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PRELIMINARY STUDIES ON THE PREPARATION AND FEEDING OF CRUSTACEAN DIETS*

GEORGE H. BALAZS

Hawaii Institute of Marine Biology, University of Hawaii, Kaneohe, Hawaii, 96744 (U.S.A.)

ERNEST ROSS and COY C. BROOKS

Department of Animal Sciences, University of Hawaii, Honolulu, Hawaii 96822 (U.S.A.)

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An inexpensive, simple and effective method is described for preparing water-stable diets suitable for aquatic crustacean feeding experiments. Seven representative diets ranging from 25 to 40% total protein were prepared and tested for their dry weight loss in water for 1-, 3- and 5-hour periods. Dissolution was influenced by diet composition, and loss was greater in fresh water than in sea water. Diets were subsequently fed to both marine shrimp (*Penaeus* sp.) and fresh water Malaysian prawns (*Macrobrachium rosenbergii*) held under experimental conditions. Results of these trials showed that diets were capable of producing a 106–329% increase in weight in juvenile Penaeid shrimp over a 25-day period. Growth appeared to increase with amount of protein in the diet. Juvenile Malaysian prawns held under mass culture conditions for 60 days increased in length from 98 to 112% on three different diets. Juvenile *Penaeus japonicus* held under mass culture conditions for 75 days increased in weight 1877% on a 35% protein diet. In general, all prepared diets produced good growth with low mortality when compared with data reported by other workers.

INTRODUCTION

The development of experimental diets for crustaceans is complicated by the need to bind the feed ingredients into a water-stable form. This is

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true particularly if conventional feedstuffs are to be utilized. With the increased interest in aquaculture, especially in intensive shrimp farming, knowledge of how to bind feeds to prevent disintegration in water has become increasingly important. Standard pellets manufactured for domestic animal and fish production disintegrate rapidly when placed in water, thus fouling the medium and rendering nutrients less available. For juvenile and adult shrimps and crabs, feed particles need to be bound tightly enough to prevent undue wastage during the mastication process, and to ensure that ingestion of a diet can continue for at least several hours after introduction. In addition, it is highly desirable to be able to store animal rations without refrigeration, which is the major objection to a moist form of pellet.

Ingredients that have been tested for their binding ability include agar, alginates, carageenan, guar and locust bean gums, gelatins, celluloses, and various combinations of agents manufactured under brand names. Some success has been obtained with each of these additives when incorporated at the proper level in a ration. Although these additives are suitable for small batches, the large scale production of experimental diets bound with any of the above-mentioned additives is limited by: (1) costs, (2) availability, and (3) machinery which can be readily utilized in the manufacturing process.

In developing test diets for the fresh water Malaysian prawn (*Macrobrachium rosenbergii*) and marine shrimps (*Penaeus* sp.), the Hawaii Institute of Marine Biology has given priority to the binding of ingredients into a water-stable form by exploiting the binding properties of natural feedstuffs themselves. Both Hastings *et al.* (1971) and Szumiec (1969) have reported good levels of water stability in processed pellets containing only natural feedstuffs. Such techniques currently offer inexpensive alternatives to the use of exotic binding additives.

This paper describes a method of preparing experimental diets for aquatic crustacea. In addition, the paper presents the results of preliminary feeding studies which were carried out under several environmental conditions to evaluate the growth-promoting abilities of the diets.

MATERIALS AND METHODS

Feed preparation

Representative experimental crustacean diets of differing protein levels and sources are listed in Table 1. The ground ingredients were first weighed out according to formula and thoroughly mixed in a dry feed mixer. All formulas contained 20% high-gluten durum wheat (*Triticum*

TABLE 1

Percent composition of prepared crustacean diets

Ingredient	% Protein	Diet						
		1	2	3	4	5	6	7
Soybean meal	46	35.5	8.0	8.0	21.0	29.0	67.6	8.0
Hawaiian fish meal	57	–	7.0	9.5	20.3	29.0	–	18.0
Shrimp meal	45	–	18.5	29.0	20.0	–	–	45.0
Brewer's yeast	43	5.0	5.0	5.0	–	–	–	5.0
Corn, ground	9	36.5	40.5	27.5	17.3	20.6	9.0	13.0
Wheat flour, high gluten	16	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Tricalcium phosphate		2.0	–	–	–	–	2.0	–
Iodized salt		–	–	–	0.4	0.4	0.4	–
Microingredient mix*		1.0	1.0	1.0	1.0	1.0	1.0	1.0
% Protein, calculated		25	25	30	35	35	35	40

*Microingredient mix provided the following in mg or international units/Kg: vitamin A, 8,818 IU; vitamin D_3, 2,205 ICU; vitamin E, 8.3 IU; riboflavin, 4.4; *d*-Ca pantothenate, 8.1; niacin, 33.1; choline chloride, 440.9; thiamine, 2.2; folic acid, 0.33; vitamin B_{12}, 0.011; butylated hydroxytoluene (BHT) 125; menadione sodium bisulfite, 2.2; pyridoxine, 10; inositol, 100; biotin, 1; ascorbic acid, 2.2; *p*-amino benzoic acid (PABA) 100; Mn, 60; I, 1.2; Fe, 19.8; Cu, 2; Co, 0.2; and Zn, 44.1.

durum) flour. Water (30–40%, by weight) was added to the dry mixture. The exact quantity was dependent upon the particular combination of ingredients in the formula; e.g. diet 6 used 40% and diet 7, 30%. The mass was thoroughly kneaded to wet all particles and to form a stiff dough. Kneading was readily accomplished by passing the material two or more times through a heavy-duty meat chopper fitted with a die having large (10-mm) holes.

The resultant dough was shaped into final form by extrusion, with the same machine, through a die with small holes. Diets in this study were prepared using a die 17 mm thick with 3-mm diameter holes, although larger diameter holes could also have been suitable. As the spaghetti-like strands formed, they were easily spread out on flat trays. When correct water additions were made, extruded material was stacked as deep as 3 cm without sticking together. Extruded material was subsequently dried at 80°C for 10 h in a forced air oven and stored in heavy-duty plastic bags.

Water stability tests

All prepared diets were tested for stability in both sea water and fresh water by placing triplicate 10-g samples in a 270-liter flow-through aquarium which measured 0.6 m by 1.5 m by 0.3 m deep. Flow rate was maintained at 6 liters per min; sea water temperature was 27°C and fresh water, 25°C. Samples were placed in the aquarium for 1- ,3- and 5-hour periods, respectively. After soaking, each sample was drained, redried at 80°C for 10 h and reweighed. The difference between the initial and final weight was expressed as the percent dry weight loss. This technique is similar to that developed by Hastings (1964) and used by other workers for measuring the dry weight loss of aquatic diets in water.

Laboratory feeding studies

Controlled laboratory feeding experiments were conducted using 730-liter fiberglass aquaria measuring 0.7 m by 1.3 m by 0.8 m deep with a flow rate of 6 liters per min. Tank bottoms were left bare. Sea water averaging 27°C was passed through a 5-micron cartridge filter (Aquapure No. P110, Cuno Engineering Corp., Meridian, Conn., U.S.A.) before entering each tank; filter elements were changed and tanks cleaned daily. Illumination was adjusted to 10 h of light per day and moderate aeration was provided. Animals were fed the assigned diet morning and evening at a rate that ensured an excess of food present in the tank at all times. Uneaten food was completely removed from each tank just prior to feeding.

One hundred and fifty five juvenile *Penaeus aztecus* averaging 0.58 g each were held in one tank under the described condtions and fed diet 2 (Table 1). In addition, four groups of 15 juvenile *Penaeus japonicus* with average weights of 2.12, 2.27, 1.66 and 1.50 g, respectively, were each placed in adjacent tanks and randomly assigned either diet 1, 2, 3 or 7. These studies lasted 25 days.

Mass culture feeding studies

The prepared diets were examined under pilot-scale commercial conditions. Approximately 5000 juvenile *Penaeus japonicus* averaging 0.40 g were stocked in a 195 000-liter circular rubber-lined tank which measured 5.4 m in diameter and 1.2 m deep. A flow rate of 290 liters of sea water per min was maintained throughout a 75-day experimental period. Heavy aeration was provided from four perforated plastic pipes located on the bare tank bottom. Diet 4 was fed twice daily for the first 3 weeks and once daily, in the evening, for the remainder of the study. An excess of food was present on the tank bottom at all times. Triplicate random samples of not less than 20 shrimp each were taken for weekly weighings.

In another study three adjacent rectangular 117 000-liter freshwater tanks, each measuring 1.8 m by 9.1 m by 0.9 m deep, were respectively stocked with 14 200, 9 200 and 9 200 postlarval *Macrobrachium rosenbergii*. Animals averaged 9.3 mm in length (eye socket to tip of telson). Diets 4, 5 or 6 were assigned randomly to the tanks, and were fed in excess once daily in the evening. Water was changed as necessary, usually once a week. Moderate aeration as well as partial shade were provided for each tank. Increase in length was used as a measure of growth, and samples of 100 animals were measured at the middle and end of the 60-day experimental period.

RESULTS AND DISCUSSION

Preparation of feed by this technique was simple and rapid. The dry mixture could be readily prepared in large quantity, although preparation of the dough stage was limited by the capacity of the equipment available. When dry, the finished particles were approximately 2.5 mm in diameter and tended to break into sections which averaged 250 to 500 mm in length. This size and shape proved satisfactory for the animals used in the study.

Results of soaking tests conducted on the prepared diets (Table 2) show that binding efficiency is affected by diet composition. Stability in sea water was consistently better than in fresh water; nutrient dissolution from each particle is apparently slower because of the higher ionic concentration of sea water. Diets which contain brewer's yeast displayed better water stability when compared with the other diets. At the end of the 5-h tests all particles were still firm, retained their original shape and could be handled without falling apart. Loss of specific nutrients was not

TABLE 2

Dry matter weight loss (%) of prepared crustacean diets in sea water and fresh water

Diet	1 hour		3 hours		5 hours	
	Sea*	Fresh**	Sea	Fresh	Sea	Fresh
1	6.7	10.7	9.8	11.6	11.3	12.4
2	6.4	11.9	8.2	11.9	8.4	12.3
3	5.9	13.1	9.5	13.2	11.4	13.4
4	11.4	12.6	13.0	17.0	15.0	18.8
5	8.8	11.4	11.0	15.8	12.7	16.6
6	8.6	12.5	13.4	18.4	16.4	22.1
7	5.8	14.3	9.1	16.3	10.5	16.6

*27°C flowing at 6 liters per min.
**25°C flowing at 6 liters per min.

measured. In processed corn and sorghum grain, Hastings and Muller (1961) found percent loss of soluble protein to be less than that of soluble starch and reducing sugars. The weight losses listed in Table 2 were considered acceptable for aquatic crustacean feeding. Under intensive farming conditions it may be advantageous to feed growing shrimps using automatic feeders every 3–4 h, making a longer diet stability time unnecessary. In fact, the present cost of producing a more stable particle may well be uneconomical in terms of the benefits derived.

Several factors in the preparation procedure contributed to the stability of the food. The addition of 20% of high-gluten wheat flour to the blended feedstuffs of the diet aids in dough formation when water is added. The superior binding characteristics of durum wheat flour are well recognized in the manufacture of dried paste products such as macaroni. The high percentage of gluten protein in this wheat acts as an adhesive, and is in part responsible for the ability of dried paste products to retain their shape and integrity after extended periods in water. Gluten protein acts to form a matrix which holds other constituents. In addition to the action of this protein, the flour contains a large exposed surface area of endosperm starch which is readily subjected to gelatinization, along with other starch present in the diet, under the heat and abrasion created in the die. Gelatinized starch also acts to bind feed particles together (Pomeranz, 1964; Frey and Holliger, 1972).

When the dough material is processed through the meat chopper, high pressures are produced immediately behind the die. Pressure in this area is inversely proportional to the die hole size and the amount of water in the mixture. Too much water added during preparation decreases this pressure and decreases the heat, abrasion, and extent of gelatinization. In addition, the area occupied by any excess water will be exposed after drying, producing gaps or holes in the food through which water can enter when the material is immersed in water. Dough containing an insufficient amount of water will lack cohesion, and insufficient wetting of starch particles will take place for adequate gelatinization to occur. When the correct conditions for extrusion had been established, dense, highly compacted particles of food were produced in which a high percentage of available starch had been gelatinized. Subsequent baking in the drying oven caused further gelatinization, and removed enough moisture so that the material could be stored without refrigeration.

Results of feeding studies (Table 3) show that all prepared diets produced good growth with low mortality when compared with findings of other workers conducting similar studies (Forster and Beard, 1969; Sick *et al.* 1972; Deshimaru and Shigeno, 1972).

The 25% protein diet (2) produced the same rate of growth (107%) in

TABLE 3

Summary of feeding studies using prepared crustacean diets

Species	Diet	No. of Animals	Experimental period (days)	Mean weight (g) Initial	Final	Increase (%)	Survival (%)
Laboratory controlled							
Penaeus aztecus	2	155	25	0.58	1.20	107.0	95.5
Penaeus japonicus	1	15	25	2.25	4.63	105.8	93.3
	2	15	25	2.27	4.70	107.0	86.7
	3	15	25	1.66	5.62	238.6	93.3
	7	15	25	1.50	6.44	329.3	100.0
Mass culture							
Penaeus japonicus	4	5 000*	75	0.40	7.91	1877.5	N.D.
				Mean length (mm)			
Macrobrachium rosenbergii	4	14 200*	60	9.3	19.7	111.8	N.D.
	5	9 200*	60	9.3	17.9	92.5	N.D.
	6	9 200*	60	9.3	18.4	97.8	N.D.

*Numbers estimated from initial total weight of animals.
N.D. = not determined.

the two species of *Penaeus* to which it was fed, even though one tank contained 10 times the number of animals and represented close to 2.5 times the stocking density (90 g/m^2 *vs* 37 g/m^2). The all-vegetable 25% protein diet (1) promoted growth almost as well as diet 2 which contained two animal sources of protein. In these controlled laboratory studies, growth rate appeared to increase as the protein content in the diet increased. The slightly lower initial mean weight of *Penaeus japonicus* on diets 3 and 7 (Table 3), compared with those receiving diets 1 and 2, may have been partly responsible for the greater percent increase obtained. These data suggest that the optimum protein level may exceed 40% for young shrimp. None of the diets used by Deshimaru and Shigeno (1972) contained less than 60% protein.

Diet 4 containing 35% protein produced good growth in *Penaeus japonicus* under mass culture conditions. However, under these conditions some nutrients may also have been derived from algae, zooplankton and detritus, since water entering the tank was not filtered. An accurate estimate of percent survival could not be made in this study; several times during the course of the experiment malfunctions caused water to overflow, and an unknown number of animals were lost.

The experiments with *Macrobrachium rosenbergii* showed that the all-vegetable diet (6) gave results somewhat superior to diet 5 (fish-soy protein) but inferior to the 3-protein source (fish-soy-shrimp, diet 4). Some nutrients also may have been obtained from algae in this study. No mortality were observed during the experimental period.

Penaeid shrimp and the Malaysian prawn are exceptionally promising species for aquaculture, and large scale nutrition experiments will continue with diets prepared by the method described.

SUMMARY AND CONCLUSIONS

Seven crustacean diets of differing composition were prepared by blending each with 20% high-gluten wheat flour, adding water, and kneading the material into a stiff dough. This dough was subsequently extruded through a die having small holes, using a commercial meat chopper. Formed particles were then oven dried at 80°C and stored in plastic bags. Diets were tested for their dry weight loss over a 5-h period in both sea water and fresh water. Feeding studies were conducted using both marine and freshwater crustacea to evaluate the growth-promoting abilities of diets prepared in this manner.

Under the conditions of this experiment, the following conclusions appear justified:

(1) The feed preparation method described offers a simple, rapid and relatively inexpensive technique for producing experimental crustacean diets with acceptable levels of water stability.

(2) Diets prepared by this method are capable of producing good growth in penaeid shrimp and Malaysian prawns reared under both controlled laboratory and mass culture conditions for short periods of time.

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