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Growth factors in Penaeid shrimp feeding

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Abstract. — *Some nutrients known as non essential in vertebrates could be essential in invertebrates and could be related to some « unknown growth factors » which were evidenced or suspected, mainly in mollusc or crustacean flesh or meal, by several authors working on different species of crustaceans. Since 1982 a set of experiments was carried out in our laboratory in order to isolate one of these factors found in squid meal. In all these trials, meal (prepared from fresh lyophilised squid) or extracts were tested in isoproteic and isolipidic casein based semi-purified diets. The first experiments showed that a growth enhancement amounting to 30 to 50 % per month in* ***Penaeus japonicus*** *factors (**sensu stricto**) by stimulating cell hypertrophy and not hyperplasy. The postprandial level of glucose and aminoacids in haemolymph was found to be increased by the « squid factor ». This effect is not explainable by a better digestion of feed, since no clear effect was found on digestive enzymes activities; furthermore no effect was found on the absorption of glucose or aminoacids neither* ***in vitro*** *nor* ***in vivo****. Therefore the action of squid factor seems to be related to metabolic phenomena and remains to be elucidated. The practical interest of this factor and that of feedstuffs containing possibly similar factors is discussed.*

INTRODUCTION

It is well known that the first authors who studied the nutrition of penaeid shrimp using mixed diets, i.e. Drs Deshimaru and Kanazawa took pattern by the knowledge in fish (Chinook salmon), insect (silk worm),and artemia nutrition. This very clever step allowed fast success in many fields. The good efficiency of shrimp semi-purified diets now used in many laboratories demonstrates that the essential nutrients taken into account in feed formulation fit well those actually required by shrimp. However, fewer things are known concerning so called « unknown growth factors » that can be found for example in fish meal, fish solubles or distillery by

products; their activity was partly explained by inorganic elements supply, balance of nutrients and positive influence on feed intake (Alenier and Combs, 1981). In shrimp feed the existence of such growth promoting factors remains an open question : natural food and mixed diets made from non purified feedstuffs seem to be more efficient than semi purified diets. This higher feeding value may be related to higher palatibility, better balance or availability of nutrients, influence on gut microflora, etc but, like in upper vertebrates unknown growth factors could also be implied.

The existence of such factors was suspected by FOSTER and Beard (1973) in mussel fed to *Palaemon serratus*; Fernandez and Puchal (1979) working in *Penaeus keraturus* made a similar hypothesis. In *P. japonicus* Cuzon *et al.*(1981) supposed the existence of an unknown growth factor in spirulina and lactic yeast; however, like previous authors, they did not conduct any experiments to demonstrate that the growth promoting effect observed was not attributable to any known nutrient or to balance between some nutrients. A beautiful demonstration of the role on organic compound in the growth of a crustacean was made in *Artemia salina*, of the Utah strain which cannot synthesize purines (Hernandorena, 1987). However there is no evidence of such a requirement in upper crustaceans, though some authors use nucleic acids as possible growth promoters in *P. japonicus* semi purified diets.

THE SQUID FACTOR

— The high feeding value of squid meal

Japanese researchers were first to demonstrate the high feeding value of squid meal or squid by products in *P. japonicus* (Kitabayashi *et al.* 1971; Deshimaru and Shigeno, 1972); this result was corroborated by Faranda et al. (1983). Later on similar reports were done in other species of penaeids such as *P. aztecus* (Fenucci and Zein-Eldin, 1976), *P. setiferus* (Fenucci et al., 1980) or *P. monodon* (LIM *et al.*, 1979).

Comparing the efficiency of different mixed diets tested in our laboratory, the superiority of feeds containing squid meal was evidenced (HEW, 1983). As a first hypothesis the amino acid balance and especially the lysine/arginine ratio was supposed to be responsible for the high nutritional value of this protein source. But, the effect of lysine/arginine ratio was found to be lightly pronounced (Hew and Cuzon, 1982, HEW, 1983).

— Evidence of a « growth factor » or of a « squid factor » (Cruz and Guillaume, 1983)

In the preliminary experiment whole squid was ground and divided into 3 fractions using a process deriving from Bligh and Dyer (1959) lipid extraction method slightly modified by us. This procedure more or less similar to that of Deshimaru's one (1982) led to lipids, proteins and hydroalcoolosoluble extract (Hae).These fractions were added two by two

or all together in isoproteic isolipidic diets well fortified in minerals and vitamins and other known nutrients. The omission of one out of the three components had variable effects on the growth of shrimp :

- the omission of squid lipid, replaced by cod liver oil, had no effect,
- the omission of HAE reduced food intake without changing growth, improving therefore food efficiency,
- the omission of squid protein replaced by fish protein had a very pronounced growth depressing effect without influencing food intake.

Tab. 1. — Amino acid composition of squid meal protein, squid protein fraction and COMNAR fish meal protein, the possibility first limiting factor of fish protein is underlined.

Amino acids	Squid meal	Squid protein	Fish meal
Taurine	3.17	0.96	—
A. Aspartique	6.07	8.13	8,18
Threonine	3.48	4.65	4.49
Serine	3.17	3.80	4.28
A. Glutamique	9.30	11.45	13.63
Proline	5.33	4.69	3.91
Glycine	4.01	4.49	4.70
Alanine	3.54	3.96	5.02
Valine	2.96	4.60	4.60
Isoleucine	3.21	5.14	3.95
Leucine	5.28	7.28	7.39
Tyrosine	1.80	2.59	2.57
Phenylalanine	2.64	3.69	3.56
Lysine	4.70	6.21	7.05
Histidine	1.20	1.61	1.82
Arginine	4.68	5.99	5.77
Orinithine	0.37	—	—
TOTAL	64.91	79.24	80.92
Protein (NX6.25)	75.30	89.11	88.93

This trial was replicated and brought evidence that the high nutritional value of squid meal was due neither to lipids (phospholipids cholesterol or highly unsaturated fatty acids) nor to food attractants. Since the fraction responsible for growth enhancement was the protein fraction, attempts were made to explain its effects through its content in essential amino acids.Analyses of amino acids were performed on squid and squid fractions and chemical indexes according to Mitchell were calculated. But taking into account the most deficient amino acid in the basal diet, i.e. isoleucine, supposing it was actually limiting, comparing it to its level in the squid supplemented diet (Table 1), only a small part of the growth difference was explained. In our condition, in opposition to those of Deshimaru (1982), the amino acid balance was not the cause of the high feeding value of squid protein. Therefore the hypothesis of an unknown factor was formulated.

— Study of the squid factor in penaeids

— *General material and methods*

All studies on the squid factor were carried out in laboratory conditions. According to experiments three kinds of basal diets were used : one regime was based on casein gelatin, cod liver and purified micronutrients and called « semi purified diet »; the other regimes, called « classical basal diets » (one for *P. japonicus* and one for tropical species) contained various usual feedstuffs. All substitutions were made in isoproteic and isolipidic diets.

Squid meal or extracts were prepared from freshly caught squid (*Loligo* sp.) from Brittany (West of France) with one exception where the experiment was carried out with frozen *Nototodarus sloani* from New Zealand.

All shrimp juveniles arised from IFREMER hatcheries and weighed less than 1 g to 4-5 g. All experiments lasted 4 weeks. The environmental conditions, number of shrimp and experimental procedures were described in our preceeding publication.

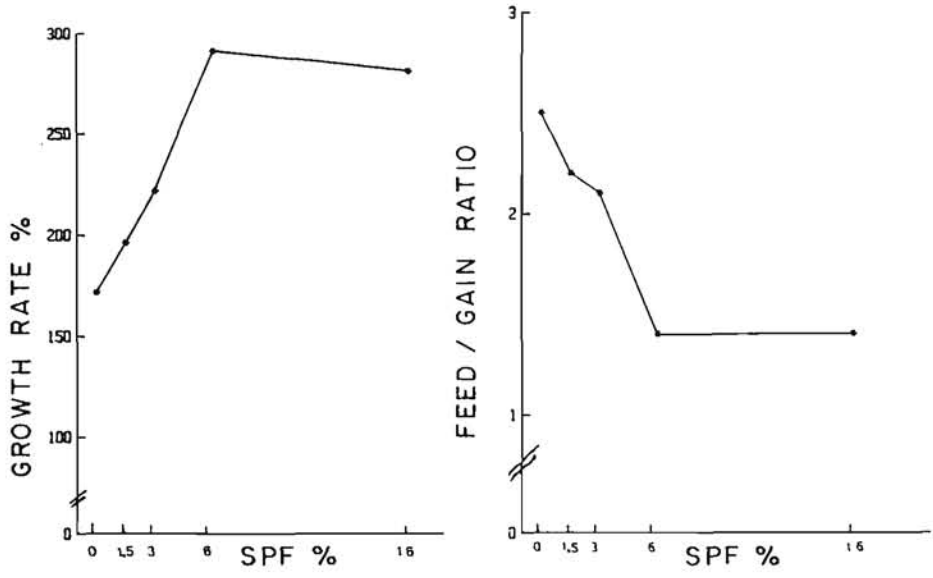

Figure 1. — Growth rate and feed conversion of *P. japonicus* in relation to the level of squid protein fraction.

— *Dose response curves in five species of penaeids* (Cruz-Ricque *et al.*, 1987b; Cruz-Suarez *et al.*, 1987).

The effect of graded levels of squid protein fractions (SPF) was tested first for *P. japonicus*, then for *P. monodon*, *P. stylirostris*, *P. vannamei* and *P. indicus*. In the first four species a very pronounced and very significant growth promoting effect of SPF appeared a plateau became visible around 6 % of SPF (Fig. 1 and 2). But the susceptibility of these species was very

different and *P.indicus* did not respond significantly to SPF supplementation; the most marked effects were obtained for *P. stylirostris* where the growth improvement was significant ($p < 0.05$) even with a supplementation of 1.5 % SPF i.e. with a very negligeable change of amino acid balance. Both series of experiments corroborate the very marked growth enhancing ability of squid protein irrespective of the composition of basal diet and even with a very low supply of essential amino acids.

— *Way of action*

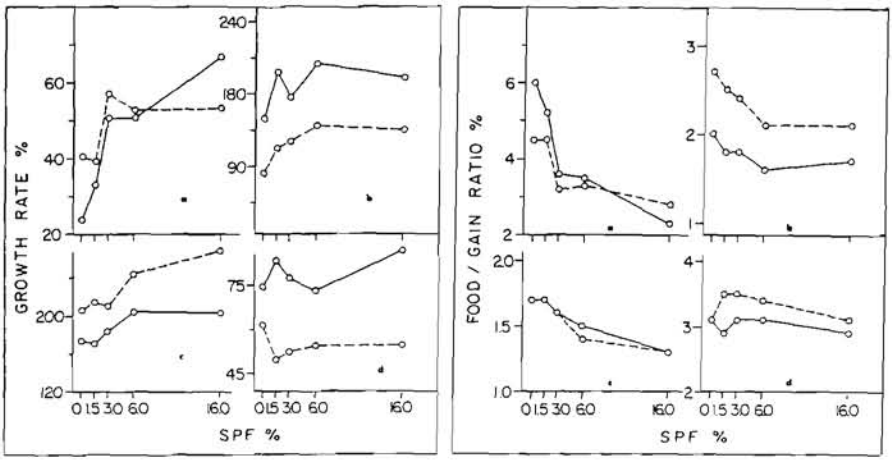

Figure 2. — Growth rate and feed conversion of four species of penaeid shrimp fed on diets containing different levels of squid protein fraction at two stocking densities.
a) *P. stylirostris*
b) *P. vannamei*
c) *P. monodon*
d) *P. indicus*
high stocking density
low stocking density

Growth factor *sensu stricto* or moulting factor - Tissue growth factors such as epidermal growth factor or nerve growth factor stimulate growth by cell division without inducing cell hypertrophy. Cell hypertrophy and hyperplasy were estimated in *P. japonicus* fed on diets containing or not SPF, using DNA and RNA content of whole shrimp as indicators of cell number and size respectively. The results clearly indicate that, DNA content being not increased, no hyperplasy was induced, while cell size was markedly increased (Cruz *et al.*, 1987 — Fig. 3).Therefore squid factor is not a growth factor *sensu stricto* (Cruz-Suarez *et al.*, 1987c).

Another way of action could be the stimulation of moulting by squid factor. This hypothesis was tested by Cruz *et al.* (1987a) in *P. vannamei.* In this trial shrimps were reared in individual tanks and weighed after each carefully recorded moulting. The experiment was conducted until all animals had moulted at least 3 times. No effect of diet (basal diet or 6 % SPF) was noticed on the duration of the intermoult periods, but the weight increment at moult was clearly improved by SPF supplementation

($p < 0.05$) (Fig. 4). Therefore squid factor does not act as ecdysones or other possible moult stimulating factor such as those tested by KANAZAWA *et al.* (1972) (without beneficial effect on weight gain).

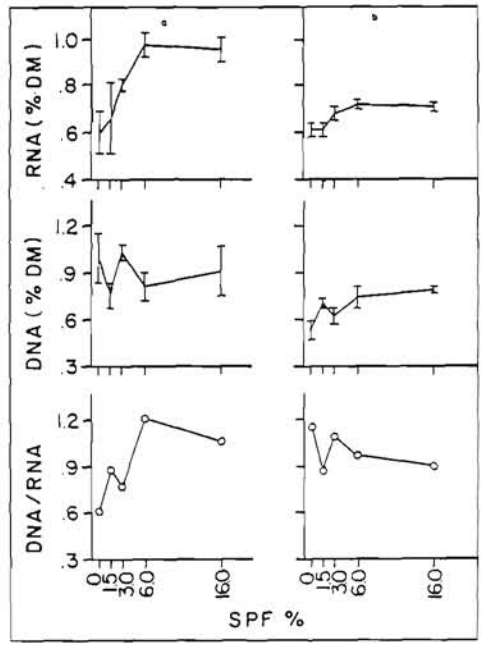

Figure 3. — Effect of dietary supplementation with various levels of squid protein fraction on RNA and DNA content of tissues in *P. japonicus*.

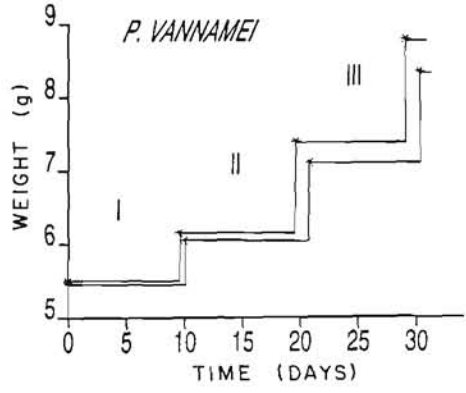

Figure 4. — Effect of the supplementation of diet with squid protein fraction on moulting frequency and weight increase at moult.

Feed attractant or related effect — In marine biology feeding behavior is often controlled or reinforced by feed activators or related compounds, at least in non predators. In the case of crustaceans, feed attractants or stimulants were shown to be free amino acids or bases

(Heinen, 1980) and may be indirect by efficient growth promotion (Deshimaru and Yone, 1978). As indicated previously, a feed activator effect was observed in our preliminary experiment when hydroalcoolosoluble extract was added to basal diet but this fraction did not enhance growth, a result in agreement with the statement of Deshimaru and Shigeno (1972) who noticed that high rate of feed-intake does not necessarily bring about rapid growth. Furthermore in all experiments where squid protein or squid extracts were tested, the increase of growth was always obtained without increase of food intake. Squid factor does not increase appetite.

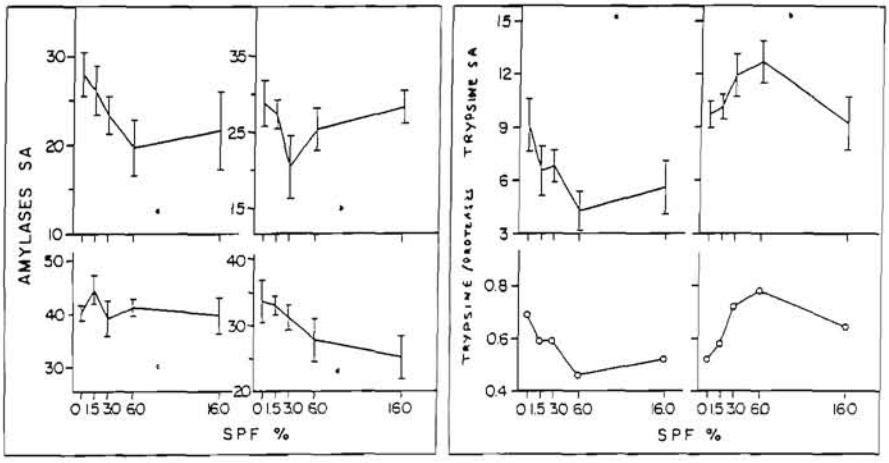

Figure 5. — Amylase, trypsine and trypsine/total protease ratio in shrimp fed diets containing different levels of squid protein fraction.
For amylase a) *P. stylirostris* b) *P. vannamei*
c) *P. monodon* d) *P. indicus*
For trypsine and trypsine/protease ration
a) *P. stylirostris* b) *P. indicus*

Improvement of digestion or absorption - In all trials feed efficiency was improved together with growth rate and, at first sight, it could be explained by an improvement of digestion or absorption of nutrients (Figure 1). No amylasic or proteasic activity was noticed on SPF or squid extracts; no effect either of SPF supplementation was observed on digestibility of food (unpublished). But these results do not allow to discard any effect on the kinetic of digestion or absorption. The activity of digestive enzymes (amylase, total proteases, trypsine) was studied in the digestive tract of shrimp belonging to four species during the day following food intake (Cruz-Ricque, 1987). Results were not quite similar in each species but, by and large, the specific activity was lower in groups fed on diets supplemented with SPF (Fig.5). The purification of *P. japonicus* trypsine was also performed and its activity was related to the actual enzyme protein; but, again, no clear relationship between growth stimulation and enzyme activity was found. However a surprising difference *in in vitro* activation of digestive enzyme activities was remarket (Fig. 6), this activation being more pronounced when shrimp had been fed on diets supplemented with squid extracts. This phenomenon has not been explained.

The time course appearence of glucose and free amino acids in haemolymph was measured in *P. japonicus* as well as in *P. vannamei*; the postprandial increase of both glucose and amino acids was more pronounced with SPF ($p < 0.05$) (Cruz-Ricque *et al.*, 1988 - Fig. 7). In a further study performed on *P. vannamei* a more pronounced increase of amino acids ($p < 0.05$) was observed when shrimp had been fed on SPF supplemented diet for 4 weeks, but was already noticeable after 4 days. It concerned both essential and non essential amino acids and was not related to the transport system (if similar to those of vertebrates) indicating that the absorption mechanism itself does not seem to be responsible for the beneficial effect of SPF (Revol and Guillaume, 1989).

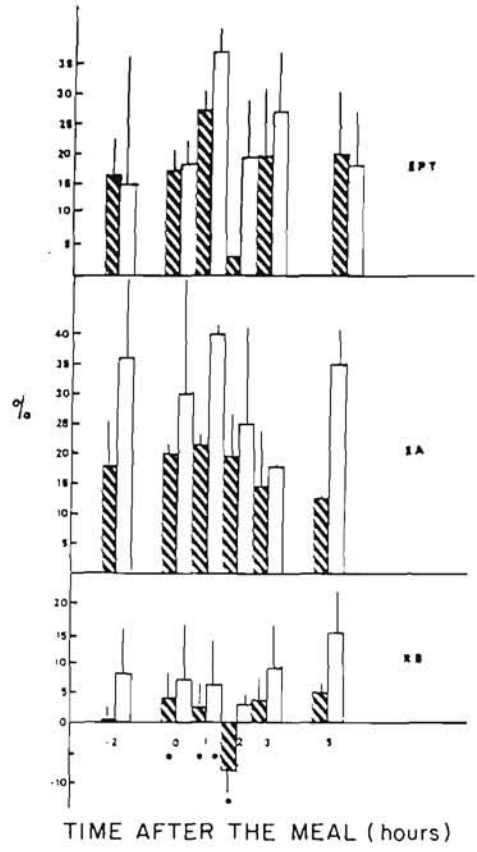

Figure 6. — Activation of amylasic and trypsic activities during incubation (in % of initial activity) in relation to time after the meal.

Otherwise attemps to estimate *in vitro* absorption of labelled amino acids either on gut or on isolated hepatopancreas cells failed to show any effect of SPF (unpublished data).

In conclusion the way of action of squid factor remains unexplained. This factor is very different from « unknown growth factors » that stimulate growth rate of chicks by 3 to 6 % only and do not improve feed

efficiency. It is different from nucleic acids. The hypothesis of its identification to a growth factor *sensu stricto*, or to a moulting hormone may be rejected. Several hypotheses remain to be tested such as a possible influence on gut microflora or an effect of hormones which may influence the organism after ingestion of animal meal such as fish meal (Pelissero *et al.*, 1988; Higgs *et al.*, 1982). Squid factor improves the nutrition of cells not their division, however the way of this improvement itself remains unknown.

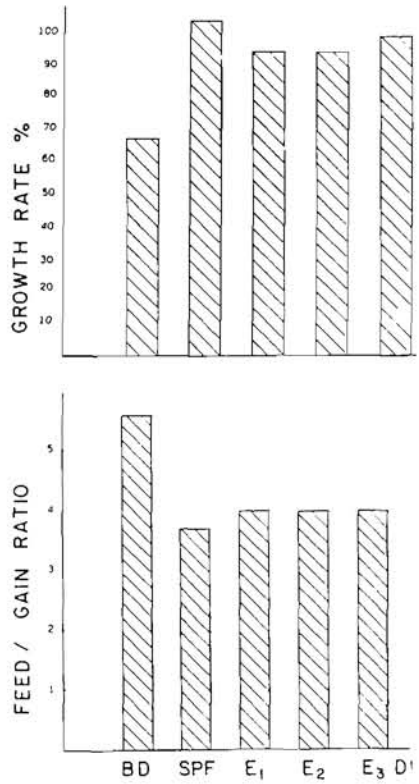

Figure 7. — Growth rate and feed conversion of *P. japonicus* juveniles fed on diets supplemented with various extracts of squid containing « squid factor » (more and more concentrated fractions).
BD = basal diet; for explanation of SPF, E1, E2 and E3 see table I.

CONCENTRATION AND PURIFICATION

Because of a patent request no detail can be given on the procedure used for purification. However fractions with increasing activities were obtained using a first procedure (Table 2 — Fig. 8). Because of unsufficient reliability of the process a second technique was developed which is still being tested.

Untill now the most concentrated fraction obtained was efficient at 100-200 ppm but it was still a mixture of several compounds.

Tab. 2. — Concentration of squid factor : incorporation level of the various fractions obtained and estimation of the concentration factor.

	Incorporation level	Concentration factor
Whole squid (DM)	10	0.6
Squide protein (=SPF)	6	1
E1	1	6
E2	0.17	35
E3	0.02-0.01	300-600

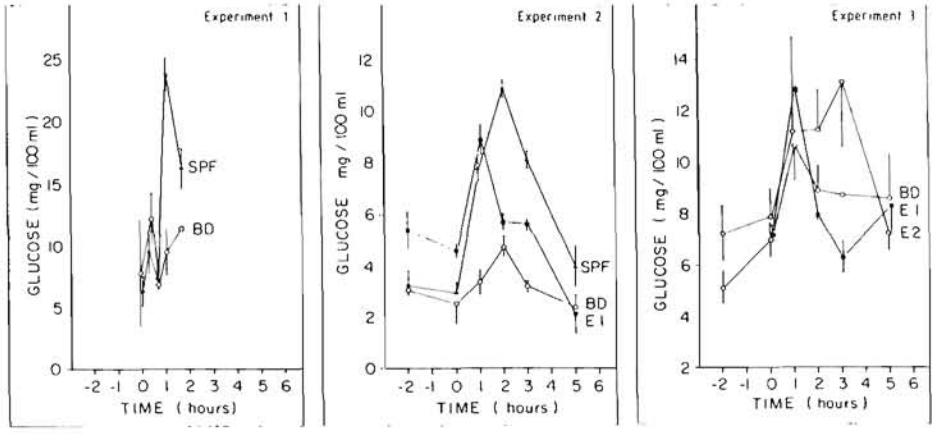

Figure 8. — Postprandial increase of haemolymph glucose level in *P. japonicus* fed on diets containing different squid fraction (same remarks as in figure 7 for explanation of symbols.

CONCLUSION

Neither the way of action of squid factor nor its chemical nature are known. But its efficiency was found in several dozen experiments or trials conducted on four species of penaeids. From a practical point of view the presence of « squid factor » in feedstuffs should be explored to gather information of their possible interest in shrimp diets. The identity of squid factor with the « unknown growth factors » suspected in mussel by Fernandez and Puchal (1979) and Foster and Beard (1973), or in lactic yeast and spirulina by Cuzon *et al.* (1981) should be verified. The very high feeding value of crab protein demonstrated by Boghen and Castell (1982) unexplained to our knowledge, could be related to a more or less similar factor.

The efficiency of squid factor in usual diets under practical rearing conditions was *a priori* not evident. An experiment of Cruz-Ricque et *al.*, (1987a) demonstrated that the effect of squid factor was at least as pronounced in a pond (with some natural productivity) rather in tanks (as sole food) when SPF supplemented versus SPF free diets were compared. But this conclusion is perhaps not valid in any rearing condition and new experiments remain to be performed before growth enhancing factors could be taken into account in shrimp diet formulation.

But from a scientific point of view the purification of this factor should be achieved and its way of action should be elucidated for a better understanding of shrimp nutrition.

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