantly lowered in the other two growth promoters used too and ranged between 3 6968 and 3 128 whereas the FCR for the control was 4 36

6 Protein efficiency ratio was found to increase when the growth promoters were added in the feed Maximum PER value of 0 9770 was obtained for feeds containing oxytetracycline The protein efficiency ratio of 0 6566 for T0 seems to indicate that the utilization of protein in the control diet was lowest when compared to other feeds

7 Inclusion of growth promoters in the feed has increased the productive protein value of the animals significantly Maximum value of 13 4763 was obtained for the feed containing antibiotic A higher PPV indicates a better conversion of feed protein to body protein which increased the

growth of the animal

8 Four levels of oxytetracycline were tried in the second experiment The different levels of oxytetracycline was incorporated in the casein based purified diet with a protein content of 36 Out of the different levels tried the optimum level of oxytetracycline was found to be 10mg /100g feed for M rosenbergii post larvae The growth increment growth rate survival food conversion ratio and protein efficiency ratio of the prawns fed at this level were found to be superior compared to other levels tried in the experiment

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EFFECTS OF VARIOUS GROWTH PROMOTERS IN THE DIETS OF MACROBRACHIUM ROSENBERGII POST LARVAE

BY
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ABSTRACT OF A THESIS

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ABSTRACT

The present study was conducted to evaluate the efficiency of various growth promoters on the growth of Macrobrachium rosenbergii post larvae The various growth promoters tried were antibiotic oxytetracycline thyroid hormone thyroxine and the enzyme papain Each growth promoter was incorporated in a casein based purified diet having a protein content of 36% In the second experiment the optimum level of growth promoter which showed the best performance in the first experiment was determined

The growth increment of prawns fed on casein based purified diets incorporated with antibiotic oxytetracycline designated as diet T1 was 43 66mg it was 35 064 mg for diet T2 containing thyroid and 32 172 mg for diet T3 containing papain The growth increment for the control diet was seen to be 26 72 mg Specific growth rate of the prawns from different treatments ranged from 1 73 to 2 52 with control diet showing the lowest and T1 the highest Incorporation of the growth promoters in the feed has markedly reduced the food conversion ratio of the animals Lowest FCR was obtained for the post larvae fed with oxytetracycline while the highest was for the control the range being 2 94 to 4 36 The protein efficiency ratio was highest being 0 9770

for the feeds containing oxytetracycline The PER values for the control diet T0 diet T2 with thyroid diet T3 with papain were found to be 0 6566 0 8541 and 0 7576 respectively Productive protein values were 8 995 for control diet 13 4763 for diet with oxytetracycline 11 7710 for diet with thyroid hormone and 10 4266 for diet with papain enzyme

The average survival rate during the experiment was 69 5 % The animals fed on antibiotic incorporated feed gave maximum survival of 72 while the lowest rate of 66 was observed for thyroxine incorporated feed (T2)

Analysis of the data on the various growth parameters in the experiment reveals that the antibiotic oxytetracycline gives better growth amongst the different growth promoters used In the experiment II four different levels of oxytetracycline were tested to find out the optimum level The result of the study showed that 10 mg/100g of feed is the optimum level of oxytetracycline which give maximum growth in Macrobrachium rosenbergii post larvae as indicated by overall growth specific growth rate food conversion ratio and protein efficiency ratio