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Effect of Dietary Starch Levels on Digestive Enzyme Activity in *Penaeus indicus*

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Abstract

Purified diets containing graded levels of starch ranging from 0 to 40% were prepared and fed to the juveniles of *Penaeus indicus* for a period of 4 weeks. The relative activities of amylases, proteases and lipases were measured in the hepatopancreatic extract. Activity of a-amylase increased with the level of starch in the diet and the maximum substrate turnover of 43.97m mol /maltose/ml/min was observed at 20% starch in the diet. However, proteases and lipases did not show any significant variations in their activity patterns. The effect of diets on mean live weight gain, digestibility coefficient and survival of prawns were also evaluated. The d.c. value for carbohydrate was the highest (81%) for 20% starch diet. Diets with starch level between 10% and 30% also provided better growth and survival than the other diets. Hence suggest that juveniles of *P. indicus* can digest dietary starch upto a level of 20% and higher level results in lowered d.c.value.

Introduction

Several reviews (Van Weel, 1970; Gibson and Barker, 1979; Dall and Moriarty, 1983) dealing with the comparative physiology of digestion in Crustacea have been published but have contained very little quantitative information. Since crustaceans particularly the prawns, are now being widely used for commercial culture, change in the digestive enzyme activities during the life cycle, and adaptation to diets are now being examined quantitatively (Hoyle, 1973; Hood and Meyers, 1977; Laubier-Bonichon *et al.,* 1977; Trellu and Ceccaldi, 1977; Van wormhoudt *et al.,* 1982a) have reported significant increase in enzyme level with respect to increasing amount of dietary constituents. Inspite of these great contributions, only very little attention was received in the dietary studies and its effect on enzyme activity in Natantians especially of Penaeids. In culture operations, the efficacy of a feed in promoting maximum growth in prawns depends not only on its nutritive profile, but also on the animals inherent ability to consume, digest, absorb and metabolise the nutrients, which the feed contains. Thus an understanding of the digestive enzyme complement is extremely important in diet formulation. With the above objectives in mind the study was carried out with respect to graded levels of starch in purified diets, so that the information if relevant can be used for the formulation of practical diets in prawn culture operations.

Materials and Methods

Formulation and preparation of experimental diets

Nine different diets (Table 1), using purified ingredients were prepared according to the procedure given in CMFRI Special Publication No. 8 (1982). The proportions of starch and α - Cellulose were adjusted to maintain the total carbohydrate contents in the diet. Chromic oxide (0.5%) was added to the diet as an inert marker to study the digestibility Coefficient of starch, the only variable component in the diet.

Feeding experiments

Juveniles of *Penaeus indicus* mean length size (30 ± 5 mm) and mean weight 100 ± 10 mg were randomly selected and stocked in 50 L circular plastic troughs in sea water of salinity 17 ± 1 ppt. The animals were fed purified pelletted diet once daily (5% of body weight). The left over food and faecal matter were collected daily dried in oven and stored in desiccator until biochemical analysis were carried out. The duration of experiment was four weeks. Survival, growth and weight gain were recorded.

Enzyme extract preparation

All the operations were carried out in the cold (0°- 4°C). Ten animals from each treatment were picked up at random and dissected live over ice after recording their length and weight. Hepatopancreas were removed pooled and total weight noted. They were homogenized in cold distilled water and the tissue extract centrifuged at 16000 x g for 20 minutes at 0°- 4°C in refrigerated centrifuge. The resulting clear supernatant (1% concentration) was used to conduct the enzymatic analysis. Few drops of totuene were added as a preservative. Total protein content of extract was determined by Lowry *et. al.,,* (1951) using Bovine serum albumin as standard.

Enzyme assay

Amylase assay was carried out by Bernfeld (1955) method using soluble starch as the substrate and the activity expressed as total activity (μ mol maltose formed per ml of the enzyme extract per minute) and specific activity (μ mol maltose formed per mg of protein per minute) in the extract.

Protease assay

Conducted following Kunitz (1947) method using Casein as the substrate. Activity represented as total *i.e.* (μ mol tyrosine released per ml/min) and (μ mol tyrosine released per mg protein/min as specific activity). Lipase assay Carried out by titrimetric method of Cherry and Crandall (1932) using Olive

Table 1. Composition of experimental diets with varying levels of starch

	Ingredients	Diet Nos								
		1	2	3	4	5	6	7	8	9
1	Casein (Fat free)	35	35	35	35	35	35	35	35	35
2	α- Starch	0	5	10	15	20	25	30	35	40
3	Glucosamine Hcl	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	
4	Sodium citrate	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
5	Sodium succinate	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
6	Cholesterol	0.5	0.5	0.5	0.05	0.05	0.5	0.5	0.5	
7	Cold Liver Oil	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	
8	Vitamins[a]	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
9	Minerals[b]	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
10	Cellulose Powder	41.4	36.4	31.4	26.4	21.4	16.4	11.4	6.4	1.4

a Vitamins	mg	a Vitamins	mg	b Minerals	g
Thiamine HCl (B1)	4.9	Menadione	4.0	K_2HPo_4	2.0
Riboflavin (B2)	8.0	B-carotene	9.6	$Ca_3(PO_4)_2$	2.72
P.aminobenzoic acid	10.0	B-Tocopherol (Vitamin-B)	20.6	$NaH_2Po_4 2H_2O$	0.76
Biotin	0.4	Calciferol	1.2	$MgSo_4. 7H_2O$	3.02
Inositol	400.0	Cyanocobalamine (B12)	0.08	$MnSo_4 5H_2O$	0.004
Niacin	40.0	Na-ascorbate (Vit. C)	2000.0	$FeSo_4 7H_2O$	0.015
Ca. pantothenate	60.0	Folic acid	0.3		
Pyridoxine HCl	12.0	Cholene chloride	600.0		
Minadione	4.0				

oil as the substrate. Enzyme activity was represented as total, *i.e.* (μ mololeic acid formed per ml of extract/min) Specific activity as (μ mololeic acid formed per mg of protein/min).

Biochemical analysis of feed and faeces for Carbohydrate content and digestibility coefficient was performed according to procedures outlined in CMFRI Special Publication No. 8 (1982).

Data Analysis

All the experiments were replicated three times. Assays for each experiment were done in triplicates. Mean plus standard deviation were computed. Data were statistically analysed using analysis of Variance (ANOVA).

Results and Discussion

Hepatopancreatic amylase, protease and lipase activities on prawns fed the experimental diets are given in the Table 2. The activity pattern of amylase showed on increase with respect to the increasing level of starch in the diet, in prawns fed the control diet (Starch - free), the activity of amylase was highly insignificant. Thus it is very evident that, as the percentage of starch in the diet increased, the amylase showed a corresponding increase with the optimum at 20% level. Beyond this the activity showly declined attaining significantly ($P<<0.01$) low level at 40%. Proteases, however diet not show any significant variation in the activity pattern with reference to starch level in the diet. The values remained more or less steady without wide fluctuations (Table 2). The maximum recorded was between 15% to 30%. Starch in the diet; and optimum between 20% to 25%. Lipase activity was also found to be relatively steady without any significant variations.

The result of feeding the purified diet on weight gain and survival of prawns showed significant ($P<<0.01$) results on animals fed 20% starch diet, and survival was 93%. Again the digestibility coefficient data also showed highest value of 81% for Carbohydrates in experrimental diet (20% starch).

Hoyle (1973) who studied the digestive enzyme secretion associated with dietary variation in the lobster *Homarus americanus* reported that on feeding 0.5 and 20% starch diets for periods upto 37 days, the specific activity as well as the absolute amounts of proteinase, amylase, and lipases increased, when compared to the base line or true fasting enzyme level. He attributed the increase in enzyme activity for two reasons. (1) Supply of necessary amino acids. (2) Induction of enzyme production of specific components or digestion products of the diet. Such kind of adaptation of enzyme secretion with changes in the dietary components are well evident in fishes too. However there are only few studies in crustaceans. Van Wormhoudt and Sellos (1980) and Maugle, *et al.*, (1982) in *Palaemon serratus* and *Penaeus japonicus* noted that some nutritional variables did influence the enzyme activity. Since the above studies were conducted feeding the animal using natural diet, the components in the diet responsible for stimulation of enzyme activity could not be identified.

The information generated from the present study clearly establishes that dietary starch level in feed had significant influence on secretion of amylase: and had no noticeable effect on proteases or lipases. This may be expected as the diets were isoproteic and isolipidic, with starch being the variable. Since diets giving rise to the most enzymatic activities have the highest nutritive value, and monitoring enzymatic activities can be a good means of checking the physiological state of the culture animals, it may be suggested that, optimum level of the nutrients may be incorporated in the diet, to get optimum - enzyme turn - over in future culture systems.

Table II: Digestibility of Dietary carbohydrates, Weight gain, Survival, Digestibility coefficient value, Total protein content and Enzyme activities on *Penaceus indicus* fed on experimental diets.

Diet No.	Stach Level %	Carbohydrate content (%)	Weight gain (%)	Survival (%)	Digestibility Coefficient of Carbohydrate (%)	Protein content in the extract mg/ml	Amylase T.A. - (S.A)	Protease	Lipase
1	0	12.24	6.08	86.6	24.12	8.61±2.6	5.13±5.82 (0,66±0.03)	201.41±1.22 (23.39±2.65)	3.93±0.07 (0.456±0.88)
2	5	16.75	4.35	83.3	35.90	7.31±2.4	25.18±2.68 (3.44±4.2)	205.27±2.19 (28.08±3.07)	3.96±0.05 (0.541±0.72)
3	10	15.42	5.22	76.6	65.29	5.44±3.6	40.42±5.41 (7.43±3.6)	204.58±2.30 (37.60±6.61)	4.04±0.01 (0.742±0.64)
4	15	15.18	2.61	90.0	81.00	5.65±4.2	38.00±12.96 (6.73±2.4)	208.19±1.05 (36.83±1.18)	4.16±0.05 (0.736±0.28)
5	20	16.0	25.22	93.5	87.73	7.73±3.4	43.97—10.02 (5.61±1.8)	209.57±1.22 (27.11±2.54)	4.26±0.08 (0.55±0.09)
6	25	19.5	20.87	83.5	68.36	6.89±4.5	24.40±2.60 (3.54±2.8)	210.22±1.06 (30.51±1.08)	4.15±0.06 (0.60±0.02)
7	30	18.8	14.78	90.0	50.45	8.12±2.6	25.28±4.2 (3.13±3.0)	208.11±2.01 (25.62±3.18)	4.16±0.05 (0.51±0.23)
8	35	15.0	11.30	86.6	52.49	6.85±3.4	21.01±4.60 (3.06±2.0)	206.97±1.62 (30.21±4.59)	4.14±0.06 (0.60±0.43)
9	40	19.30	18.26	90.0	38.57	6.29±4.6	18.36±4.13 (2.92±1.08)	204.58±1.26 (32.52±2.46)	4.09±0.07 (0,65±0.24)

T.A. Total enzyme activity - μ mol product formed per ml of extract per min.
S.A. Specific enzyme activity - μ mol product formed per mg of protein per min.

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