The Effects of Animal or Plant-Based Diets on Energy Partitioning in Selected Ontogenetic Stages of the Shrimp *Litopenaeus vannamei*

Gérard Cuzon[1], Abelardo Brito[2], Luis Jiménez-Yan[3], Roberto Brito[4], García Tomás, Gabriel Taboada, Gabriela Gaxiola[5]

1.- COP-Tahiti, IFREMER, Francia
2.- Posgrado de Ciencias del Mar y Limnología, UNAM
3.- Universidad Juárez Autónoma de Tabasco
4.- Universidad Autónoma del Carmen
5.- Unidad Multidisiplinaria de Dcoencia e Investigación de Sisal, UNAM.

Abstract

L.vannamei is considered as an omnivorous species with a trend to be herbivorous. Some attempts to raise juveniles at low dietary protein under laboratory conditions showed a feasible way at least in terms of weight gain (0.60 versus 0.47g live weight for PL52 raised on animal and plant diets respectively). *L.vannamei* is a promising species from this point of view to examine ontogenetic variations of its performance whether receiving animal or plant-based diets. Also, it is interesting to follow the performances of juveniles on a monotonous dietary treatment or with a drastic change between plant base (2.5g weight gain) and animal base diets (3.1g weight gain). It is the purpose of this work because data are presented at larval and postlarval stages. Performances can be produced in terms of recovered energy for larvae (5.2 J/larvae/day), and it helps to propose the range of variation larvae exhibited while fed both dietary treatments and measure in particular digestible energy intake (1.1J/larvae/day at Zoea I), feces production on a basis of 82% digestible energy, ammonia excretion (0.02 J/larvae/day) and respiration (0.48J/larvae/day). Postlarvae required between 1 and 3J/Pl's /day that is in agreement with findings on other peneid species. Final assessment is made on a possible improvement in terms of energy expenditure in the two situations (PA and PV) and for the main periods of the life cycle (larvae, Pl's, juveniles and pre-breeders). Energy demand is increasing with development stages and values will be used to explore a model of feed distribution which should be adequate to maintain animals in optimum growth with a minimum wastage and a maximum water quality. Moreover, it is of importance through the energy expenditure in both cases (PA and PV) to see up to which extent animals are going to sustain on all plant diets while keeping performances (recovered energy in a range of 10-20% for juveniles) to get enough growth rate with a minimum of animal marine protein utilisation as a main source for this species.

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Introduction

Omnivory is the most common feeding habit of peneids. Examples are common *with P. monodon, L.vannamei, P. indicus*, etc, and among species one is particularly *carnivorous (P. japonicus*) , two close related (primitives) species *L.stylirostris* and *L. vannamei* seems to differenciate in carnivory trend (*L.stylirostris*) or herbivorous trend (*L.vannamei*) based on previous work (Cousin, 1995): Regimen for *L.stylirostris tend to be* carnivory *(*DE 15kJ/g and protein-30-35) and *L. vannamei* more herbivory with 16-17kJ/g and dietary protein<25-30. Those requirements where determined with diets based on a large animal protein fraction (squid-casein based dietes) ans did not forecast of the ral potentialities of both species to do with plant protein (soybean, wheat, peas, etc.). However, in experimental tanks it is known the relative good acceptance of soybean in large proportion (30-40%) in *L.vannamei* diet. In ponds, it has been reported from stomach contents observation, a presence of benthic diatoms (*Navicula sp*.). That is one of the reasons why *L.vannamei* was choosen to stydy the potential replacement of fishmeal in the diet, a maximum acceptance level of starch and to study ontogenetic variations in digestive enzymes. Such work came on top of polymorphism consideration, according to the level of domestication of the species (Arena, 2004). Finally, progress made in feeding at early stages with plant protein sources and continuation up to breeders size could lead to select strains which possess good ability to grow under feedig condtions including high-carb diets (cbh) coming from plant protein sources.

L. vannamei and other related species received more attention in recent years first at larval stages to relace *Artemiai* (Jones et al., 1997; Gallardo et al., 2000; Gaxiola et al., 2000; Durruty, 2001) and following the work of Lovett and Felder, (1990a and b) on ontogenetic variations of digestive enzymes came to the point where live food sequence was to be replaced successfully with inert particles. From those experimentations several concepts arose such as the secretagogue effect and the level of polymorphisme that larvae or juveniles could reach without affecting their physiological status. Moreover, it came as a rule to make an assessment on energy budget in spite of the current complexicity to get a correct estimate of food intake for example with *L. brasiliensis* (Gaxiola et al., 2004).

Among peneids species *L.vannamei* is probably the most suited for utilization nutrients from vegetal origin as well at larvae stage or at juvenile stage.

Plant materials will provide starches and proteins. Ingredients such as wheat gluten, peas, canola, soybean, etc; there is a wide range of substances under native state or processed, and in this case inder meal, concentrate or isolate, with a variant represented by hydrolysates (gluten, rice, soybean, etc). Future possible impact on formulations for larvae and juveniles is more than expected in face of so many constraints regarding environment.

It is interesting to find a relation between food replacement in larvae or juveniles and chages in physiology leading to a possible variation in energy expenditure and in consequence in digestible energy (DE) requirement. It means to produce evidences at several levels such as protein sources, protein digestion, amino acids degradation, level of energy derived from protein, ratio of protein to energy in the diet, latitude of action on digestive enzymes at early stages, possiblity to induce a shrimp selection towards a higher utilization of cbh as an energy source.

This paper reports with preliminary work done on *L.vannamei* at several stages of its life cycle with some comparative aspects.

Development

Regarding variations in energy requirement in selected ontogenetic stages, the consideration of plant sources will lead to examine two major nutrients, starch and protein and their effect in association in the diet. Energy partioning during larvae development is a function of feed intake and also a function of temperature that is going to influence basal metabolism (Hem). Temperature is an important factor. Water temperature in a hatchery is set at around 27°C but it is common to elevate temperature up to 29°C for example with *L.stylirostris* larvae production to shorten larvae period (7 instead of 10 days). If one considers an increase in basal metabolism (Hem) there is a need for higher energy intake. This can be done by increasing feed distribution (amount of feed) or multiplying the frequency of feeding or the energy density of the microcapsules. However, in the following results, the temperature will remain at 27°C to avoid an additonal source of variation.

1. Starch

Starch should be considered as a primary energy source for shrimp and if it is considered formulations for grower for example (Devresse, 1988) it is regularly found a substantial percentage around 20%. It is necessary however to analyse the differences between starch of several plant sources, variations according to the stage of development and to evaluate the ability of organisms to digest raw or gelatinized starches.

1.1 Apparent Digestibility Coefficient according to starch presentation (i.e. native starch or precooked starch, (Cousin, et al., 1996).

The ADC values were around 92% for at inclusion level of 35% in diet as found in a previous work indicate for *L.vannamei* juveniles raised during 28 days on two different experimental diets. No significant difference appeared between native or gelatinized wheat starch.

α-Amylase hydrolyze more readily the α-1-4 link, easily accessible in starch rich in amylopectin, such as wheat starch. Starch such as corn starch rich in amylose is less digestible (63%) than wheat starch (92%) because of its high amylose content (Cousin et al., 1996).

Specific activities values are given for α-amylase: with a substantial higher values in shrimp taken from the wild (Culiacan) compared to domesticated ones (Table 1) despite a variation in size.

Table 1 Specific enzymes activity (U/mg protein) measured on *L.vannamei*, three strains, 2 diets.

	Culiacan	Sisal	Tahiti
hcbh	40	14	4
lcbh	30	20	5

One can assess than the digestion of starch by shrimp taken from the wild is improved and glyceamia is regulated enough (even though the postprandial value remained high for a longer time than with the lcbh diet), regulated enough to avoid a down-regulation of amylases. Then, at metabolic level, one can expect a satisfactory level of phosphorylation of glucose to enter the metabolism and provide enough G 6-P whether for glycogen synthesis, or glucosamine formation or energy yielding through the glycolytic pathway.

1.2. Dietary starch levels

As previously said, routine level of incorporation in a grower diet was used to be 20% and for digestiblity studies it raised up to 35% then in a way to go to the limit, diets were formulated with up to 40% starch. (Arena, 2004). Comparison between 4/40% levels and effect on growth were examined on *L.vannamei* and in relation with energy expenditure.

1.3. Starch and its protein-sparing effect

Energy expenditure could be evidenced at the level of heat increment (HiE) with a large difference between the two treatments 4/40% starch levels in relation with a possible higher energy demand in the deamination process. This is a 60 year-old theory but it is probably untrue. Several explanations are given such as (i) a more rapid growth will lead to a lower maintenance level (ii) retained energy is mainly used to synthesize body protein then at this point, amino acids efficiency is larger than the one of lipid or carbohydrate (Guillaume, pers. com.). A contradiction still remains between the fact that HiE is higher in case of a load of protein; and energy of amino acids is better utilized as amino acid net energy is higher?

Arena (2004) found a protein sparing-effect of energy in form of starch primarily in an attempt where dietary protein was low (30%) and wheat starch was high (40%). This result achieved only on a wild population will receive attention in the future.

1.4 starches as energy source during development

a) Larvae

In spite of a possible improved response in development stages in larvae fed inert diets, the composition of particles were made with raw starch instead of gelatinized starch (Gallardo et al., 2001) and apparent development was not so different of the control receiving live food as far as carbohydrate was concerned. There is a trend however for higher specific activity of alpha amylases (0.1mU mg protein^{-1} µg dw^{-1} versus 0.03 with live food sequence ;Gallardo et al., 2004) if *L.vannamei* Mysis received inert particles with up to 68% cabohydrates (cbh) because it include some complex cbh with a gum used for the µcapsules formation.

b) Postlarvae

Postlarvae *L.vannamei* showed similar performances (Fig.1) during a comparative growth trial ($p<0.05$) with a range of size slightly higher (120µg dry weight versus 95µg dry weight with PA and PV respectively); however with PA, itcan be attributed such a benefit in weight gain to a higher feed intake than with PV producing a more favorable recovered energy (RE) for protein synthesized in muscle tissue.

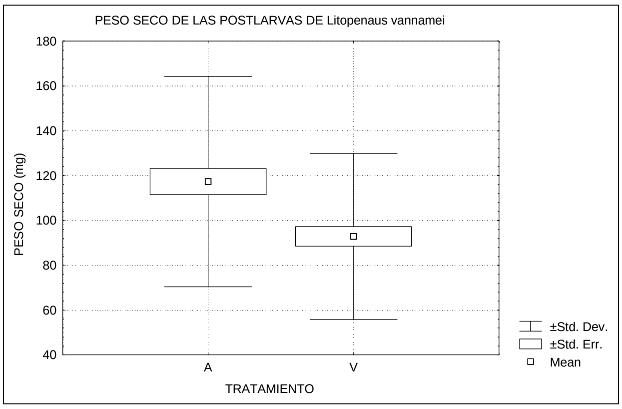

Fig. 1.Performances of postlarvae fed 52 days whether on PA or PV diets.

c) Juveniles

Rosas et al., (2002), developed a conceptual model for cbh utilization by shrimp with a metabolic approach. An indication of flexibility of shrimp to use pathways according to the requirement in energy or metabolites in a precise moment of the intermoult cycle. It focused on the importance of cyclic metabolism and the pervasive influence of the moult cycle. Is this metabolism influenced equally by the nature of the diet? A comparison of two distincts diets (Table 2) showed that to a certain extent and with the large enzymatic equipment juveniles can rely on, there was a significant difference in weight, on a short period of assay (Table 3) if diets are compared, and in the same time it gives an indication on the potential for cbh utilisation as an energy source in spite of the fact that diets were not strictly isoproteic.

Table 2. Diets composition of low cbh (PA) and high cbh (PV) for juveniles *L.vannamei*.

Diet	(PA)	(PV)l
carbohydrate	3	30
lípid	11	11
DE kJ/g	16	16

Table 3 Results on growth performances. Average weight initial and final in g. PA: low cbh diet; PV: high cbh diet. Superscript letters give a significant difference ($p<0.05$).

diets	replicate	inicial wet weight (g)	final wet weight (g)
PA	1	3.62 ± 0.09^{a}	6.9 ± 0.19^{a}
PA	2	3.5 ± 0.08^{a}	6.65 ± 0.14^{a}
PA	3	3.7 ± 0.087^{a}	6.72 ± 0.14^{a}
PV	1	3.56 ± 0.087^{a}	6.14 ± 0.14^{b}
PV	2	3.54 ± 0.09^{a}	5.88 ± 0.16^{b}
PV	3	3.54 ± 0.077^{a}	6.14 ± 0.14^{b}

These diets were consumed in a similar manner whatever composition and gave acccess to weight gain because no particular cannibalistic tendency was observed that could be linked to a severe deficiency or imbalanced diets.

1.6. Starches as energy source

There is no carbohydrate requirement properly speaking; glucose can be produced through certain amino acids or lipids. Is is clear however, that growth is influenced not onlyby carbohydrates level (starch at 4 or 40% in the diet for example) but more evidently by the nature of carbohydrates. For example glucose can inhibit growth, while starch let shrimp grow (Pascual, 1988) and trehalose was mentioned to promote growth (Kanazawa, 1986). Calculation from an energy point of view was conducted on a basis of digestible energy with an average value 15 kJ/g (Cuzon and Guillaume, 1997) used to control isoenergetic level of any series of experimental diets.

1.7 Metabolic utilization of diets with plant protein and starch

From actual results, there is evidence that *L.vannamei* larvae or postlarvae possess a real potential of adaptation to the regimen (Brito et al, 2004) because comparative feeding trials showed a similarity in performances between diets PA and PV. In case of a diet without starch such as diet PA, at metabolic level, a shift to neoglucogenic pathway from pyruvate and/or lactate with the lactate dehydrogenase (LDH) is observed:

pyruvate ⟶ lactate (with influence of an enzyme LDH)

Lactate plays as an energy source while replacing glucose (Fig.2). Glucose derives, in case of feeding on diet PA, from glycogenic amino acids (non-essentiel amino acids principally) to

keep glycemia at around 0.3mg/ml of hemolymph in conditions out of post-prandial stage during intermoult period C).
In case of important load of glucose (case of diet PV) glycolytic pathway predominate, tissues enzymes activities increase, glucose goes along successive steps leading to Krebs cycle with energy supply (Fig.2).

$C_6H_{12}O_6+6O_2 \longrightarrow 6CO_2+6H_2O+673$ kcal/mole of heat (H_2 and O_2 release energy)

In case of a low carbohydrate diet, that is the case with diet PA, shrimp metabolism takes glycolysis pathway in reverse direction to bring glucose necessary for tissues. In this way, glucogenic amino acids take Krebs cycle to transform in lactate, pyruvate, substrates absorbed in neoglucogenesis pathway. Recent results on *L.vannamei* Pl's (Brito et al, 2004) indicate an important presence of lactate (180 mg/ml in hepatopancreas). This level is slightly higher than the one found in crab (38 mg/ml) or the one found in shrimp fed with the diet PV (120 mg/ml).

It translates correctly the adaptative level of postlarvae to a given feeding regime. Once again it shows the panel of possibilities that this species exhibit to adapt to a quality feed (essential amino acid composition, digestibility of starch) that is given in culture.

During intermoult cycle in stage C, energy substrate via glycolysis, predominate as it happens in other Crustacea; however, in *L.vannamei* Pl's lactate in animal fed PV is lower than with PA. Diet rich in animal protein sources (PA) there is a lack of dietary CBH that enhanced probably the neoglucogenic pathway (Fig. 3) to maintain glycaemia (Fig.2). Glycaemia is quite similar in intermoult stages with both species except in late premoult ($D_{1'''}$) where HMP pathway for ribose formation (precursor of nucleic acids) could be the main pathway. Of course, tissue enzymes activities would come to confirm those hypotheses in a further work.

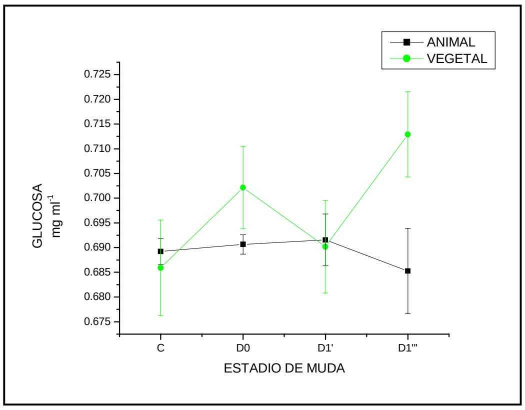

Fig 2. Glucose from last sampling early juveniles and 4 intermoult stages. Notation C=intermoult, D_0, $D_{1'}$, $D_{1'''}$=premoult. (+ -) signs different represent significant difference between treatments or moult stages (p<0.05).

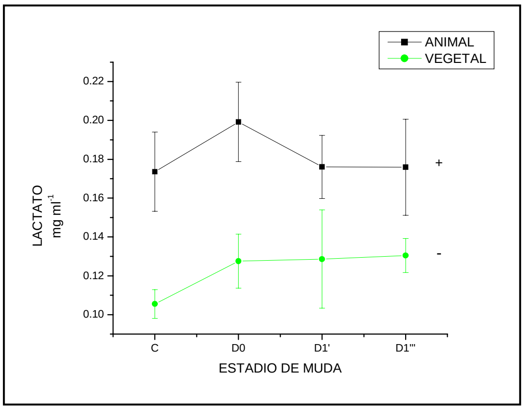

Fig 3. Lactate from last sampling early juveniles and 4 intermoult stages. Notation: C=intermoult, D_0, $D_{1'}$, $D_{1'''}$=premoult. (+ -) signs different represent significant difference between treatments or moult stages (p<0.05).

2. Protein

Protein digestion rely on a proteolytic equipement similar to the one described in more evoluted species. Trypsic activity is linked to carboxypeptidases A and B and Leu-aminopeptidase to hydrolyze protein substrate as it is common to vertebrates.

Protein are generally well-digested by shrimp, there is a range of 90-92% for squid, casein in *L.vannamei* or *L.stylirostris* (Cousin et al.,1996) or values from Akiyama et al.(1991) on soybean meal(SBM), and it is not possible to give a large difference between both categories of protein sources. One point with SBM could derive from antitrypsic factor present in this ingredient depending on the level of cooking.

2.1 Digestibility

The enzymatic equipment is efficient to digest protein from animal or plant origin and the only limitation would come from the presence of antitrypsic factor brought with soybean meal for example in case of unsufficient processing (that rarely occurs in practice).

Protein digestion is completed with proteases; enzymes such as trypsin is the major one, carboxypeptidases, aminopeptidase and a protease of low molecular weight (MW=11000da) previously examined (Ceccaldi, 1987).

Within hepatopancreas cells, endopeptidases produced a change of trypsinogen in trypsin and under enterokinase action chymotrypsinogen to chymotrypsin through trypsin action and elastases leading to peptides bonds and in hepatopancreas lumen, exopeptidases including carboxypeptidases A and B also aminopeptidases and dipeptidases from enterocytes release amino acids.

2.2 Comparison animal / plant based diets

Animal and plant protein sources were tested for their effect on *L.vannamei* PL's. On the following basis: a diet PV with soy protein (30%), gliadin and glutenin from wheat gluten (6%), wheat flour (9%), algae protein from Spirulina (21%) and a diet PA based on squid (31%), shrimp meal (15%) and fish meal ((9% plus $CPSP^{70}$, 9%) were formulated.

The question was aised concerning a possible negative incidence of plant protein on growth and survival at different stages of the life cycle? A contrario, the mixture of marine protein sources would benefit to the shrimp because of an excellent amino acid profile and a high ADC protein plus a good appetibility.Lipids are not going to be considered as a source of difference between the two formulations because of the range, 6-11% that is acceptable for normal growth and there exits a balance between neutral (4-5%) and polar lipids (2%) and n-3HUFAs would reach 0.8%; minor ingredients such as $CPSP^{70}$ with low molecular peptides acting as an attractant like fish solubles (WAS 2004) is recommended at 5% in PV diet.

A difference in composition do not represent a major impact on the final result due to the range (8 points of protein).Amino acid profile of dietary protein cannot evidence major difference at least on basic amino acids; for PV; PA respectively in g as fed: LYS; 1.9; 2.2 ; ARG; 2.2; 2.4; HIS; 0.8; 0.8; THR; 1.2; 0.4; LEU; 2.7; 1.4; ILE; 1.6; 0.9; VAL; 1.8; 1.4; PHE; 2.1; 1.1; MET; 0.9; 1.4; TRP; 0.4; 2.2. a difference do not interfere with the overall evaluation of protein sources. Basic amino acids :are important for shrimp nutrition and in particular Arg with a role in osmoregulation, as a phosphagen in muscular contraction and

Arg is also considered for its secretagogue properties (on secretions of insulin and hGH) and immunoregulator, linked to its precursor quality for radical nitric oxide. Branched amino acids given as large neutral amino acids (LNAA) comprise Thr that is important in fast growing animals.Limiting factor for formule.PV tend to fall on Lys especially if wheat gluten is present in large amount but in the same time that leaves place for eventual supplementation provided that amino acid be coated in fish gelatin for example. Concerning fatty acids, the n-3/n-6 ratio=0.84; no requirement in carbohydrates makes the difference between the two diets (20 and 7 %) of little significance.Gross energy (GE) was expressed in kcal/100g: 305 and 325 for PV and Pa respectively and digestible energy (DE) showed diets more or less with the same energy density: 11 and 12 kJ/g for PV and PA respectively.

a) Larvae

Average results on respiration (R) calculated for two nutritional regimes gave a mean value of 0.20 J larvae^{-1} day^{-1} for µcapsules MDKH and 0.23 J larvae^{-1} day^{-1} for live food sequence (LFS). Such values were higher than those reported by Kumarly et al (1989b), with an average value of 0.11 J larvae^{-1} day^{-1} but lower to those reported by Lemos and Phan (2001) for *F. paulensis* (average value of 0.48 J larvae^{-1} day^{-1}). Assimilation values (ME) between M_I and PL_1 were almost 50% lower in larvae fed with MDKH (1.12) and LFS (1.34), than reported values (2.5 J ind^{-1}) for *F.paulensis* (Lemos and Phan, 2001).

On the other hand, production values (RE) required between M_I and PL_1, of larvae fed on MDKH (0.36 J ind^{-1}) and LFS (0.43 J ind^{-1}) are in the same order of magnitude to those reported by Kumarly et al, (1989b) for *P. monodon* (0.55 J ind^{-1}), while lower (1.51 J ind^{-1}) than for *F. Paulensis* larvae (Lemos and Phan, 2001). Finally, and in relation to average net production efficiency (K_2) per substage both larvae fed on MDKH (23%) and those fed on LFS (27%) maintained very low values compared to *F. Paulensis* larvae (62%, Lemos and Phan, 2001) or *P. monodon* larvae (48%, Kumarly et al, 1989b), which could indicate that *L. vannamei* mysis were less efficient in assimilated energy (ME) to transform it into growth (RE). Insufficient growth rate under our laboratory conditions this time was observed. However, energetic balance points out that artificial food could supply equivalent DE, as supplied by living food, to achieve a whole replacement of live food to larvae in a *L. vannamei* commercial hatchery. Microcapsules formulated with a marine hydrolysate (krill or

CPSP[70]) led to a successful replacement of *Artemia* nauplii from mysis to PL's stage under the rearing conditions set in this study (Gallardo et al., 2004).

b) Postlarvae

Gaxiola (1994) working on *L. setiferus*, described a series of casein-based diets varying in protein /energy ratio to demonstrate at high dietary protein levels (40-60%) a better growth when dietary energy remained lower than at high energy level (Fig. 4). By and large, Pl's showed an improve weight gain from a P/E ratio equal to 32 mgprotein/kJ or above and such value is achieved with 40% CP and more.

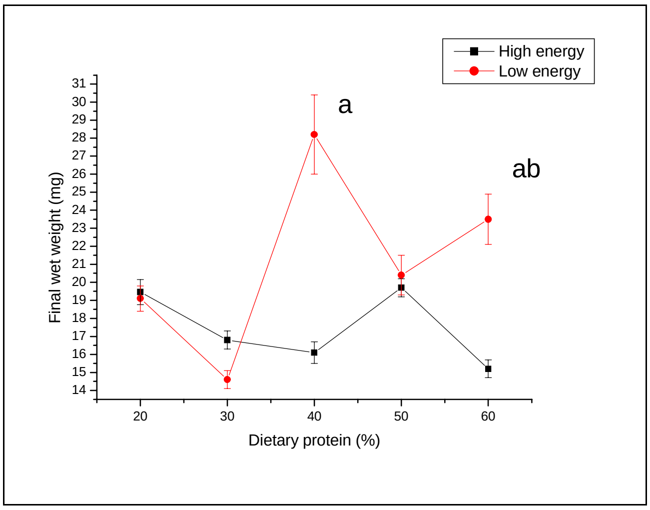

Fig.4. Protein energy ratio on Pl's *L. setiferus* in experimental conditions (Gaxiola, 1982).

This experiment was feasible because carried out on postlarvae that accept a rather high level of dietary lipid. However, it contrasts with experiment results on juveniles where Cousin et al. (1992) could not find a possibility to raise DE above a certain level (18kJ/g) and faced a constraint linked with a high dietary lipid level. It led to some shadow areas (high DE and low protein) in the three dimensions graph with isopleths of growth or contour lines (Cousin et al., 1995). This example with Pl's giving an optimum protein-energy ratio at high lipid level (probably not fully digested) and an animal protein source (casein) should be duplicated with a plant protein source such as soy concentrate or native wheat gluten.

c) Pl's and juveniles

Postlarvae (noticed (2) on the Fig,) during their development did not show difference during early stages as far as digestives enzymes was concerned (Fig.5) and only at last sampling at juvenile (3) there was a difference between the two treatments. Values of chymotrypsin in hepatopancreas concurred with the two diets but contrasted with data from previous work on *P. monodon* and *P.penicillatus* (Tsai et al.,1986) giving much lower values (23mU/mg protein) than on *L.vannamei* (4000 U/mg protein). Trypsin values found with PA and PV diets were 15 and 30 U mg protein-1 respectively. Juveniles raised with plant protein sources had a higher trypsin activity possibly in relation to hydrolysis sites that are less accessible to enzymes.Different explanations could be produced at this stage of the study. Increase in trypsin activity while other diets are substituted to *Artemia* may be interpretated as a response to substrate scarcity or indigestibility (Harris et al, 1986).

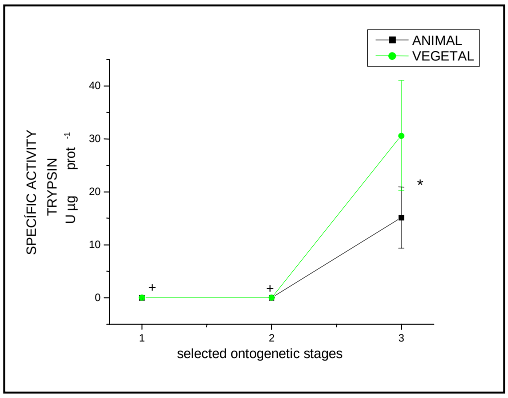

Fig 5. Trysin specific activity (U µg protein^{-1}) in selected ontogenetic stages of *L.vannamei* fed PA or PV. (Brito Bermudez, 2004)

Juveniles do not present any lipid accumulation and it means that whatever diets given to juveniles, there is a use of nutrients in an adequate way and no energy wastages because it is neither shown lipid depot in tissue except in hepatopancreas. It means that lipid even in excess is metabolized or expulsed in feces, and carbohydrates transformed to energy or precursor of glucosamine for chitin synthesis without deposition in neutrallipids. It is a useful predisposition of shrimp and another aspect of its flexiblity regarding nutrients utilization with a great prominence for protein utilization, whether from animal or plant source.Energy partitioning is an adequate tool to evaluate amount of joules remaining for protein deposition enoug (Table 4), and enzymatic system showed an adaptation according to protein sources.

On the long term additional aspects should be consider such as organoleptic properties of ingredients for succulence of the diet. Level of immune response is of concern with reductino of FM in PV diet but the achievement of a breeders production and successful reproduction for further selection approach seems one of the main point actually to obtain new strains that can be raised on PV type diets (high cbh content and low Fm content).

Table 4.-Bioenergetic values (Joules h^{-1} mg^{-1} wet weight of juveniles *L. vannamei*.

	DE	respiration	excretion	SE	RE
PA	4.6	0.14	0.02	0.2	4.2
PV	4.7	0.14	0.01	0.2	4.2

Notation. DE=digestible energy, respiration=routine energy and heat increment of feeding, ammonia excretion in starved condition and post-feeding ammonia excreción energy, RE=retcovered energy, SE= exuvia energy (5% RE).

2.3 Level of replacement of fishmeal (FM) by plant protein in 20g juveniles during a laboratory trial in experimental tanks.

There is during this experiment of all fishmeal replacement (trial ttvk, 1999) a post weighing mortality with large size shrimp that received in their diet not a single gram of fishmeal. In this case, the hypothesis is made for a situation of "fragile" animals due to several reasons that need to be investigated. One of it can be a lack of energy intake in a situation near inanition (Comoglio et al., 2004). Juveniles were analyzed for condition: moulting stages and physiological parameters (CO) and growth, and final appearance (Table 5). Definitely, feed intake was not the same in both treatments and performances differ; also tissue texture and fragility of animals after weighing operation were noticed (Aquacop, 2000). This preliminary result set the ground for aspects to be improved at formulation level to get enough energy to be channelled into growth with animals receiving a diet without fishmeal content.

Table.5. Trial for comparison .(pm: mean weight, CO for osmotic pressure +/-SD, HSI for hepatosomatic index, moult stages) on *L.stylirostris* pre-breeders fed one month in individual indoors tanks on a regular practical shrimp diet (PGN) or an all-plant diet (ttvk).

	PGN	ttvk
mean wt(g)	16+/-5	14+/-4
CO	192+/-13	216+/-25
HSI	3.9+/-0.9	3.4+/-0.7
molt stages(n)		
B_2	1	3
B_2-C	1	1
C	4	3
C-D_0	1	
$D_{1'}$	1	1
$D_{1''}$		1

2.4 Metabolic utilization

2.4.1 Brief background:

Most shrimp are omnivorous with some carnivorous habits. They deaminate much of their dietary protein using carbon residues as an energy source (Cowey, 1994). For example, about 40 % of dietary protein is retained by the fish, of that 60% is lost or oxidized. It means that whole body composition as a basis for eaa pattern is questionable. But especially, much of protein synthesized in fish or in shrimp is broken down. The rate of protein synthesis in the muscle is much lower in shrimp than in mammals. Turnover of protein is low (Mente et al., 1998). But is there an incidence of dietary protein on tissue enzymes? At the difference with mammals where formation of enzymes increases with lot of dietary protein in the diet, fish or shrimp do not show any adaptation of enzymes in response to protein level in the diet. Tissue enzymes (deaminases, urocanase) activities are probably limited by substrate concentration (Amouroux et al., 1995). Utilization of dietary energy in shrimp compare to fish with excreted products and heat lost leading to a recovered energy (RE) that is in a range of 10-20%. Heat lost is the main variation component of the energy budget with HiE (heat increment of feeding) which can vary largely (see paragraph below) and maintenance making for amout 20% of heat lost. All other parts of the budget, in terms of digestible energy and metabolizable energy are, by and large, in the same order of magnitude of what was found in trout.

2.4.2. Incidence of the nature of the protein

Teshima (1995) explained that protein sources had an influence on energy flow and for example between animal protein sources (crab, squid, casein, white fishmeal) and plant protein (soybean concentrate) the difference came not so much from DE but from heat production (HiE+Hem+activity). Values of 43-59% with animal protein and 65% with soybean concentrate (SBC). Apparently shrimp fed with SBC ate less, were more active in search for food, triturated pellets many time prior to ingestion….There is a connotation which proceed more from behavior rather than metabolism even though incidence on energy budget appeared clearly. RE (recovered energy) that is in a range of 9-20% can easily become nil if feeding activity is exacerbated in relation to one component of the diet. Rosas et al. (2002) made similar observations with *L.vannamei*. Finally, whether under experimental conditions or in ponds, each time consumption of feed is poor, feed intake is low, shrimp stimulated in search of food increase activity. Activity increase heat production (HP) and reduce the part of energy normally allocated for growth (RE).

3. Energy budget

The energy partitioning of a Crustacea is of course largely dependent on energy intake. Energy intake that paradoxically is the parameter controlled with a low accuracy due to the behavior of the species. However it is suggested to take an example of a crab (Table 6) to show a case of species eating all the time minute amounts of food at the difference with cultured species that receive given fixed amount at regular intervals.

Table 6 Cumumative energy budget in crab larvae in joules and relative value (%).Dawirs,1983).

	joules	%
intake	28	
egestion	22	77(DE)
excretion		
metabolism	53	19(ME)
growth	0.9	3(NE)
exuvia	0.2	1(SE)

Energy channelled to growth (about 4%) with a Crustacea that presents a constant feeding activity and drawn according to the same model of presentation as above indicates a relative ly low percentage derived for protein synthesis.

a) Larvae

Larvae development depend directly as well on temperature and it is common to raise up to 30°C water temperature (Goguenheim, 2004, com.pers.) in order to shorten the duration increase metabolism and get Pl's at $J_{9\text{-}10}$ on *L.stylirostris* instead of J_{12} at 27°C (Aquacop, 1984). Metabolism is increased and protein deposition follows this elevation with in final, provided that feed is given in sufficient amount, recovered energy (RE) can increase but there is no report on energy expenditure (Aquacop, pers. com.).

An estimate of energy intake at 27°C was calculated from a standard larvae rearing procedure in small flasks (Gallardo et al.,2004) in order to assess the metabolizable energy (ME) that is around 1J/larvae/day (Table 7). It is quite difficult to calculate the partitioning of energy in larvae, and the example below is given for a mixture of algae and animal protein sources in order to obtain an idea of the proportion derived from energy intake? Approximations are the rule in absence of precise intake measurement and in final there is an indication of the budget. It adds to the complexicity if microparticules are added to the food sequence. Table 7 gives the range of variation of DE requirement to maintain a development leading to survival (>70%) and development index (DI) comparable to live food sequence used as control (Table 7). Order of magnitude on values for respiration (0.13J/mg d wt/h) and energy content of the whole larvae (22J/mg) can give a possiblity to buckle the budget.

Table 7.Energy utilized by larvae *F.brasiliensis* during hatchery from a digestible energy point of view (Gaxiola et al., 2004), fed mixte food (PV, PA).

day	stage	*Chaeto* 12 J/mg	*Tetra* 18 J/mg	*Artem.* 17 J/mg	growth mg fresh
t_0	N				
t_2	ZI	80000	6000		0,05
t_3	ZII				0,1
t_4	ZIII				0,3
t_5	MI			0,03	0,5
t_6	MII			0,06	0,6
t_7	MIII			0,09	0,7
t_9	PL			0,15	0,8

day	stage	reqt DE	J/larva/day Hem + HiE	J/larva/d (UE + ZE)	J/larva RE	allowance J/mg/d
t_0	N					
t_2	ZI	0	0,001	0,02	0	
t_3	ZII	0			0,024	0,003
t_4	ZIII	1			0,06	0,06
t_5	MI	0,2	0,002	2	0,12	
t_6	MII	3			0,18	0,012
t_7	MIII	3	0,018		0,18	0,014
t_9	PL	4			0,24	0,02

b) Postlarvae

Energy cost consecutive to *Artemia* ingestion (ART+MCD+algae) is too high (1.233J/larvae) and RE is not increased in proportion giving a negative balance (-3joules). Finally, a best ratio feed/metabolism is obtained with MCD or MCD+algae thanks to a low metabolic expense (0.3J/larvae) leading to a balance–1 more favorable than with other test diets (Table 8).

The energy budget let to control some feeding situations in hatchery and help to correct the feeding amout and so the water quality; for example there exists some calculations showing an input in energy from the diet around 200 times higher than what the larvae really needed in experimental flasks ((Brito et al.,2004)).

Table 8. Energy partitioning on postlarvae fed various regimen (Brito, R. et al.,2004).

brito		Ab	U		As	R	Ex	P	DE	
Bureau	GE feces	**DE**	**UE+ZE**	**ME**		**HiE+Hem**	**SE**	**RE**	requirement	*balance*
ARTemia		1,4	0,015	1,40	*1,386*	0,619	0,186	0,595	**2,8**	-1
ART+algae		2,3	0,023	2,30	*2,272*	0,917	0,243	1,134	**4,6**	-2
MCD		0,6	0,037	0,53	*0,493*	0,328	0,092	0,202	**1,2**	-1
MCD + algae		0,7	0,042	0,61	*0,572*	0,326	0,096	0,193	**1,3**	-1
Art+MCD		2,1	0,019	2,06	*2,043*	0,816	0,218	1,027	**4,1**	-2
Art+MCD+algae		2,7	0,032	2,68	*2,644*	1,233	0,36	1,772	**6,1**	**-3**

c) Juveniles

Juveniles procure easier situation to make the budget and get precise data. Juveniles can be starved for Hem measurement, they can ingest a single meal, feces can be collected in sufficient amount, and ingestion of various set of diets can be done without too much of a problem (Bureau et al., 2000; Gauquelin, 1996).

Moreover this is the period where feed composition whether plant based or animal based or mixte can take allits signification. Ocampo (1984) found RE values on *Farfantepenaeus californiensis*. Temperature has a direct influence on metabolism of juveniles (Ocampo, 1998) and an elevation of maintenance (Hem) with increase from 22 to 27°C. Comoglio et al., (2004) obtained maintenance level on *F. paulensis* .Starvation gives the situation with DE=0 and it is simply the way to measure the basal metabolism (Hem) in the partition of energy for an individual or a group of shrimp.however there is a constraint due to a risk of moulting during the measure. Gauquelin et al., (2004) produced energy partitioning on *L.stylirostris* raisied in tanks and controlled in microcosmes. Most of these studies with a use of mixte diets provided an RE in the range of 10% or below. (Table 9).

Table 9 Growth performance and carcass characteristics of *L.stylirostris* fed diets A to F (22-58% CP) over a 7-weeks period (Gauquelin, 1996).

	Diets					
parameters:	A	B	C	D	E	F
feed g/shrimp	35	35	35	35	40	35
gain g/shrimp	3.0[a]	5.5[b]	5.0[b]	5.9[b]	7.0[c]	8.0[c]
feed efficiency, feed: gain	13[a]	7.9[b]	7.2[b]	6.4[b]	5.2[b]	4.9
daily growth coefficient, %	0.25	0.44	0.41	0.47	0.55	0.62
hepatosomatic index %	3.6	-	3.2	-	-	3.0

Recent work on *L.vannamei* lead to estimates that do not differ from one diet or another and from the work on larvae and Pl's and juveniles. Following comments can be made: (Brito Bermudez, 2004): survival and growth rates are similar with PA or PV as well as energy ppartitioning with Pl's. Growth is slightly inferior to live food sequence as control whatever inert diet used; juveniles fed 40%CP diet plant based or animal based protein sources get similar weight gain after 20days in experimental indoor tanks.

If growth on any inert diet is inferior to live food sequence, it means that RE is inferior with µcaps compared to live food. Live food is highly digestible, has a well-balanced aa profile, then essential amino acids arrive in aa pool for protein synthesis with a probable lower oxidation of aa than in other feeeding situations. Higher protein synthesis, lower aa oxidation with live food sequence or from an energy point of view, more energy channelled to growth (because of less expense at protein digestion level, less heat increment, less N-ammonia excretion).

By and large, it should lead to a more favorable energy budget near 20-25% instead of 10-15% for example in case of an inert particle presenting imperfections (in ADC protein, ratio P/E, essential amino acid balance).

If no apparent difference in energy metabolism was found between Pl's receiving either PA or PV (Brito Bermudez et al, 2004) there seems to exist a difference in intermediary metabolism. Pl's receiving PA tends to change substrate using lactate to produce glucose and maintain its glycaemia. Such a change does not occur of course in Pl's fed PV because the load of cbh is enough and glycolysis is the main route for energy production (this is in case of intermoult stage because during premoult stages there is a possibility animals shift glucose to HMP pathway leading to ribose and NADPH for unsaturated fatty acids.

It will be studied further the tissue metabolism in juveniles to see the impact of plant sources as main ingredients in diets for animals in growth phase.

4. How to fit increase in plant protein sources with energy expenditure for larvae, Pl's and juveniles.

a) Larvae

Larvae with stimulation of amylases in early stages by high load of starch native or pre cooked.Larvae were fed in early 70's with glucose and starch, but considering the level of amylases during zoeal stages it can be consider possible to provide pre-gelatinized starch insteas of raw starch. However along the course of the study most of the time raw starch, wheat starch were incorporated to the diet without apparent adverse effect. It is not neede probably to go up a supplementation purified amylase in the microcapsules as previous workers attempted for on juveniles of *P.japonicus*.

To the question of supplementation of the diet with phytases to increase the availiability of phosphorus that is under complexed form in plant sources, again, no indications lead to make such an adjustement at least in larval diets. In fact, there is the mineral mixture aside plant sources that should provide soluble phosphates such as monosodium phosphate. Besides that, up to wich level alkaline phosphates at intestinal level can hydrolyze phytates to inositol and phosphorus? Some data let incline to an ability of shrimp to make such hydrolysis.

b) Postlarvae

Postlarvae transition to plant materials with increasing starch load leading to average DE 17kJ/g in experimental diets

Those Pl's are normally resistant to manipulation and from PL_{12} to the variations in abiotic factors. Observations led to think that survival difference between the two diets tested could be due to an imbalance with the plant diet because of a protein index (not enough protein supply? or a limiting factor in relation with the load of plant sources? or a simple explanation can be found with an unsufficient feed intake of a diet that succulence drop too drastically?

All those points will be reconsidered at the final rough reading of the results; consequences deducted from feeding of Pl's on all-plant diet. It is worth noting that trials with Pl's coming from 8th generation in captivity gave a particular interest in regard to wild Pl's behavior and further comparisons in the scope of this project look promising.

c) Juveniles fed an animal or plant protein sources.

Juveniles should receive a balanced diets in tanks, decreasing portein to reduce catabolism of aa, maintaining starch up to 20% to spare protein, keeping energy level at ~15kJ/g DE or above without overpassing a necessary limitation in lipid content. But those nutritional considerations should be slightly different in pond conditions. As said before, with a species such as *L.vannamei* and according to stocking density, shrimp can have access to meiofauna, benthic algae and bacteria that are going to complement the pellet. This complement is such that a farmer can afford to use in production some pelleted feeds containing as low as 20%CP. And for memory, *L.vannamei* raised in intensive systeme with a bacterial folck in earlu 70's received an extruded feed MR20 allowing good performances ans today in Belize it operates a kind of extension of this technique.

5. Conclusions

How amino acid and energy demand for growth can be covered with alternative protein sources?

----diet formulation and husbandry

---- In larvae, stimulate amylases with starch load and high polymorphisme and frequent feeding

---- In postlarvae considering protein/energy ratio and frequent feeding

---- In juveniles, while replacing FM by plant protein, increase plant protein, maintain low % CP in case of *L.vannamei* considering a frequent feeding and a good attractiveness of the feed.

Would natural productivity from intensive rearing system or zero water exchange contribute in a significantly way to energy supply for shrimp?

Variations were observed during ontogenetic stages of the shrimp and there is a permanent adaptation and mechanism of digestion is complex but efficient. Amylases are produced under different isoenzymes forms. It helps primarily to digest complex plant carbohydrates or glycogen that participate to the bulk of food. A change from herbivory to omnivory with carnivory trend is then manifested. Production cycles made necessity for an early «weaning» and it was reported in 90's a possible use of inert diet at Zoea stage in large volume at commercial hatchery level. It does not mean a total absence of contribution from microalgae and bacteria however. But it enlights the fact that balanced dry diets can replace live food during the whole cycle. Major progress with larvae was done using microencapsulated feeds containing casein, then marine protein sources such as krill, squid, CPSP[70] (Gallardo et al, 2004) And during this project plant sources (gluten, lysamine, spirulina, canola,). All information on replacement of marine protein sourceswith plant protein showed that it is feasible (i)-larvae with survival and DI not different from diets including conventional protein source (ii)-postlarvae with performances identical between PA and PV and a similar partition of energy but an incidence on tissue metabolism and a neoglucogenic pathway (use of lactate) in case of PA diet.(iii) -juveniles with similar performances PA/PV and potential for adaptation to a load of starch and plant protein sources.

Implications were analyzed on energy partition with an aim at RE to examine the % generally found between 10-20% for RE but also the range of protein utilization that remains low (20%) with a significant contribution for maintenance and heat increment of feeding (including metabolic wastes, biosynthesis and digestive process). However, starch should lead to a current supply of dietary energy to limit to a certain extent amino acid catabolism. And if protein-sparing effect on protein can be evidenced (Arena, 2004) in juveniles it remains the attribute of individuals with a high degree of polymorphisme on digestive enzymes (wild animals).

The course of the research project aim at demonstrate a better adaptation to a cbh-rich diet and based on plant protein sources provided to shrimp as early as possible in its life cycle. Such preparation of animals with a stimulation of genes would lead to a good adaptation of juveniles receiving all-plant diets to sustain growth performances up to marketable size. Such premisses examined not only performances, but physiological aspects, body reserves and energy partioning and also tissue metabolism to give evidence of proper activation of key enzymes under diet variations and set the ground to further selection program. *L.vannamei* species present the best potential to study such potentialities while using previous data accumulated at all stages of the life cycle.

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