Some Steps in Shrimp Nutrition «a linear way»

AQUACOP, Cuzon[(1)] G. and Gaxiola[(2)] G.

[(1)]Ifremer BP 7004 Taravao Tahiti French Polynesia

[(2)]UNAM Umdi-Sisal, Mérida Yucatán

E-mail corresponding author: cuzonster@gmail.com

Abstract

In the early 70's, applied research was proposed to support pilot stage of penaeid shrimp farming. Later, some nutritional requirements could be addressed with an increased demand for a grower feed through a study on recommended Protein/Energy ratios for P. *vannamei* and P. *stylirostris*. The work on squid factor gave an indication on a growth factor. Many attempts to mimic Nippai feed led shrimp mature under captive conditions. COP was pioneering on molt stages and its application to penaeid. The IWGCN was an opportunity to exchange information with the industry on ingredients such as wheat or amino acids, vitamins, fatty acids, growth factor. Feed technology and formulation contributed to feed texture, presentation, and stability in sea water. Shrimp in earthen ponds receive natural productivity as part of their food intake, and techniques with stable isotopes gave values on respective contribution of feed and meiofauna. "Floc", investigated in the early 80's, was another option to promote weight gain at high stocking density, the shrimp being surrounded by kind of an external rumen. Recently breeders maintained in "floc" tanks have spawned 6 times per female/month while females from earthen ponds gave 2spawns/female/month. Immune response via β-glucans was tested. Recently, nutrigenomics dealt with digestive enzymes gene expression in early stages and on juveniles. A collaborative work on several species was dedicated to replace fish meal with plant-based protein. These lines of research coupled with bioenergetics will contribute to minimize wastes.

Key words: nutrition, shrimp, physiology, culture systems, wastes, immunity, nutrigenomics

Cuzon, G. 2010. Some Steps in Shrimp Nutrition «a linear way». En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J. (Eds), Avances en Nutrición Acuícola X - Memorias del Décimo Simposio Internacional de Nutrición Acuícola, 8-10 de Noviembre, San Nicolás de los Garza, N. L., México. ISBN 978-607-433-546-0.
Universidad Autónoma de Nuevo León, Monterrey, México, pp. 8-40.

Introduction

It was fortunate to work in a great team. In early 70"s it was sure that very few people had a piece of knowledge on shrimp nutrition, and in fact it was biochemists who took the lead well before pure nutritionists who came in the field well after in mid 80's with, in our case, the arrival of Dr Guillaume who brought such a deep background acquired in chicken nutrition; It is clear to see that at difference with chicken nutrition we started in early 70's with a focus on ingredients instead of on requirement; in fact there was nearly nothing on such topic. Dr Shigeno and Dr Deshimaru were experts in this field and in a more or less empirical way they obtained quite solid results with *Penaeus japonicus* thanks to an adequate sourcing of quality ingredients. The inspiration came basically from data on insects nutrition and for physiological responses on data from zooplankton. It is common to say that progress was very slow at that time and 20years later I recall the comment of Dr L V. Sick who was flabbergasted to see after so many years leaving research work on *Macrobrachium* to see things with so little change. Seen 15years later he was astonished by so low progress in nutrition requirements determination and level of improvement of the knowledge in shrimp nutrition. The first book on crustacean nutrition appears only in 1998 after an effort of the members of the IWGCN as reported by Lou D'Abramo. In our Aquacop team certainly the evolution and progress in the field of shrimp farming came first of all from the success in reproduction in captivity, however, this domain when mastered opened the way to many studies on nutrition and helped to develop a whole program on "floc system" aa program called Ecotron that boosted the comprehension of the productive system acting as an external rumen and benefiting so much in terms of nutrients input for juveniles. The extension work was not so much successful mainly because of a scale problem and the shift from a particulate biomass system to a system where algal bloom prevailed. In Tahiti, there was a progressive evolution of the genetic status of our original strain issued from Mexico and that is in mid 80's only that we started to take into consideration the genetic approach. After 15years of reproduction in captivity of *P. vannamei* and *P. stylirotris* (Table 0). cooperating with the Concarneau laboratory it was possible to show a loss of 95% allelic frequency of the population for the alpha-amylase gene! This revelation directed some studies on the relation between genetic status and nutrition later on nutrigenomics. *P.vannamei* was the main model, followed by *P. stylirostris*.

Cuzon, G. 2010. Some Steps in Shrimp Nutrition «a linear way». En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J. (Eds), Avances en Nutrición Acuícola X - Memorias del Décimo Simposio Internacional de Nutrición Acuícola, 8-10 de Noviembre, San Nicolás de los Garza, N. L., México. ISBN 978-607-433-546-0.
Universidad Autónoma de Nuevo León, Monterrey, México, pp. 8-40.

Table 0. Reproductive performances of penaeids at COP

origin	breeders raised at COP		wild Ecuador	breeders raised at COP	wild Ecuador	breeders raised at COP		
species	*P.mono.*	*P.vanna*	*P.vanna*	*P.styli.*	*P.styli.*	*P.merg.*	*P.indicus*	*P.japon*
P_m φ g	60-80	30-50	60	40-70	70	10-20	15-25	40
#φ/12m^3	30	30-40	30	30	30	100	100	30
P_m male g	25	20-30	50-60	40-50	50-60	10-15	10-15	27
#m/12m^3	30	60	60	60	60	100	100	30
epedoc.φ	yes	yes	yes	yes	yes	yes	no need	yes
#spawn	1	1	1	2-3	2-3	1,5	2,5	1,7
#ω/ φ/sp	175 000	130 000	165 000	180 000	200 000	40 000	80 000	120 000
%fcdn	60	5-10	25-50	40	65	70	80	80
%hatch	70	2-3	60	70	75	90	90	80
#NA/φ/sp	73 500	-	50 000	50 000	97 500	25 000	58 000	67 000
%surv.	60	90	90	80	80	90	90	50
#P^3/φ/sp	44 000	-	45 000	40 000	78 000	22 000	52 000	33 000

Recent concerns came with the replacement of fish meal that is now a topic largely covered by many laboratories and COP maintained cooperation with UNAM to keep track on this topic, part of a more general field of alternative protein sources for the future.

This presentation summarizes the way shrimp nutrition progressed during this period in a tropical environment with a constant effort to transfer the knowledge to the private sector and abroad in order to develop what was a new activity especially in French territories such as Polynesia or New Caledonia. In summary there is a documentation on feeding in captivity that changed to nutritional approach, and rapidly there was a technological aspect to cover to produce feeds for farmers, meanwhile research efforts was maintained on some physiological aspects of nutrition and the comprehension of nutritional adaptation of shrimp strain with a reduced genetic variability in a given environment to end up with genomics. Health and nutrition received relatively minor attention because environment in Tahiti was free of main pathogens and *L. stylirostris* presented a character SPR (specific pathogen resistant) for IHHN; in final one of the complexity of the shrimp nutrition came from the mixture of sources of nutrients, feed on one side and natural productivity (meiofauna, biofilm, floc) on the other.

1. Kagoshima University

As an initial step when in AQUACOP team, a two month stay at Kagoshima University gave us the chance to work with Dr. Kanazawa and Dr. Deshimaru, and to realize all the benefit from a well balanced artificial diet for kuruma shrimp (*P. japonicus*) with an exceptional weight gain over a month of nutrition test. Working with a burrowing species, it was striking to see the difference, at the end of a one month trial, between shrimp receiving a well-balanced diet such as the Shigeno diet and a purified casein-based experimental diet.

In 1976 we had a chance to receive prof Drach who explained its classification of molt stages and we prepared for the first time the transfer of this concept, develpped for thick shell species such as crab, to soft shell species (Drach, 1939). Two soft shell species served for the determination of intermolt stages, *P. merguiensis* for peneids and *Macrobrachium rosenbergii* for Palaemonidae. Unfortunately we never published such key information for physiology studies on penaeids and it remained as an internal report from AQUACOP. One interesting point was the morphology of the cone in the setae that differed noticeably between *Macrobrachium* and *P. merguiensis* and also between *P. merguiensis* and *L. stylirostris*. Many results on nutritional physiology were conducted on this basis. That permitted to lower the variability of results and to stick on the metabolism of penaeid in relation with molt stage C. This stage was characteristic with the cone formed in the middle of the setae contrasting with the morphology of cone for *P. merguiensis* (Aquacop *et al* ., 1975). A large part of the variations in blood parameters or HP contents was directly related to the molt stages and could find interpretation solely on the basis of the metabolism proper to a given molting stage.

2. The "floc"

The bacterial "floc" is a very complex ecosystem that works in a multifactor context where each parameter needs to be considered. The bacterial microflora played initially the major role; it could stand on a unique role in total darkness. However, outdoors tanks getting larger and larger dimension induced an additional factor with the presence of phytoplankton. At start, a few programs were held in order to comprehend how such a "black box" would function, some people speaking of an "external rumen". Implication are coming from different fields such as the evolution of each bacteria communities in the medium, feed composition, management of wastes, sizing for a commercial operation. How shrimp performed in such environment? How shrimp could derive so many nutrients for its growth; would such a culture system be explained through a model?.

First very positive results came from a zootechnical approach with considerable improvement in weight gain, healthy animals at harvest compared to regular earthen ponds production; and recently it was evidenced a potential for reproduction of *Litopenaeus stylirostris*.
That is the purpose of this paper to present a comprehensive approach of the use of "floc" ecosystem in a tank structure called also "moulinettes" (Cuzon *et al* ., 2008). Today there is such a great attention from farmers willing to go into intensive or super-intensive culture system that a comprehension of the trophic chain is necessary. Finally, there are great expectations for a biosecurity aspect with the disappearance of diseases outbreaks, the possibility to develop culture out of the constraints of the seashore and overall an application of the concept of sustainability linked with a profitable operation.

This is one of the most breakthrough that we together with Ralston Purina produced in the field of shrimp culture. The concept was so positive and the first zootechnical results so easy and consistent that in early 70's that was a real big interest for the small team of researchers concerned about it. Unfortunately, the concept of shrimp culture in ponds, with large available areas and no limitation to take sites on the mangroves areas, was such a biff in Central America and south America, that the "floc" system lost its advantages. Ifremer and the COP team found the way to mobilize a team of French scientists to generate a whole bunch of data on “floc” and attempted to find a way to produce a model. The model in reality was too complex with so many parameters that it was impossible to reach this goal? Nevertheless many data obtained during this period (1988) are still useful to give answers on the management of such ecosystem and find new strategies related to it.

- Ralston Purina field laboratory designed the shape of a 300 L tank in a hoof shape; the concept was maintaining the water in constant movement with a system to eliminate wastes easily, and such volume allowed to set a row of tanks indoors and consequently to run trails in total darkness. In total darkness it was possible to get a “pure floc” acting in shrimp uniquely with a bacteria population and representing what could be assimilated to a external rumen. It was the initial step of “floc”

-Ecotron (1979-80) activity of microbial communities (Martin and Bianchi, 1980).
The heterotopy and autotrophy communities were described taking into account the two main compartments water and shrimp. (Bianchi and Bianchi, 1988). Denitrification process occurred in the water and in shrimp. And nitrites were transformed in nitrates by the autotrophic community. The index of exo-enzymes was respectively 28% and 50% in water and shrimp. There is little attack on big molecules of organic matter in the water; oxidation is at a higher rate in the digestive tract in relation with a higher index of exo-enzymes. In both cases, ammonium is taken in charge by bacteria from autotrophic community to transform NH_4 in nitrites then in nitrates. Nitrates peak in the water tank signed a stability of the system.
Two “moulinettes” were monitored one in NH_4 and the other in NO_2 for primary production associated with bacteria in the “floc”system using two different substrates, glucose and glutamate, a sugar and an amino acid. Respiration and assimilation could be measured and an evolution of the two systems evidenced the succession of populations which was clearer with ^{14}C glucose than with ^{14}C glutamate and assimilation and respiration which contrasted between the two substrates. Definitively, glucose allowed a better growth of primary production and autotrophy community than glutamate did. By extension, that explains the trend for use of molasses, a carbon source to initiate and/or maintain the “floc” system (Samocha *et al* ., 2007).

In summary our colleague of Aquacop (Alain Michel) summed up the problem related with floc: "I think we can discuss for ever of the stability and variations of the floc system sometime going as the theory says and sometime the opposite. As for me, this is the characteristic of a chaotic system very sensitive to the initial conditions; among them, water quality and chemistry and light intensity are the major points, plus the built up of some components as phosphate in the system. Clearly it is site and culture system dependent, which prevents to find a general recipe. The first problem is to avoid at the start the development of green algae. It should be done by a very low water renewal and the development of a phytoplancton bloom which will allow to the community of small organisms as rotifers, ciliates and other bacteria to establish. They will begin to control the phytoplancton and to turn the system to floc. According to the nutrients of the site water, the light intensity the period you start, the nature of your sediments if in ponds the evolution should and will be very different. The kind of populations you want to rely on as rotifers and ciliates have cycles and fluctuations of chaotic types (see papers of Robert May on chaotic populations of rotifers and papers in Monterey bay describing the variations in organic particulate matters. My experience on flocs was in the late 70's deriving from what the team of Ralston Purina (Harvey Persyn) was doing in tanks in Crystal River where I saw foams of more than 10 cm covering the surface of the tanks due also to a strong aeration. The advantage was to remove some of the metabolism products in the foam and to keep the water quite dark. With the Ifremer AQUACOP team in Tahiti we apply this technique in our environment which was coral reef. The water in the coral reef area specially in lagoon is always similar to a light floc with a lot of suspended organic particles in the water dead (mucus from corals) or alive (many appendicularans, ctenophores, siphonophores, small medusae and salpa. You rarely get colour water (phytoplancton bloom made of chlorophycea or diatoms) because of the lack of phosphorus in the water (taken fast by the zooxanthellae of the corals).This water with no phosphate and already quite a lot of floc particles was a good inoculum for flock system. We run the experiments in 1000m^2 tanks with liners on *P.vannamei*. For sure the adding of the feed modify rapidly the nutrient content of water and the phosphate increase (any product able to block the phosphate should be good). At that time the only way to maintain the floc was to play with the water exchange (low on general but sometime very heavy to flush-out the bottom waste and start again the floc. It is really a problem of daily observation and great reactivity. We apply this technique in a small commercial farm in Tahiti (Sopromer), which with 10 tanks of 1000m^2 was able to produce 20tons/crop in a floc system with AIR-O_2 aerators to maintain the floc in suspension. The main problem was the starting to avoid the green filamentous algae development. We apply also this system in small tanks of 10m^3 to constitute on a routine basis our broodstock of *P.vannamei*. We have done like that more than 20 generations of *P.vannamei* derived from a small population of post larvae introduced in the Center late 70's. **The maintenance of a stable bacterial floc is easier in well aerated small tanks, which you can easily cover to control the light, than in bigger tanks**. In both cases the growth of the shrimp on the same feed was much better in floc system than in clear water or phytoplancton system due to the participation in the food of the rich particulate matter; gut are always full. Even in this context of high bacteria level the shrimp health was very good with no one black spot on the carapace. it is for all these reasons that the bacterial floc is interesting but there is so many black boxes and the chaotic system will be always there. Each one has to find his own way in his variable environment".

3. Ingredients

Large concern, because of a lack of data on requirements (Cuzon *et al* ., 1994): "so far, it is rare to find a formulated feed producing growth comparable to the combination of natural foods such as clam, shrimp, or squid meal coupled with pond productivity. The research remains to be done, notice that there probably are several thousand meaningful permutation waiting to be tested in this realm alone. Unless some lucky investigator experiences a revelation from above, the road to truly effective formula feeds is likely to be long and expensive. By truly effective, of course, we mean formulated feeds that are inexpensive, reliable, non-polluting and which result in growth rates at least comparable to those obtained with natural foods in extensive grow-out systems". Among ingredients one can cite shrimp meal but also some plant protein such as *Leucena leucocephala* or a fish hydrolysate (CPSP).

The hassle of water stability: that is another problem linking ingredients and technology. Many papers were written on technology for shrimp feeds and one of the greatest concern was the achievement of a water stable feed. A considerable number of binders were used. Among the first was the wheat gluten because it fitted well with the wet way of processing, though with the advent of cooking extrusion there was still a room for this ingredient-binder.

STD program: collaboration with the UANL (Cruz & Ricque, 1998).

Fishmeals made of fresh, medium and stale raw material were compared for their use regarding weight gain and survival rate for juveniles of several shrimp species. Among protein sources which are selected for the formulation of practical diets for penaeid shrimp, fish meal is the most widely utilized. Practical diets and commercial feeds can incorporate up to 50% fish meal. Several studies already underlined that fish meal inclusion in a shrimp diet is not only a matter of percentage, but also a question of quality protein, and lack of peroxidized fish oil. Initially there was a preference for white fish meal instead of brown fish meal for lipid quality reason. This paper refers mainly on quality of fish meals which were produced from material qualified as fresh, medium and stale, according to the maturation time of the product before being processed to fish meal. Three species (*P. monodon*, *P. vannamei*, and *P. stylirostris*) were selected for the study on growth, survival and FCR, when animals received an extruded feed during 30 days. Specific answers are summarized in Fig.1): it comes out that fish meal from fresh fish is promoting better growth rate than the two other qualities of fish meal, while medium or stale couldn't be discriminated. Survival rates are not significantly different. The importance of quality protein from fish meal is discussed in comparison with other protein sources, some native ones providing maximum growth rates in tanks.

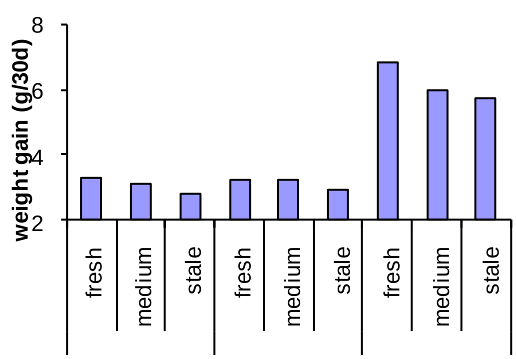


Fig.1. Compared growth response to diets including sardine meals prepared from raw material of graded freshness (fresh, medium or stale)

Cuzon, G. 2010. Some Steps in Shrimp Nutrition «a linear way». En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J. (Eds), Avances en Nutrición Acuícola X - Memorias del Décimo Simposio Internacional de Nutrición Acuícola, 8-10 de Noviembre, San Nicolás de los Garza, N. L., México. ISBN 978-607-433-546-0.
Universidad Autónoma de Nuevo León, Monterrey, México, pp. 8-40.

Plant protein sources and attractants.

Amino acids make an important contribution to the flavour of fish and prawn (Carr *et al* ., 1978). Absence of glycine and alanine reduced the sweetness and enhanced the bitterness of the meat; a combination of GLU with nucleotides also produced a meaty taste (Nose *et al* ., 1974).

Pigments are needed for the coloration of shrimp, mainly astaxanthin (Howell and Matthews, 1991). A wild *P. monodon* exoskeleton contained 26ppm total carotenoid, a normally pigmented farmed shrimp had 25ppm, contrasting with "blue" farmed shrimp (4-7ppm), and such a deficiency was observed also when shrimp were fed a maturation feed that was stored in poor conditions and presenting a high level of peroxides (Aquacop, *com.pers*.), as an indication of the degree of oxidation of carotenoids.

All the work on animal protein versus plant protein is briefly summed up in the following table:

Table 1. Animal *vs* plant protein

•plant protein	•animal protein (e.g. Fish Meal)
•low protein digestibility	•digestiblity 90^{+}
•low DE	•high DE
•biodisponibility of essential aa	•good balance in aa
•antinutritional factors	•growth factor bound to $vitB_{12}$, Ca, P, carnitin
•low appetibility	• high appetibility

To supplement a plant protein based diet with aa , with an emphasis on lysine, is difficult for technical reasons (encapsulation), as well as physiological reason (intestinal absorption), and to a lower extent for economical reasons. The case of methionine was emphasized recently always in case of "all-plant based diets" (Davis *et al* ., 2009; Fox, 2008).

4. Feed technology

Moist way, pelletisation, cooking-extrusion (Cuzon *et al* ., 2001): technology for feed was a part of the research work and it started with what we called the moist way using a regular meat-mincer and formulations including wheat gluten as a binder and also as a source of protein. Such equipment was limited in size and capacity and then another machine (Kustner, see Aquacop, 1984) served to produce starter feeds with a good stability and adequate shape and length. Meanwhile other techniques were tested at experimental scale with an extruder Brabender and then an expander Anderson $3^{1/2}$ inches mono-screw (Moe Williams) set in a factory called Huilerie de Tahiti. This workshop had a capacity for several metric tons a week. The other technique for feeds wad the pelletization, which was used to produce large quantities of shrimp feeds 2mm diameter, sinking pellets. The technique has diverse concept; the example of Mauritius and its pelletiser running with formulations without binder (wheat flour only or factories in Taiwan with a conditioner and post conditioning, up to three conditioners prior the pellet mill. The experience included also a twin screw extruder Clextral that seemed to be more adequate for shrimp feeds with optimum characteristics than other types of extruders; Taiwan survey of 7 shrimp feed factories dedicated to P.*monodon* gave a clear indication on the way to make a water stable feed by pelletisation and to select ingredients to match the requirements during the growing period and by an adequate management of the pond to maintain an FCR low enough to cope with a sustainable production.

5. Feed production

Expertise for crustaceans compounded feeds

This expertise started in 1972 with the set up of a workshop for experimental preparation of feeds for fish, shrimp and others marine animals (publication at a meeting in Hawaii 1978). After 10 years of management of this workshop with one technician and two workers, there was a transfer to a feed mill in Papeete to produce larger quantities at a scale of one metric ton per day. Meanwhile we developed and applied the following technologies: pelletization with a CPM

Masters; extrusion with a Brabender and extrusion with a Anderson mono-screw 3 ½ inches and all the technical aspects of cooking extrusion (see paper with Mau Williams in Feed Intl: Cuzon *et al* ., 2001). The process of pelletization was used for many years to make feeds for two shrimp farms. Technical controls were made routinely for water stability. Back to France, we contacted a small feed miller in Montfort-sur-Risle in Eure (France), with a veterinarian as a manager who helped us considerably to manufacture for several years a starter feed for shrimp and a grower feed with a technology called moist way meaning that the process was done by adding a large amount of water and drying afterwards and this system guaranteed the good water stability of the feed for many years and supported shrimp farms development in the southern area of France (Mediterranean region). Then different expertises for feed mills were achieved in the area; we approached our colleagues of INRA in order to work on cooking extrusion with a Clextral apparatus, with co-rotating co penetrating twin screws. This collaboration led to several papers (Melcion *et al* ., 1983) and gave the opportunity to experiment with floating feeds (for the flatfish such as sole or turbot), sinking feeds for shrimps and what we called re-hydratable feeds that allowed to coat the collets with oils in order to increase its overall digestible energy content. But the main transfer for shrimp feeds production in collaboration with NRM was done in New Caledonia where two large feed mills for poultry and pigs were in operation and the purpose was to install a line of aquatic feeds production. It was an experience of several years to set up proper equipments, select ingredients and formulate at low cost on Mixit-1. Meanwhile a survey fully dedicated to shrimp feed production was conducted in Taiwan with a visit of 9 different feed mills in the area of Tainan (Report Taiwan, 1988). Another expertise was conducted in Mauritius island to comfort the production of water stable feeds at industrial level and guarantee water stability. Other advises were done to different places such as Colombia, Ecuador, Thailand, Back to Tahiti, there was the management of the Huilerie of Tahiti for many years to produce shrimp feeds; an affiliate of Ifremer, France Aquaculture, send these feeds to many places to contribute to shrimp farming around the world and then the collusion with SANOFI opened up a new area with the production of larval feeds and especially ACAL, and what will become the Frippak line of micro-particles using a special technology of atomisation.

Then, there was a large amount of work done in Latin America, especially in Mexico, Yucatan to survey feed mill factory production shrimp feeds. Such expertise was very useful to enlarge the level of knowledge on feeds production. Recently a feed miller in Mérida Yucatan required an expertise to set a line of production through pelletization and extrusion to manufacture shrimp feeds and a whole bunch of advice help to overpass the stage of feed manufacturing with poor water stability that is a great prerequisite for shrimp feeds especially (Fig 2).

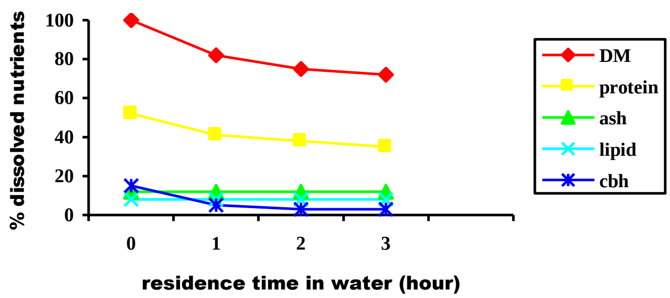


Fig.2. Percentage of feed nutrients remaining undissolved at crescent residence times in water

Afer some international expertises and/or recommendations for Dubai, Koweit, Fiji, NC (SICA, MSV), Mauritius, Ecuador, H.de T., Sangue SA., Malta Cleyton, Higashimaru feeds Cie, Madagascar, Barlas feeds, Singapore, Peru Nicovita, NRM feeds Nelson NZ, Taiwan, Kaoshiung, Montfort-sur-Risle (Dietevet France), Huilerie de Tahiti, Anderson (Ohio), INRA/LTAA, Nantes, France for extruded feeds. In New Caledonia (congress of Martinique, 1989), there was a transfer of technology from Tahiti to New Caledonia for the shrimp farming. The production of *L. stylitrostris* was the objective of this territory. According to the previsions of production it was urgent to set up the production of local shrimp feeds in order to avoid a constant dependence from the Taiwanese feed called “President”. This feed was manufactured by pelletisation and the alternative for New Caledonia was to produce with this technology or to shift for extrusion. Advantages from feed quality view point was similar but the pelletisation had more background and then the choice went for pelletisation. This process imply whether a special conditioning or the incorporation of a binder in the formulation. The first steps were difficult because of this problem of stability of the feed in the water. The track for binders led to a selection of a polymer which was very efficient and then the process went right for many years. In the same time there was side problems to solve such as the fine grinding and the low cost feed. Linear programming (Barbieri and Cuzon, 1980) helped find low cost feed and a solution was achieved. However, there was a constant questioning about the effect of natural productivity and the feed.

Pressure for a feed

Pressure for a feed was a permanent situation especially in a remote place such as ours in a tropical area in the middle of the South Pacific.

- Larvae: collaboration with the team of Galveston on larvae culture in 500L conical tanks. Sequence algae+*Artemia*, and later attempts to replace completely algae and partly *Artemia*. Then participation to the concept of microbound particles, with the epoch-making ACAL, a microbound diet for *Macrobrachium rosenbergii* larvae. ACAL was the result of many observations and adaptations of the technique of feeding *Macrobrachium* larvae in 500L larvae tanks. Based on daily observations for many years, it was possible to adapt a formulation with alginate to produce an alginate particle easily sieved in different sizes: 100, 300 and 500µm. The formulation given in table 2 and the process described above gave a result that allowed to save 50% of the amount of *Artemia* nauplii provided during a whole larvae cycle. It allowed to replace definitively the bonito meat that was sieved daily to distribute in larvae tank as an additional food to *Artemia* nauplii for maintaining larvae with a low level of cannibalism.

Table 2. ACAL formula (Aquacop, 1975)

19/10/2007								
	ACAL							
				CP	CL	cbh	kJ/g	
œufs		30dz						
calmars entiers	10kg			69				
laitance poisson	3kg							
crevettes congelées	9kg			18				
	22kg	21600	44					82.5
lecithin	100							1%
huile rouge	300							4%
alginate Na	860							11%
vit HL	20							0.3%
Phosol	20							0.3%
	1300		1					16%
pâte	44kg		45					
sec	8kg			87	9	11	25	**99**

-Postlarvae: postlarvae were fed adequately with a feed particle produced by moist way (Aquacop, 1989) and operating the Kustner to prepare one of the best diet for postlarvae in the centre called Mocal and presented under the form of spaghettis of 1mm diameter.

-Grower: this type of feed took an enormous amount of time to set the formulation according to the species, to control the process, to maintain the stability in the water and to keep a good appetability meanwhile maintaining a constant quality in spite of the remote place of Tahiti in a tropical environment.

A «magic» formula»?

There has been a constant search for an optimum formulation of a dry feed especially for the grower phase, leading rapidly to another search for a least cost feed. During several years, the attention was put on ingredients and such trend led to a multi ingredients formula, but an attempt to simplify combined with more and more data on requirements ensured the manufacturer to propose grower feeds with a minimum of ingredients, cf following quote: «the industry is also unlikely to get terribly excited about new wonder formulas developed in non-profit labs unless these formulas are inexpensive to produce, not only in terms of their ingredients, but also in terms of the capital investment required for facilities and machinery. To the extent that aquaculture becomes a large formula feed market, this will turn around gradually. But, for the immediate future, the commercial shrimp grower faces a situation similar to that of the original buyers of model T Ford: he can have any formula he wants so long as it is one of those already in production - or he can build his own".

Table 3. Formula of a feed produced at "Huilerie de Tahiti"

27/06/2009					
	%	CP %	CL%	cbh%	DE kJ/g
lactic yeast	10	5		2	
corn distillers	10	2		3	
corn steep	5				
autolysates PNF	5	3			
CPSP 70	5	4	3		
krill hydrolysate	2	1			
Phosol	2				
vit HL	1				
Stay C					
capelin oil	3		3		
AQUAlipid lecithin	1		1		
fermented soyabean	20	10			
whole wheat	25	3		15	
white fish meal	11	6			
	100	33	7	20	13

In initial stages, some of the key ingredients were corn distillers, corn steep, CPSP, wheat, soybean in reasonable amount, autolysates from Lorientaise, …today a search for protein sources is such that it is going up to the recuperation of papaya skin to add to the shrimp feed in Hawaii (2010) and macroalgae too as described in several papers (Cruz-Suarez *et al* . 2009).

6. Zootechnia

An 80 tanks room was installed at Aquacop with indoors 225L fiber glass tanks in order to run tests in triplicate, within periods of 30days and a daily control. Many experiments were conducted on requirements, on ingredients, on feeds, most of the time with juveniles of 1g at start, sometimes with larger species. Tanks were usually equipped with a double bottom, first with a sand layer, later without substrate. Once a piece of PVC on the bottom, trials were conducted with a perforated plate……such false bottom could be used with or without a sand layer. In the second case the apertures of the perforated plate could let collets go through the layer and sediment on the tank bottom. Observations made led to consider that the daily ration distributed each night was fully consumed. However, after 15 days of such procedure, there was a mortality rate that increased day per day to finally led to total mortality. This phenomenon was strictly due to soluble substances produced by the decomposition of the remaining feed on the bottom tank and the diffusion of probably compounds such a H_2S was sufficient to produce a severe mortality. The curve was like a standard curve over a month. Those proved simply the severe degradation on a medium without substrate and only due to the degradation of the organic matter contained in the feed and a rapid development of bacteria and fungi, but an action of soluble toxic substances as shrimp were never in direct contact with the material in decomposition but 10cm above it.

Approximately a hundred trials were conducted in such area of investigation (1982-91) and among the most interesting ones one can cite the comparison between feeds from different origins, the effect of pollution on survival rate (curve of mortality *vs* time), the squid factor, the comparison between Japanese feeds and locally produced feeds, the influence of water stability of extruded or pelleted feeds, the role of vitamin C, the incidence of multiple meals in a day on weight gain, the protein/energy ratio in the feed, comparison of performances between practical diets, effect of supplementation with crystalline amino acids, …among nutrients, after many attempts for protein determination of optimum content in the diet, there was two lines of research one on lipids, the other one on carbohydrates.

Lipids

This class of nutrients had been mostly studied by Dr Kanazawa team (Kanazawa *et al* ., 1979). Among classes of lipids, sterols were of particular interest. Phospholipids were specially studied for larvae, and fatty acids represented one of the most important constraint in shrimp feed formulation. Rapidly, it was assessed an amount of total lipids that in respect of constraints from the different classes would not overpass around 8%. Deshimaru showed the refrain in weight gain in case of high lipid concentration in the diet. And several authors found similar trend concerning a maximum dietary lipid. Phospholipids were essential at all stages of life cycle and went in formulation with ingredients such as Aquagran 60%, de-oiled lecithin containing the following: PC 26%, PE 20%, PI 14%. Cold acclimation is invariably associated with an increase degree of unsaturation of the fatty acid moieties of phospholipids (Hazel and Prosser, 1974) and an application of this concept could be found in the culture of *L. stylirostris* in NC (Chim *et al* ., 2001). Andrews (1972) found an adverse effect of corn oil on *P. duorarum* and this may be explained as a competition of n-6 fatty acids series with n-3 HUFA's for the Δ6-desaturase; desaturases seemed more active than enzymes implicated in elongation process (Leger *et al* ., 1975).

Nutrition and reproduction: maturation feed

Shrimp breeders were much studied in the 80's and *L. vannamei* breeders in particular were observed in ponds, transported to maturation unit (MU), eyestalk ablated and did not spawn and the process was screwed up. Hopefully, *L. vanna* raised in «floc» conditions, transferred to MU gave quality spawning. Quality medium and a maturation feed+live food were optimum in «floc» conditions, which allowed the production of better animals than in earthen ponds with algal bloom. A whole chapter (Cuzon *et al* ., 2006) was written about this topic.

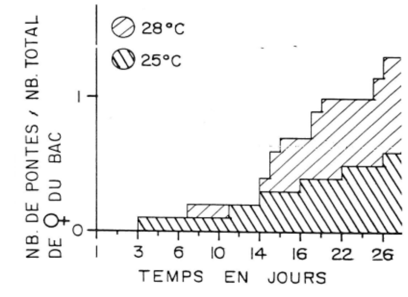
Table 4. Species and their ability to stand in captivity.

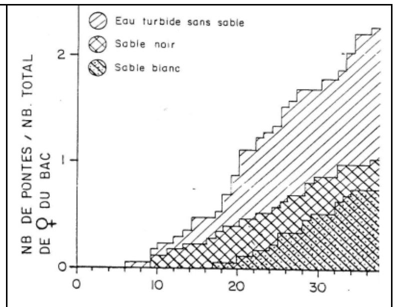
	larvae	nursery	grow-out	maturation	nutrition
P.merguiensis	x	-	--	xxx	x
P.indicus	xx	-	-	xxxx	x
P.semisulcatus	x	-	x	-	-
P.monodon	xx	x	xx	xx	x
P.japonicus		xx		x	xx
P.vannamei	xxx	x	xxx	xx	xxx
P.stylirostris	xxx	x	xxx	xx	xx

x signs indicate the level of easiness to achieve each step of the life cycle (xxxx for *indicus* that is so easy to reproduce,..)

Many experiments indoors in 220L or 30m^3 tanks were conducted with *stylirostris, monodon, japonicus; semisulcatus, plebejus, chinensis, monoceros, and vannamei* (Aquacop, 1975) but among all, *P .indicus* revealed to be an excellent species for reproduction in captivity and nutritional requirements of breeders. Reproduction of *L. vannamei* under laboratory conditions was influenced by several factors (in Mente *Eds*). Previously with *P. japonicus* it was shown that females responded to a variation in temperature and photoperiod. Under tropical environment the response to an elevation of temperature on spawning was evidenced on a 30d period with only 3°C difference. It means that is was preferable to get spawners coming from a "cold season" (austral winter) and start operations to prepare the females just during the increase in water temperature.

Fig. 3.Influence of abiotic factors on spawning during 30days (Cahu *et al* ., 1994)





Influence of water temperature	Influence of substrates

Another factor such as the color of the sediment was quite effective on spawning as coral sand seems less effective than volcanic (black) sand but the striking difference leading to a concept of breeders contention so much easier was the absence of substrate in a turbid water (2 spawns per female instead of 1 spawn with substrate). Therefore the food quality given to the breeders do not explain all the variation and can produce animals in good physiological conditions for reproduction but abiotic factors seemed to be active on the short term, after eyestalk ablation and during a period of one month.

Free amino acids and the comparison between fresh food and compounded feed led to conclude about the real benefit of **free amino acids** as well for reproduction as for weight gain but the real benefit was evidenced during the reproductive period in captivity. Animals placed in “floc” conditions or in clear water with fresh food such as mussel flesh, squid,….showed performances in spawning and the main role of free amino acids was evidenced (Table 5).

Table 5. Free amino acid and proteic amino acid contents in diet and body of shrimp growers fed fresh food vs compound feed

	fresh food				compound feeds			
	NEAA		EAA		NEAA		EAA	
	FAA	AAprot	FAA	AAprot	FAA	AAprot	FAA	AAprot
Diet	36	180	13	140	3	230	3	200
body comp.	61	284	37	230	75	300	22	230

«squid factor» (E.Cruz, 1983-84)

Elizabeth Cruz was at the initial step of the work on squid factor (Fig. 4) initiated at Ifremer Brest in the 80´s. The idea came from the details on formulations issued in particular from South East Asia and particularly in Japan to make feeds with excellent performances for juveniles as well as for breeders. Following this established fact it was decided to investigate on the active compound in squid meal which would bear such a property (Cruz-Suarez and Guillaume, 1983). There is a resurgence of the question of growth factor today with the attempt to find an explanation of the properties linked with the particulate biomass in “floc” system.

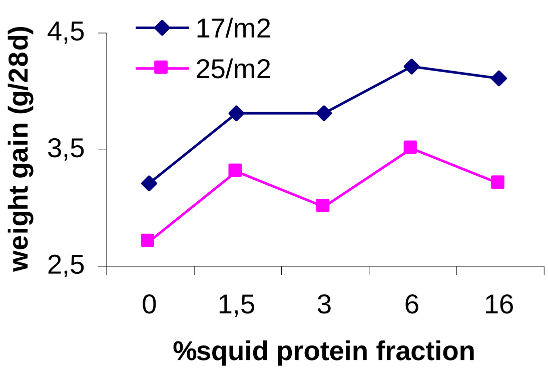


Fig.4. Effect of crescent dietary inclusion of a squid protein fraction on shrimp growth a two different densities

IWGCN 1991-93 and book on crustacean nutrition

Louis d'Abramo, wrote "in the spring of 1988 on the island of Tahiti a workshop was organized with Ifremer funded by the French Ministry of Research and Technology. The primary objective of the 'Nutrition of crustaceans" workshop was to assemble experts, working with either tropical or temperate climate crustaceans, who would synthesize the state of knowledge by particular topics and who would define future research directions and goals. At this workshop the IWGCN was formed and G. Cuzon was elected as the first chairman. The primary mission of the IWGCN is to ensure a constant and regular exchange of information. Since then, the IWGCN has sponsored several symposia which serve to inform scientists, producers and representatives of the private sector by providing a synthesis of current research, and presentations for new developments and topics of current interest. The most recent symposium was held in Kagoshima, Japan in the spring of 1995 where crustacean nutrition studies began almost forty years ago". One of the main contributions was the edition of the book on crustacean nutrition (WAS, 300pp).

Collaboration with Roquette on native wheat gluten

This collaboration gave the opportunity for a thesis work on the relation protein/energy (Cousin, 1995). That is one of the most rigorous experimentation ever conducted in the laboratory which led to a comprehensive approach of requirements for two species simultaneously in conditions of clear water. Still today, such bunch of results is a guideline for similar studies on other species and a reference to the set up of new formulations for juveniles of shrimp particularly non grooved species. The only regret was to be unable to set a respirometer during this period which could have added a point of bioenergetics to confirm the results in terms of DE requirement.

Cooperation with Mexico (E.Cruz *et al* . 1992) on fishmeal quality (fresh, stale, medium) and IFOMA

Fish meal is a complex compound and aside from its crude protein content there are several others components such as amines, nucleotides, ammonia,…that contributes to the (Nx6.25).

True protein (myosin) could represent around 79% of the total. This product faces different qualities depending on the mode of processing, especially the mode of drying. This mode could severely affect the digestibility of the final product. Finally the freshness of the raw product could be directly related to the presence of biogenic amines. Such amines were the main research concern of a project under IFOMA, UANL and Ifremer. The response of shrimp was not so different among three species tested with a susceptibility level more pronounced with *P. monodon* compared to *L. vannamei* or *L. stylirostris*.

U of Guelph and bioenergetics

Several species were studied for their protein requirement; response parameters were survival and growth rate at first, and later on NPU or protein retention based on analytical data on shrimp at initial and final of the trial. Then ammonia excretion was measured according to level of dietary protein (Gauquelin *et al* ., 1999) and more parameters were measured from the moment a respirometer was set up and produced reliable data. The work led to a calculation of DE with an extrapolation for the whole period of growth of juveniles. Then it was possible to produce a table of feed distribution in accordance with the daily gain (Bureau *et al* ., 2000). RE was obtained from the energy partitioning of the energy of the feed and compared to the previous energy balance based on calculation of body energy gain. The level of knowledge did not reach obviously the one on baby chick (Hill and Dansky, 1950), which consume easily an amount of energy in proportion to its basal metabolism. Proportionality exists between calories consumption and live weight^{0,76}.

By and large it was, at end, enough to answer the following questions about shrimp (Bayley, 1994): what is the diet of shrimp in the wild? What is the proximate composition of shrimp? How is the growth of penaeid shrimp? What kind of feeds is currently used? How shrimp behave with temperature? What is the proportion of chitin in whole body? Can shrimp utilize efficiently carbohydrates? How can shrimp utilize protein from the diet? What is the metabolic rate in kJ/h for 100g shrimp rate of protein synthesis?

ECOS-Nord and utilization of carbohydrates by shrimp (1998-2002)

The objective of this program was to better understand the utilization of carbohydrates (cbh) by shrimp. Many years ago during the early seventies, a few trials reported that glucose fed to juveniles were giving a growth inhibition when compared to other cbh sources such as dextrose, starch, glycogen,...and it was stated that a need for complex cbh was to be met instead of simple sugars (Aquacop,1989). More recently (1996-97), growth of *P. stylirostris* was good (2g/week under experimental conditions) when fed practical diets including up to 30% starch (practical diets formulated with squid meal helped increase the palatability of the feed). A study was conducted to determine the optimum protein/energy ratio for growth of juveniles. The utilization of carbohydrates tended to be optimal for a 30% protein and 20% cbh diet. Energy retention was low compared to values found with trout for example (20% versus 40% for trout) with a possible explanation from the composition of feed. Recent results (Gauquelin *et al* ., 2007) showed that shrimp utilize protein better than cbh for growth as a significant growth promoting effect was monitored on protein dense diets; the utilization of amino acids was done both from a synthesis (accretion) point of view as well for energy purpose coming from the decarboxylation of essential amino acids (eaa); and it is possible that shrimp benefit from energy from eaa more efficiently than from sugars simply because of a more progressive release of the energy coming from eaa (according to Szent Gyorgii who explained «the liberation of energy by small coins»). There is another way of expressing the benefit from eaa catabolism with the statement on a higher metabolizable energy (ME) derived from eaa rather than from glucose or dextrins (Cowey, 1992;com.pers.). And interestingly, ammonia production from protein metabolism was higher (0.6 mg/g dry weight of shrimp compared to 0.4 with low dietary input) for shrimp receiving more dietary protein, indicating a catabolism of protein in excess of the requirement, but the energy-yield seemed to favor protein synthesis in the same time, in regard to the growth performances registered (7-8g/50days versus 2-3g for the same period which means that a growth metabolism was compared to a maintenance metabolism).

Consequently, from a nutrient point of view, one statement can be made: «*protein exhibits the best digestibility and the best metabolic utilization by shrimp, lipids are well digested especially* *lipids from marine origin but little is derived for fuel, part being utilized for membranes build-up and an excess of dietary lipids (above10-12%) leading to a reduction of growth; cbh are digested fairly well but there is a possible problem with metabolic utilization»* (in fish, when there is a ^{14}Cglucose load, about 49% is recovered as $^{14}C\ CO_2$ which demonstrated a utilization as a fuel source). Lobsters fed low protein diets demonstrated an increased dependence on carbohydrate metabolism for energy production. The reduced growth rates of lobsters fed the two lower protein diets were the result of differences in the amounts of food consumed and not increased energy expenditures or reduced assimilation efficiencies (Capuzzo, 1979).

Digestive physiology (Gaxiola *et al* ., 2009)

Proteolytic enzymes in the hepatopancreas or HP or midgut gland according to van Weel (1974) are very powerful (Cowey and Forster, 1971) and it is recalled the aspect of fingers completely black when workers were decorticating shrimp heads on a line of shrimp conditioning in Sri Lanka.

-specific activity of digestive enzymes was measured at all stages of the life cycle for different species of shrimp in order to express the level of carnivore or herbivore status not only from an ontogenetic viewpoint but also after a drastic change in type of feeding including a shift from animal to plant based diets or the reverse (Maldonado *et al*., 2009).

-K_m of amylase and hexokinase were studied partially with *L. vannamei* and needed to be completed. A whole revision of the article of Ceccaldi (1978) was updated for the second edition of this book issued from an initiative of the members of IWGCN and later by Gaxiola *et al*. (2009).

-Vmax should have been measured as for phosphatase alkaline in HP of *Squilla mantis* (Principato *et al*., 1988) "The partial purification of the soluble cholinesterase from *Squilla mantis* is described. A single of cholinesterase is present in the soluble fraction of *Squilla mantis* homogenate. The isoelectric point is 5.3. Although propionylthiocholine was hydrolyzed at a higher rate than other substrates, V_{max}/K_m values indicated substrates containing acetic acid (acetylthiocholine and acetyl-beta-methylthiocholine) showed a better affinity for the enzyme".

7. Pathology and nutrition

-Nutritional diseases

There are some cases of what could be called nutritional diseases. For example the imbalance in Ca/P or the source of phosphates can produce a "soft shell" as described in *P. japonicus* juveniles or other species (Lightner *et al* ., 1979). There was a case of molt death syndrome observed on the first species. Soft animals were observed also in case of severe starvation. But one of the most striking evidence of nutritional deficiency was described with the "black death disease" with an identification by previous author of a lack of dietary vitamin C. Another problem went with "blue shrimp" in *P. monodon* and the reason was a rancid feed that definitely changed the carotenoids metabolism: there exists also a modification of pigments in hemocyanin due to excessive Zn level (61ppm). Zn interferes in multi-enzymatic systems of organisms. Artificial feeds prepared for *P. monodon* were containing 157-357ppm Zn for an admitted concentration of 35ppm (G.I.P.M, 1973).

Other symptoms were not clearly identified for a nutrition origin such as "cauliflower aspect" or "white periopodites". That is about even though short antennae or cannibalism could be signs for sub-deficiency from the diets as well as poor weight gain and obviously mortality. A more recent problem appeared with *L. stylirostris* and *P. monodon* which revealed with what is called "red heads". This problem was analysed biochemically and an interesting result appeared at the level of fatty acids in polar lipids with a significant difference between red heads animals and control (Table 6). Such difference could be due to an episode of poor fish meal quality incorporated in the feed and a risk of auto-oxydation with an enhancement of peroxide activity. Such deterioration at fatty acids level could explain a weakening of the HP membrane (Aquacop, Sydney WAS meeting, 1988).

Table 6. Comparison of Pls from HP of *L. stylirostris* (Robin, 1988). ET=standard deviation.

	control		red heads		
	%	ET	%	ET	t test
C14-0	1.8	0.2	3.1	0.4	**
C18-1n-9	12.3	1.4	16.6	2	*
C20-4n-6	1.8	0.4	1.1	0.2	*
C18-3n-3	1.4	0.1	1	0.1	**
C20-5n-3	11.4	2.4	7.4	0.8	*
C22-6n-3	9.8	2	7	0.7	*

-Immune response and nutrition

β-glucans were tested on the blue shrimp in Tahiti where there was no pathogenic *Vibrios* and in New Caledonia where severe mortalities occurs due to the presence of *V. peneicidae* and *V. nigripulchritudo* and in final, it was not possible to demonstrate any protective effect of such polysaccharids, putting forward that administration of such compounds in the diet would not resist to the action of laminarinases present in the digestive tract.
Protein content of the feed seemed to have an incidence on immune response of *L. stylirostris* juveniles. Hemocytes varied from 20 to $45x10^{-3}$ at high protein content versus 15-30 $x10^{-3}$ at low protein content. However, there is little evidence of real impact of dietary protein on the immune response. And more investigation is needed along the area.

8. Genetics and nutrigenomics

Our strain P. *vannamei* is by far the oldest domesticated strain in the world (at least since1978). The dominant opinion that inbreeding is bad, if proved for species with low fertility, seems more than questionable in highly fecundity species such as shrimps. The proof is the results obtained in New Caledonia on a strain where inbreeding was maximum. One hypothesis could be that interest for domestication variability is created by genetic recombination at meiosis and the

Cuzon, G. 2010. Some Steps in Shrimp Nutrition «a linear way». En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J. (Eds), Avances en Nutrición Acuícola X - Memorias del Décimo Simposio Internacional de Nutrición Acuícola, 8-10 de Noviembre, San Nicolás de los Garza, N. L., México. ISBN 978-607-433-546-0.
Universidad Autónoma de Nuevo León, Monterrey, México, pp. 8-40.

selection is based on the combinations best suited to the environmental context and culture techniques and that adequacy between strain and rearing system in place is refined at each generation. see combinatorial chemistry techniques to develop medicines better adapted. (A. Michel, com.pers.). Before the consideration on nutrigenomics there was many studies conducted at COP on the enhancement of the SPR strain of *L. stylirostris* (Bedier *et al*., 1996) and the determination of the level of heterosis (Bierne *et al*., 2000). Such preliminary works help to assess the responses to genetic selection for growth under different diets and to control the level of digestibility of such diets (Dao *et al* ., 2002).

Studies on variations of digestives enzymes activities and the change in isoforms gave information on the level of adaptation to a diet after so many years of domestication (Arena, 2004). For the future, there is a perspective for more accurate level of comprehension on the role for example of animal protein vs vegetable protein sources. Transcriptomic response with the microarray method should provide an idea on the quantity of genes reacting according to a change in nutritional status as clearly shown already for a variation in environmental conditions in molluscs (LeMoullac, 2010; pers. com.). There is an open door for a more accurate knowledge on shrimp nutrition.

9. Texture and feed composition

Preliminary results on shrimp muscle texture have been obtained with a texturometer. The measure is quite delicate; therefore an attempt for analysing collagen content gave results that could be in relation to feed composition. The muscle proline could be measured as well, taking into account the level of ascorbic acid as shown before on the collagen formation in the muscle. This approach is promising because it goes for a better control of shrimp quality during this period of drastic changes in feed formulation due to the replacement of fish meal with plant protein sources (Ezquerra-Brauer *et al*., 2003; Maldonado *et a* ., 2010).

Cuzon, G. 2010. Some Steps in Shrimp Nutrition «a linear way». En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J. (Eds), Avances en Nutrición Acuícola X - Memorias del Décimo Simposio Internacional de Nutrición Acuícola, 8-10 de Noviembre, San Nicolás de los Garza, N. L., México. ISBN 978-607-433-546-0.
Universidad Autónoma de Nuevo León, Monterrey, México, pp. 8-40.

Conclusions

During those years we benefited largely from the team spirit and a constant impulsion to "make things happen" as the motto of Ralston Purina fiercely shown by Bill Mc Grath on his suitcase. Cnexo, then Ifremer, placed us constantly in a situation of R&D and the paradox was at start to be sent to Tahiti to work on shrimp in an environment completely deprived from species of commercial interest!…..It was a big challenge that pushed us in all directions of zootechny, pathology, water quality and feeding and nutrition. We started with feeding, before going for nutrition. It took time to get consistent results, hopefully we worked with open minded concept and collaborate with many institutes such as U o Kagoshima, SEAFDEC in Iloilo, RP in Crystal River, Galveston in Texas, and some colleagues in France (CNEXO Brest) in order to raise results and produce the transfer from experimental stage to pilot stage then demonstration for the field application at an economical level. The original idea was to export our know-how to the Tuamotu archipelago where huge places out of pollution were available….but our techniques at this time militated for earthen ponds system. So as there was not enough land space in French Polynesia we exported the technique for raising shrimp to New Caledonia. It is amazing how things changed so smoothly because it needed about 40 years to find that blue shrimp could be cultured in floating cages and then, today, there exists a kind of second chance for French Polynesia to develop some shrimp culture in lagoons of Tuamotu. In this context nutrition studies were permanently done during 5 years under laboratory conditions and as a "benedictan monk" some people ran and ran experiments in small volumes to raise some useful data to improve growth performances in different systems of culture and innovate in terms of larvae feeds, breeders feed as well as a unique opportunity to follow the change in adaptation of highly inbred strains of *L. stylirotris* and *L .vannamei* to a lower extent in the same environment. All those aspects are part a huge heritage for the benefit of local aquaculture and some papers would probably help future scientists dedicated to nutrition of penaeid shrimp in different parts of the tropical areas. However the new prospects are there and there is a real need for intensification as FAO claimed in recent San Diego WAS meeting and its corollary with active research dedicated to wastes management and control.

Cuzon, G. 2010. Some Steps in Shrimp Nutrition «a linear way». En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J. (Eds), Avances en Nutrición Acuícola X - Memorias del Décimo Simposio Internacional de Nutrición Acuícola, 8-10 de Noviembre, San Nicolás de los Garza, N. L., México. ISBN 978-607-433-546-0.
Universidad Autónoma de Nuevo León, Monterrey, México, pp. 8-40.

Acknowledgements

First of all this summary of a work mostly dedicated to shrimp nutrition would not have been productive without the fabulous start-up given by the team AQUACOP. This acronym once accepted produced a phenomenal team spirit that guided us all along those 40years or so.

References

Andrews, JW and Sick, LV. (1972) Studies on the nutritional requirements of penaeid shrimp. *Proceedings. World Aquaculture Society*, **3,** 403-414.

Arena, L. (2004) étude des populations de crevettes peneides; aspects génétiques du métabolisme des glucides chez L.*vannamei* (populations sauvages et domestiquées). Thèse EPHE, Paris Sorbonne, 195pp.

Barbieri, MA. and Cuzon,G. (1980) Improved nutrient specification for linear programming of penaeid ration. *Aquaculture*, **19,** 313-323.

Aquacop, Bourgeois, B. and Cuzon, G. (1975) sur le méthode de détermination des stades d'intermue et son application chez M.*rosenbergii* et P.*merguiensis*. Internal report CNEXO, 40pp.

Aquacop, (1975) Maturation and spawning in captivity of penaeid shrimp:*Penaeus merguiensis* de Man, *Penaeus japonicus* Bate, *Penaeus aztecus* Ives, *Metapenaeus ensis* de Haan and *Penaeus semisulcatus* de Haan. *Journal World Mariculture Society*. **5**, 123-132.

Aquacop (1979) Penaeid reared broodstock:closing the cycle of *P. monodon*, *P. stylirostris* and *P. vannamei.* Proceedings of the 10th Annual Meeting of *World Mariculture Society* **10,** 445-452.

Aquacop (1984) Review of ten years of experimental Penaeid shrimp culture in Tahiti and New Caledonia (South Pacific). *Journal World Mariculture Society* **15,** 73-91.

AQUACOP, Bédier E. and Bianchi M. (1988) Maintenance of water quality by bacterial systems in intensive tropical penaeid culture ponds : preliminary investigations. Présenté à *World Aquaculture Society* 1988, Hawaii.

AQUACOP, Bédier E. and Soyez C. (1988) Effect of dissolved oxygen concentration on growth and respiration of *Penaeus vannamei.* Présenté à *World Aquaculture Society* 1988, Hawaii

Aquacop and Cuzon, G. (1989) Selected ingredients for shrimp feed. In Advances in tropical Aquaculture, 20feb-4march, Tahiti, Aquacop/Ifremer, Actes Colloque, **9,** 405-412.

Bautista, MN. and Baticados, MCL.(1990) Dietary manipulation to control the chronic soft-shell syndrome in tiger prawn, P.*monodon* Fabricius. 2nd *Asian Fisheries Forum*, Tokyo (Japan) 17-22April 1989.

Bédier E., Patrois J., Aquacop (1996) Genetic enhancement of the *Penaeus stylirostris* Aquacop SPR43 strain. Divergent selection for growth: preliminary results. In Books of Abstracts, SICCPPS, SEAFDEC/AQD, Iloilo, Philippines, May 1996:p60.

Bianchi, A. and Bianchi, M. (1980) Participation des communautés bactériennes aux problèmes poses par l'élevage de crevettes peneides au COP/Tahiti.

Bierne N., Beuzart I., Vonau V., Bonhomme F., Bédier E., Aquacop (2000) Microsatellite-associated heterosis in hatchery-propagated stocks of the shrimp *Penaeus stylirostris. Aquaculture* **184,** 203-219.

Bureau, D.P., Azevedo, P.A., Tapia-Salazar, M. and Cuzon, G. (2000) Pattern and cost of growth and nutrient deposition in fish and shrimp: potential implications and applications. In: Cruz-Suárez, E., Ricque-Marie, D., Tapia-Salazar, M. and Civera-Cerecedo, R. (Eds). Avances en Nutrición Acuícola V. Memorias del V Simposio Internacional de Nutrición Acuícola, 19 al 22 de Noviembre , 2000, Mérida Yucatán, México 2000, 111-140.

Cahu, C.L., Guillaume, J.C, Stéphan, G., Chim, L., (1994) Influence of phospholipid and highly unsaturated fatty acids on spawning rate and egg tissue composition in *Penaeus vannamei* fed semi-purified diets. *Aquaculture* **126**, 159-170.

Capuzzo, JM and Lancaster, BA.(1979) Larval development in the American lobster:changes in metabolic activity and the O:N ratio. *Canadian Journal of Zoologie*., **57,** 1845-1848.

Carr, W.E.S. (1978) Chemoreception in the shrimp *Palaemonetes pugio*:the role of amino acids and betaine in the elicitation of a feeding response by extracts. *Comparative Biochemistry and Physiology.,* **61**A, 127-132.

Ceccaldi, H.J., (1978) La nutrition des crustacés. *Oceanis*, **4**, 55-62.

Chim,L., Lemaire, P., Delaporte, M., LeMoullac,G., Galois, R., Martin, JLM. (2001) Could a diet enriched in n-3HUFAs be considered a promising way to enhance the immune defences and the resistance of peneids to environmental stress? *Aquaculture Research*. **32,** 91-94.

Cousin, M. 1995. Contribution à l'étude de l'utilisation des glucides et du rapport protéine/énergie chez P.*vannamei* et P.*stylirostris*. Thèse INA/PG, Paris, 201pp.

Cowey, CB. and Forster, JR. (1971) the essential amino acids requirements of the prawn *Palaemon serratus*. Growth on diets containing proteins of different amino acid composition. *Marine Biologie*, **101,** 77-81.

Cruz and Ricque, (1998) Etude de la digestibilité des farines de poissons. Rapport final sur les critères des farines de poissons pour l'aliment crevette. STD program.

Cruz-Suarez, E. and Guillaume, J. (1983) Facteur de croissance inconnu de la farine de calmar pour la crevette japonaise: localisation de ce facteur. ICES. Meeting, Comm. Maricult.,CM 1983/F:**14**, 13pp.

Cruz-Suarez,E., Tapia-Salazar,M., Nieto-Lopez, MG., Guajardo-Barbosa, C. and Ricque-Marie,D.(2009) comparison of *Ulva clathrata* and the kelp *Macrocystis pyrifera* and *Ascophyllum nodosum* as ingredients in shrimp feeds. *Aquaculture Nutrition* **15,** 421-430.

Cuzon G. *et al* .(1994) Composition, preparation and utilization of feeds for *Crustacea*. *Aquaculture* **124,** 253-267.

Cuzon G., M. Williams, G. Gaxiola and C. Rosas (2001) Shrimp ration for the future. *Feed International*, June 2001, 4 pp.

Cuzon, G., Gaxiola, G. and Rosas, C. (2006) Nutrition in relation to Reproduction in crustaceans. Science Publishers, ed. E. Mente

Cuzon G., Goguenheim, J.,Gaxiola G. and Aquacop (2008) “Floc” contribution to peneid intensive culture. IX SINA, Ensenada, Mexico,

Dao T., E. Goyard, G. Cuzon, D. Bureau, G. Seynave and AQUACOP (2002) Assessment of the responses to genetic selection for growth in *P.stylirostris* under different diets

Davis, DA. (2009) Fishmeal extension and use of alternative proteins in shrimp diets. Presented at nutrition session in*World Aquaculture Society,* Veracruz,

Drach, P. (1939) Mue et cycle d’intermue chez les crustacés Décapodes. *Annales Institut Oceanographique,* Paris, **19,** 103-391.

Ezquerra Brauer, J., Salazar, JA., Alvarado, LB. and Rouzaud-Sandez, O. 2003. Effect of dietary protein on muscle collagen, collagenase and shear force of farmed white shrimp (*L.vannamei*). Europ. Food Res. technol., 217(4)277-280.

Fox, J. (2008) Estimation of methionine requirement of the Pacific White shrimp, *Litopenaeus vannamei,* using protein-bound and crystalline sources. Presented at nutrition session in*World Aquaculture Society,* Veracruz,

Gauquelin, F., Cuzon, G., Gaxiola, G., Rosas, C., Arena, L., Bureau, DP. and Cochard, JC. (2007) Effect of dietary protein on growth and energy utilization by *L.stylirostris* under laboratory conditions. *Aquaculture,* **271,** 439-448.

Gaxiola G, Cuzon G, Ceccaldi. (2009) Digestive physiology. In Browdy G and Jory D editors. The rising tide: proceedings of the Special Session on sustainable shrimp farming. World Aquaculture Society, Baton Rouge Lousiana USA ISBN: 978-1-888807-08-0.

Gaxiola, G., Ceccaldi, H.J. and Cuzon, G. (2010) Food *vs* feed: what makes the point with shrimp? J. Nut. Metabolism, *submitted.*

GIPM, 1973.

Hazel, JR. and Prosser, CL. (1974) Molecular compensation of temperature in poikilotherms. *Physiological. Review* **54**, 620-677.

Hill, FW. and Dansky, LM. (1950) Studies of the protein requirement of chicks and its relation to dietary energy level. *Poultry Science* **29,** 763.

Howell, BK. and Matthews, AD. (1991) The carotenoids of wild and blue disease affected farmed shrimp *P.monodon*, Fabricius. *Comparative Biochemistry and Physiology* **88,** 375-379.

Kanazawa, A., Teshima, S., Tokiwa, S., Kayama, M. and Hirata, M. (1979) Essential fatty acids in the diet of prawn: effect of DHA on growth. *Bulletin Japanese Society Scientific Fisheries* **45,** 1151-1153.

Leger, C., Luquet, P. and Boudon, M. (1975) Effects of cbh suppression in diet of RT kept at 10°C. evolution of body compartments w/ special reference to FA n-9, n-6 and n-3 series. *Annales Hydrobiologie.* Inst. Natl. Rech. Agro. Paris, **6,** 71-93.

Lightner, R., Hunter, R., Magarelli, PC., Colvin, LB.(1979) Ascorbic acid: nutritional requirement and role in wound repair in penaeid shrimp. Presented 10th Annu. Meet. *World Aquaculture Society*; Honolulu (HI) USA, 29 Jan. 1979.

Maldonado, C., Cuzon, G., Guzman, E., Brito, R., Soto, L., Arena, L. and Gaxiola, G. (2009) Effect of an herbivorous diet on energy balance of *L.vannamei* at selected ontognetic stages. *Aquaculture*, **296,** 123-128.

Martin Y.P. and Bianchi, M.A. (1980) *Microbiology Ecology* **5,** 265-279.

Melcion, JP., Guillaume, J. Méhu, J., Metailler, R. and Cuzon, G. (1983) Préparation d'aliments pour animaux marins par cuisson-extrusion. *Alimentation Animale*, **371,** 26-31.

Mock Corny, (1980) comm. pers.

Nose, T., Arai, S., Lee, DL. and Hashimoto, Y. (1974) A note on amino acids essential for growth ot young carp. *Bulletin Japanese Society Scientific Fisheries* **40,** 903-908.

Principato, GB., Talesa, V., Giovannini, E., Pascolini, R., Rosi, G. (1988) Characterization of the soluble cholinesterase from *Squilla mantis*. *Comparative Biochemistry and Physiology* **90**C, 413-416.

Robin, J. 1988. Comparison of Pls from HP *L.stylirostris.* Proceedings Meeting *World Aquaculture Society,* Sydney.

Samocha, TM., Patnaik,S., Speed, M., Ali, AM., Burger, JM., Almeida, RV., Ayub, Z., Harisanto, M., Horowitz, A., Brock, DL. (2007) Use of molasses as carbon source in limited discharge nursery and grow-out systems for L.*vannamei*. *Aquaculture Engineering* **36,**184-191.

van Weel, PB. (1974) "Hepatopancreas?" *Comparative Biochemistry and Physiology*. **47**A,1-9.