EVALUATION OF CERTAIN SUBSTANCES AS GROWTH-PROMOTING AGENTS FOR THE PRAWN *PENAEUS INDICUS*

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ABSTRACT

Seven substances, namely oxytetracycline (antibiotic), ethyloestrenol (synthetic steroid), thyroid (hormone), alfalfa extract (plant material), glucosamine (chemical), prawn shell (animal material) and testosterone (hormone), were selected for evalution as growth promoting agents to add in the diet of the prawn *Penaeus indicus*. These substances each was incorporated in a purified diet consisting of casein, starch, sucrose, cod-liver oil, cholesterol, vitamins, minerals and cellulose. The diets supplemented with testosterone, glucosamine, prawn shell and alfalfa extract, respectively at levels 2.5 mg, 0.8 g, 0.8 g and 2.0 ml per 100 g of diet, produced faster growth, higher survival and better food conversion ratio than the control diet did. Testosterone gave the best results, the other substances in the order of efficiency being glucosamine, prawn shell and alfalfa extract. The diets supplemented with oxytetracycline, ethyloestrenol and thyroid, respectively at levels 10 mg. 0.5 mg and 1.0 mg per 100 g of diet, gave no better result in growth, survival rate and food conversion ratio than the control. Presenting the details of the evaluation results, the effect of these seven substances on the body composition of the prawns is discussed.

INTRODUCTION

The use of certain substances as growth promoting agents in the diets of homeothermic land animals such as poultry and pigs has been known since long. The growth promoting agents, when incorporated in small quantities in feed, enhance growth and improve the food conversion efficiency and thus help in reducing the rearing period as well as the cost of production of the cultivated animals. A variety of substances such as hormones, antibiotics, synthetic steroids and plant preparations have been in use for this purpose. In recent years, though some of these growth promoting agents have been used in diets of finfish all over the world (Donaldson et al 1979) with varying results, there are only a very few studies made (Corliss et al 1977, Kitabhyashi et al 1971) on the use of growth promoting agents in the diets of crustaceans, especially prawns. In the present study the substances oxytetracycline (antibiotic), ethyloesternol (synthetic steroid), thyroid (hormone), alfalfa extract (Plant material), glucosamine (chemical), prawn shell (animal material) and testosterone (hormone) were selected as growth promoting agents and their relative efficiency evaluated in the diet of *Penaeus indicus* for the first time and the results presented.

MATERIAL AND METHODS

Formulation and preparation of the diets: For the purpose of studying the effect of the selected growth promoting agents in the diet of *Penaeus indicus,* a basal purified diet was used to which the identified substances were individually added. The basal purified diet was formulated based on the formula developed by Kanazawa et al (1970) for *Penaeus japonicus,* with slight modifications according to the availability of the ingredients. The diet was formulated so as to contain 40% protein, 40% carbohydrate, 5% lipid and 3% vitamin and mineral mixture. The detailed composition of the basal diet is given in Table 1.

TABLE 1. *Composition of the basal purified diet*

Ingredient	Quantity (g)
Casein	40.0
Cod liver oil	5.0
Sucrose	10.0
Starch	30.0
Vitamins and minerals*	3.0
Sodium succinate	0.3
Sodium citrate	0.3
Ascorbic Acid	0.5
Choline	0.6
Inositol	0.2
Sodium carbonate	1.0
Cholesterol	0.5
Cellulose	5.6
Agar	3.0
	100.0
	+
Distilled water	100 ml.

* 100 g of the diet contained:

Vitamins: Vitamin A (as acetate) 15000 I.U., Vitamin D 1500 I.U., Thiamine mononitrate (B1) 9.0 mg, Riboflavin (B2) 9.0 mg, Pyridoxine hydrochloride (B6) 3.0 mg, Cynanocobalamin (B12) 6.0 mg, Calcium pantothenate 15.0 mg, Niacinamide 60.0 mg, Vitamin C (as sodium ascorbate) 150.0 mg, Vitamin E 15 I.U., and Folic acid 0.3 mg.

Minerals: Calcium carbonate (equivalent to 300 mg Calcium) 750 mg, Ferrous sulphate (equivalent to 30 mg Iron) 102 mg, Potassium iodide (equivalent to 0.45 mg Iodine) 0.6 mg, Pottassium sulphate (equivalent to 15 mg Potassium) 33.0 mg, Copper sulphate (equivalent to 3.0 mg Manganese) 7.4 mg, Zinc sulphate (equivalent to 4.5 mg Zinc) 19.8 mg, and Magnesium oxide (equivalent to 16 mg Magnesium) 30 mg.

Seven substances identified for evaluation as growth promoting agents and their description and sources are as follows:

(1) Oxytetracycline (OCN), obtained as capsules from Pfizer Ltd., Thane.

(2) Ethyloestrenol (Orabolin), a pharmaceutical preparation, widely used as an anabolic factor for human beings, obtained in the form of tablets from Organon (India) Ltd., Bombay.

(3) Thyroid (TRD), obtained as tablets from Burroughs Welcome & Co., (India) Pvt. Ltd., Bombay.

(4) Alfalfa Extract (AFA), an alcholic extract (10% W/V) of the forage legume, *Medicago* spp. (composition on dry basis protein 19.90%, fat 1.81%, fibre 29.51% and ash 11.73%, *Wealth of India;* CSIR Publication, New Delhi, 311-318), procured as a homeopathic mother-tincture from Mahesh Laboratories (Pvt.) Ltd., Calcutta.

(5) Glucosamine (GMN), D (±) glucosamine hydrochloride, obtained from the British Drug House Ltd., England.

(6) Prawn Shell (PS), prepared in the laboratory by the following method: The exoskeleton of *P. indicus* was dried, crushed and defatted using methanol-chloroform (1 : 2 ratio) mixture. It was then dried in the oven at 60° ± 2°C for 12 h. The material was then boiled for 1 h with 4% NaOH twice and filtered. The residue was washed well and then boiled with distilled water to get rid of traces of alkali. The material thus obtained was dried in the oven at 60° ± 2°C for 20 h and powdered This material contained approximately 43% of chitin.

(7) Testosterone (TSN), obtained as testosterone propionate (a liquid preparation) with the trade name, 'Perandren', from Ciba Geigy of India Ltd., Bombay.

The substances OCN, Orabolin, TRD, AFA, GMN, PS and TSN were each separately incorporated in the plain diet (which acted as control. D0) and seven different diets, D1, D2, D3, D4, D5, D6 and D7, were formulated. The level at which each of these substances was included in the diet is shown in Table 2.

The dry ingredients, casein, starch, sucrose, cholestrol, cellulose and vitamins and minerals, were powdered in an electrical grinder and were passed through 200 micron sieve. These ingredients were mixed thoroughly to get a dry homogenate. Sodium citrate, sodium succinate, ascorbic acid, choline, inositol, sodium carbonate and agar agar were dissolved in 100 ml of distilled water. To this solution, cod-liver oil was added and finely emulsified in an electric blender. The test substance was added at this stage, to the emulsion, homogenised and

TABLE 2. *Composition of the experimental diets*

Component	D0	D1	D2	D3	D4	D5	D6	D7
Bsal diet (g)	100	100	100	100	100	100	100	100
OCN (mg)		10						
Orabolin (mg)			0.5					
TRD (mg)				1.0				
AFA (ml)					2.0			
GMN (g)						0.8		
PS (g)							0.8	
TSN (mg)								2.5

then mixed with the dry ingredients mixture and steamed for 15 min. The diet was extruded through 3 mm diameter die and dried in the oven at 60 ± 2°C for 24 h. The diet was reduced to approximately 500 micron size particles and stored in airtight polythene bag kept in plastic container until use.

Analysis of experimental animals: The animals were analysed chemically before and after the feeding experiments for moisture, protein, carbohydrate and lipid contents. Randomly selected twenty four animals were sacrificed before the start of the feeding experiment for the initial sample. At the end of the feeding experiment, the animals from the replicates of each treatment were separately pooled and analysed. Moisture content was estimated gravimetrically by drying at 60° ± 2°C for 24 h. Protein was estimated following biuret method. Carbohydrate was determined spectrophotometrically using phenol-sulphuric acid reagent and the total lipid was estimated by Bligh and Dyer method.

Feeding experiment: Hatchery-reared juveniles of *P. indicus* belonging to the same brood with an average length of about 17 mm and an average dry weight of about 8.5 mg, from the Narakkal Prawn Culture Laboratory of CMFRI, were used in the experiments. The feeding experiments were conducted in circular plastic troughs containing 16 litres of a mixture of filtered sea water and fresh water with the salinity of about 18 ‰. In each container 8 animals were stocked, and three replicates were kept for each treatment. Thus there were 24 animals receiving each diet. Three random samples of eight animals each were sacrificed, dried and weighed, and the average was taken as the initial dry weight. Intermittent aeration was provided in all the troughs. Sediments were siphoned out daily and complete water was replaced once in three days. The troughs were covered with velon screen to prevent the animals from jumping out.

The animals were separately fed on the diets D1 to D7. The diets D0 was given as the control diet for comparison. Feeding was done approximately at the rate of 30% of their initial body weight in two divided doses, in the morning and evening. The food was placed in petridishes kept at the bottom of the troughs and the left over food was removed daily before feeding. Same quantity of food was given in all troughs every day.

The salinity, temperature and pH of the water used throughout the experiment were 18.58 ‰, 30.4° ± 1.2°C and 8.06 ± 0.38, respectively. The feeding experiments were continued for a period of 28 days.

At the end of the experiment, the total lengths of individual animals of each group were measured and the final weights were taken after sacrificing and drying them.

The experimental data were subjected to Analysis of variance (ANOVA) to test the significance among the treatments with respect to the increase in length, increase in dry weight, rate of survival and food conversion ratio. The method of least significance difference (LSD) was applied to find out the significance between treatments.

RESULTS

The results of the feeding experiment are presented in Table 3. The dry weight increments recorded by the prawns fed on diets supplemented with AFA, GMN, PS and TSN were 323.23%, 342.72%, 337.81% and 473.83%, respectively, of the initial dry weight of 8.5 mg. The corresponding growth increments in length were 52.81%, 55.92%, 53.07% and 80.7%, respectively, of the initial length of 17 mm. The diets supplemented with OCN, Orabolin and TRD, however, produced growth increment of only 276.87%, 274.59% and 278.52%, respectively, in dry weight and 36.48%, 37.62% and 38.45% in length. It can be seen from FIG. 1 that the supplementation of diets with AFA, GMN, PS and TSN resulted in clearly better growth than the growth obtained in the case of the control diet as well as with OCN, Orabolin and TRD, which were similar in result to that of the control. The food conversion ratios obtained by the diets supplemented with AFA, GMN, PS and TSN were 2.76, 2.05, 2.18 and 1.61, respectively, and were also superior to the food conversion ratio obtained in the case of the control diet (2.84). While the food conversion ratio obtained by the diet with OCN (2.75) was comparable to that of the control diet, the food conversion ratios obtained by the diets with Orabolin (4.30) and TRD (4.01) were far inferior.

The results of ANOVA and LSD (Table 4) showed that the growth and food conversion ratios obtained by the diets supplemented with AFA, GMN, PS and TSN were significantly different ($P < 0.05$) from the growth and food

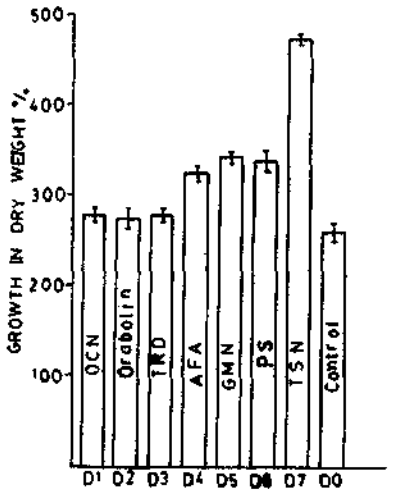

FIG. 1. Growth of prawns fed with diets supplemented with different growth promoting agents.

conversion ratio obtained by the control diet. There was significant difference ($P < 0.05$) between the growth obtained in the case of the diet with OCN and that obtained in the case of the control, whereas orabolin and TRD gave lower growth than the control. The rate of survival was the highest in the case of the diet supplemented with TSN, whereas it was not significantly different ($P < 0.05$) in the case of diets with AFA, GMN and PS compared to that of the control. Among the diets tested, the diet supplemented with TSN produced the highest growth both in length (80.76%) and in dry weight (473.83%) as well as the best food conversion ratio (1.61). The rate of survival (87.5%) was also the highest in this case. This was followed by GMN, PS and AFA in the order of their efficiency.

The body composition of animals before and after the feeding experiment is given in Table 5. The body protein of the animals fed with the diets containing OCN, Orabolin, TRD, AFA, TSN and control were higher than the protein content of the animals before the feeding experiment. The body protein of the animals fed with GMN and PS were slightly lower (51%) compared to the initial sample (53%). The body lipid in all the diet-fed animals was higher. But significant difference was found in the body carbohydrate of the animals before and after the feeding experiment. The prawns fed with diets having AFA and Orabolin had the highest protein content (64%) followed by the animals fed on diets supplemented with TSN (59%), OCN (57%), TRD (57%) and control (56%). The animals fed with TSN had the highest body lipid (17.82%) followed by those fed with GMN (17.20%), AFA (16.85%), OCN (16.48%), PS (16.42%), Orabolin (15.85%), TRD (15.85%), and the control (15.65%). The moisture content in the animals fed on the different diets was not significantly different from that of the initial sample.

TABLE 3. *Results of the feeding experiment conducted on juveniles of* P. indicus *for a period of* 28 *days.*

Description	D1	D2	D3	D4	D5	D6	D7	D0
No. of animals receiving the diet	24	24	24	24	24	24	24	24
Average initial length (mm)	16.79	16.46	16.60	16.83	16.63	16.63	16.58	16.92
Average initial dry weight (mg)	8.24	7.68	7.72	8.00	7.92	8.00	7.92	8.45
Final average length (mm)	22.92	22.65	23.98	25.72	25.92	25.45	29.98	23.44
Final average dry weight (mg)	31.07	28.79	29.24	34.25	35.06	35.00	45.44	31.15
Growth in length %	36.48^{a}	37.62^{a}	38.45^{a}	52.81^{b}	57.92^{b}	53.07^{b}	80.76^{c}	38.58^{a}
Growth in dry weight %	276.87^{b}	274.59^{a}	278.52^{a}	323.32^{c}	342.74^{d}	337.81^{d}	473.83^{e}	269.69^{b}
Food conversion ratio	2.75^{d}	4.30^{a}	4.01^{b}	2.76^{d}	2.05^{f}	2.18^{e}	1.61^{g}	2.84^{c}
Survival %	54.17^{a}	54.17^{a}	58.33^{a}	70.83^{b}	70.83^{b}	75.00^{b}	87.50^{c}	70.83^{b}

Means with different superscripts differ significantly among themseles ($P < 0.05$)

TABLE 4. *Results of Analysis of Variance and method of Least Significant Difference of the data of the feeding experiment.*

	ANOVA				LSD*
Source	D.F.	Sum of squares	Mean sum of squares	F (calculated)	
1. *Increase in length*					
Treatment	7	130.41	18.63	105.0014	0.7292
Error	16	2.84	0.18		
Total	23	133.25			
2. *Increase in dry weight*					
Treatment	7	598.71	85.53	82.0766	1.7670
Error	16	16.67	1.04		
Total	23	615.38			
3. *Food conversion ratio*					
Treatment	7	18.20	2.60	1520.5990	0.0716
Error	16	0.02	0.0017		
Total	23	18.22			
4. *Survival*					
Treatment	7	2786.46	398.07	6.7937	13.25
Error	16	937.50	58.39		
Total	23	3723.96			

* at $P = 0.05$

TABLE 5. *Body composition of the animals before and after the feeding experiment.*

Description	Moisture %	% on dry basis Protein	Carbohydrate	Lipid
Before feeding (initial)	75.84	53.00	2.97	12.05
After feeding with diet				
D1 OCN	71.89	57.00	2.97	16.48
D2 Orabolin	72.41	64.00	2.63	15.85
D3 TRD	71.96	57.00	2.83	15.85
D4 AFA	72.57	64.00	2.63	16.85
D5 GMN	72.00	51.00	2.83	17.20
D6 PS	72.04	51.00	2.97	16.42
D7 TSN	73.69	59.00	2.87	17.84
D0 Control	71.99	56.00	2.72	15.65

Discussion

In fin fishes like carps (*Cyprinus carpio*) and rohu (*Labeo rohita*) growth stimulation was achieved by supplementing the diets with antibiotics like oxytetracycline (Korneeva 1963 and 1965, Sukhoverkhov 1967), enterocycline, chloramphenicol and Hoestacycline (Sen and Chatterjee 1976). As for the prawns, Corliss et al (1977) obtained significantly higher growth and improved food conversion ratio in *Penaeus aztecus* by supplementing the diet with oxytetracycline at 100 to 1000 mg|kg diet. The average weight of prawns used in this study was 143.8 mg. Strangely, when the authors had fed the diets to prawns with an average weight of 457.1 mg, adverse effect was observed. In the present study, supplementation of diet with OCN at 100 mg|kg diet did not show any positive effect on *P. indicus* with an average weight of 17 mg. Maynard and Loosli (1969) pointed out that the response in weight gain due to antibiotic supplementation in animal diets had been varied according to the age of the animal, the antibiotic used and its dosage, type of food used and the nutrition of the receipient animal. These factors together or individually might have been responsible for not obtaining positive growth response in *P. indicus*.

Supplementation of the diet with Orabolin at 0.5 mg|100 g diet also did not result in enhancement of growth. The food conversion ratio obtained in this diet was high, compared to that of the control diet, indicating lower efficiency of food conversion. But the protein content of the animals after feeding the diets with Orabolin was the highest (64%) among the treatments, indicating that it resulted in higher protein synthesis and retention of nitrogen.

TRD, when added to the diet at 1.0 mg|100 g, too, had failed to induce faster growth and improve the food conversion ratio. The rate of survival was also low. Higgs et al (1977) had found good growth enhancement in cohosalmon (*Oncorhynchus kisutch*) fed on diets supplemented with T-3 fraction of the hormone. The failure of the hormone in the present study may be either because the dose tested was inadequate or because the TRD had had no anabolic function in prawns. Donaldson et al (1979), while reviewing the studies on the hormonal enhancement of growth in finfishes, has stated that the oral administration of TRD through the diet was not a successful means to promote growth in fishes.

Alfalfa extract when included in the diet at 2.0 ml|100 g level resulted in increased growth and improved food conversion ratio. It also resulted in increased body protein and lipid. Similar results where obtained with AFA by Rao et al (1983), in *P. indicus*. Stickney (1979), while discussing the potential feed ingradients for compounded diets for aquaculture organisms, pointed out that alfalfa meal is a potential feed stuff for this purpose.

Glucosamine, when supplemented in the diet at 0.8 g|100 g, produced positive growth enhancement, and also gave better food conversion ratio in *P. indicus*. Similar result was obtained by Kitabayashi et al (1971). However, Deshimaru and Kuroki (1974) later reported that GMN in the diet did not have any effect on the growth of *P. japonicus*, which is contrary to the results obtained in *P. indicus* in the present study.

The prawn shell preparation used in the study resulted in positive growth enhancement and in improved food conversion ratio. Kanazawa et al (1970) used chitin at 4% level in the formula diet for *P. japonicus*. There were a number of studies on the use of prawn-shell waste as useful ingredient for preparing feeds (Ahamad Ali 1982, Ahamad Ali and Mohammed 1982, Ahamad Ali and Sivadas 1983 and Venkataramaiah et al 1978). Kitabayashi et al (1971), when replaced GMN with crude chitin in the diet of *P. japonicus*, had failed to get growth enhancement, in contrast to the present results.

The supplementation of the diet with TSN at 2.5 g|100 g produced enhanced growth in *P. indicus*, improved the food conversion ratio and the rate of survival compared to that produced by the hormone-free diet. A number of natural and synthetic androgens have extensively been used in the diets of fin fishes and these have given growth enhancements of various proportions. Information on the use of steroid hormones in the diets of prawns is not available for comparison. Increased retention of nitrogen (protein) and higher lipid levels in the body were observed in fishes fed with diets containing androgenic hormones (Donaldson et al 1979 and Fagerlund and McBride 1975). The results obtained with *P. indicus* in the present study were similar to those observed in fishes in the case of body protein and lipid.

The results of the present study thus indicate that alfalfa extract, glucosamine, prawn shell and testosterone, when supplemented in the diet at 2.0 ml, 0.8 g, 0.8 g and 2.5 mg respectively per 100 g of the diet, enhance growth and improve food conversion ratio in *P. indicus*. Since these four substances are not indicated as dietary essentials, they may be considered as growth promoting agents for *P. indicus*. The results also indicate that oxytetracycline, Orabolin and thyroid have no visible growth promoting effect, at least at the levels used in the present study.

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