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MICROPARTICULATED AND MICROENCAPSULATED DIETS FOR FEEDING PRAWN AND BIVALVE LARVAE

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Food is normally the largest single item in the running expenditure in fish and shellfish farming. Hence, the suitability and cost effectiveness of the ration is of paramount importance to commercial success. Many different types of feed may require development to meet the varied needs of different species and size of larvae. Palatability and physical structure of shrimp and bivalve ration are inter-related. Both factors alone and in conjucation affect ingestion and have an important impact on prawn and bivalve nutrition. Microencapsulated diet for larval and postlarval diets have been advocated by Meyers (1973). Gelatin microencapsulated diets suitable in sea water. Until the development of microparticulated diets the recent years the study of larval nutrition was impossible. This was mainly because the larvae were semi microscopic whereas the feeds need to be microscopic and water resistant. The development of med made offering of liquid and solid nutrients in pure form made a possibility. This serve as a tool in nutritional research but also an effective way of feeding the larvae with nutrient rich food. Knowledge of the preparation of about in this form is sure to help in our efforts in developing prawn and bivalve hatcheries in India.

Teshima <u>et al</u>. (1981) have attempted to culture the Rotifers with microencapsulated diets and found to be suitable using the Nylon protein diets. However a prolongered culture of rotifers with microencapsulated diets resulted in a decreased population. Kanazawa <u>et al</u>. (1982) succeeded in rearing of prawn larvae (<u>P. japonicus</u>) using microparticulated diets. Kandasami <u>et al</u>. (1987, in Press) have studied the use of gelatin coated microencapsulated diet for the rearing of bivalve larvae successfully.

I. MICROENCAPSULATED DIETS

(1) <u>Procedure for the preparation of Nylon-Protein</u> microencapsulated diet:

- To the suspension of the artificial diet (2.5 ml),
 1.5 ml of diamine solution (0.92g/10 ml) was added and mixed with the hand.
- The mixture of diamine solution and artificial diet suspension was added dropwise to the mixed solution of 25 ml of cyclohexane and 0.5 ml of Span 85, homogenizing continuously.
- 3. Homogenizing continuously, the mixture of 10 ml of cyclohexane and 0.2 ml of sebacoyl chloride was added at a time and thus further homogenized for 15 minutes.

Reaction: Sebacoyl chloride + Diamine Nylon + Hcl

C1C0 (CH2)8 COC1 + NH2 (CH2) NH2

- CO (CH2)8 CO - NH - (CH2)6 - + HCl

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- 4. After that 30 ml. of cyclohexane was added to the reaction product and allowed to stand for 30 60 min.
- 5. Washing of the microencapsulated diets.
 - i) Remove the supernatant by decantation. Repeat with 100 ml of cyclohexan.
 - ii) Add Sucrose monolawrate (5-7 ml) and agitate by using a magnetic stirrer for 3 hours to expell the cyclohexane.
 - 111) Pour into 2 lit. water and agitate to overnight and then the capsules were collected by centrifugation (at 3700 rpm for 10 minutes).
 - 6. Storage:

The microencapsulated diet can be stored in 1.0 mol Nacl in a refrigerator (4-5°C).

- (2) <u>Procedure for the preparation of Gelatin/acacia</u> <u>capsulated diets</u> (Green and Schleicher, 1957).
 - 1. To one ml of lipid solution add 40 ml of 2% (W/v) at 40°C kept under nitrogen atmosphere in dark.
 - 2. Homogenize for 2 minutes at maximum speed (14,000 rpm) and transferred to 500 ml flask of 3 necked.
 - 3. Reduce the speed to 500 rpm for 1 minute and adjust the pH to 3.9 by the addition of 0.01 M.HCl dropwise which cause the coacervation to the walls of the capsules.
 - 4. Reduce the speed to 100 rpm and allowed to stand for 40 minutes at 40°C.
 - 5. Rise to pH to 9.3 by the slow addition of 1. M.NaOH and transferred to 300 ml water at 5°C. The content was stored in the fridge for one hour to harden the capsules.

- 6. Centrifuged in a refrigerated centrifuge at 3700 rpm at 10°C for 10 minutes washed repeatedly to remove the excess gelatin solution.
- 7. Autoclaved at 115°C for 15 minutes and stored in Nitrogen for further use.
- Procedure for the preparation of Ethyl-cellulose
 <u>capsules</u> (V. Rancken and Claeys, 1970).
 - 1. To 20 ml of 5% (W/V) ethyl cellulose in a diet solution. (degree of substitution 2.42 2.53) in dichloromethane to a 500 ml round bottom flask at 0-4 °C.
 - 2. 13 ml of 20% (W/V) aquous solution of dextrin slowly added with constant stirring at 1000 rpm in a paddle shaped stirrer.
 - 3. After 2 min reduce the speed to 250 rpm and stirr further for 13 minutes.
 - Gradually poured in to a flask containing 100 ml polyvinyl alcohol at 5°C and stirred to 1000 rpm for 1 minute.
 - 5. Reduce the speed to 250 rpm for 10 minutes.
 - Dichloromethane is removed under vacuum at 35°C for 3 hours.
 - 7. Polyvinyl alcohol removed and washed with distilled water and stored in the fridge for further use.

II. MICROPARTICULATED DIETS

(1). <u>Procedure for the preparation of Carregeenan</u> microbinding diet:

Diet ingredients were weighed and mixed well, with 25 ml of water (10 gms diet). The whole mixture was placed in a water bath at 80°C, carregeenan (5 gm. for 100 gm. diet) was added slowly with constant mixing. Then Potassium chloride was added (5 gm. for 100 gm) slowly with constant mixing. The whole diet was cooled in a refrigerator for 30 mts. The solid diet was cut into small pieces and a bit was tested for the binding effect. The diet was freeze dried and made into powder. The powder was sieved to required sized particles and the same stored in refrigerator.

(2) Preparation of agar-gelatin microbouned diet:

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To 10.0 gm of diet ingredients 7% water mix, 0.3 gm agar and 1.2 gm gelatin were added. The whole . mixture was mixed well and heated over a water bath at 80°C for 5 mts. cooled to room temperature and freeze dried. The dried diet was made into powder and sieved to adequate size. The particles were stored until further use.

III. MICRO-COATED DIETS

(1) Preparation of Zein microcoated diet:

10 gm of the diet ingredient was taken and 25 ml zein solution was added. (1.0 gm zein dissolved in 25 ml of 60% EtoH). The whole mixture was mixed well and the diet freeze dried. The dried diet was made into powder and sieved, to adequate size and stored in a refrigerator for further use. and the second states of the

(2) Preparation of Cholesterol-lecithin microcoated diet:

The diet ingredients were weighed out into a beaker and mixed well. The wet mixture was freeze dried and sieved to adequate size. The particles were coated with Cholesterol-lecithin using 10 ml cyclohexane mixture (0.08 gm. cholesterol, 0.16 gm soybean lecithin and 10.0 ml cyclohexane) for 10 g diet. The cyclobexane was dried over nitrogen gas and further dried in

a vacuum desiccator for 8 to 12 hours. The particles were stored in a freezer for further use. REFERENCES

Jones, D.A., A. Kanazawa and K. Ono 1979. Studies on the Nutritional Requirements of the Larval Stages of <u>Penaeus japonicus</u> using Microencapsulated diets. <u>Mar. Biol., 54</u>: 261-267.

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Kanazawa, A., S. Teshima, H. Sasada and S. Abdul Rahman 1982. Culture of the prawn larvae with microparticulate diets. <u>Bull. Jap. Soc. Sci. Fish.</u>, <u>48</u>(2): 195-199.

Kandasami, D. and P. Muthiah 1987. Use of gelatin coated micro-encapsulated diet as supplemental diet for rearing of larvae and spat of oyster <u>C. madrasensis</u>. National Seminar on Shellfish resources and farming held at Tuticorin, 19-21 January, 1987 (in press).

Kandasami, D. and S. Dharmaraj 1987. Effect of microencapsulated diet for the growth of pearl oyster (<u>Pinctada fucata</u>) larvae and spat as a supplemental diet, <u>Ibid.</u>, 1987 (in press)

Teshima, S., A. Kanazawa, N. Kamazawa and H. Hirata 1981. Fatty acid and sterol components of rotifer culture by a feed back system. <u>Bull. Jap. Soc. Sci. Fish.</u>, <u>47</u>(4): 515-522.

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