Utilization of carbohydrates by shrimp[1]

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ABSTRACT: Glucose metabolism was extensively studied in Crustacea during the 60's and 70's with an emphasis on decapods. In decapods juveniles can be described precisely at each step of intermolt cycle which last longer than penaeid ones (Aquacop, 1972) and qualified as diecdysis. Consequently metabolic pathways for glucose can be described accurately. This basic approach in decapods can help understand metabolism in shrimp. Comprehension of metabolic pathways lead to draw the outlines for carbohydrate utilization by shrimp: after going through main carbohydrates (CBH) sources, digestibility, glucose tolerance test, utilization by whole animal, hepatopancreas glycogen, pathways of CBH breakdown such as glycogenolysis, glucolysis, substrate cycle, minor pathways, aerobic breakdown, CBH synthesis and glucose utilisation. The diabetic like shrimp is under the pervasive influence of the molting process. Shrimp derives energy from CBH. Even though protein can easily supply energy too through gluconeogenesis. Related to the balance between protein and calories, shrimp sustain optimal growth even at high dietary protein. Maximal growth rate of juveniles can be achieved with high dietary protein level (50-60%) but CBH can play a role in sparing protein for optimal growth in practice. CBH under starch form will represent up to 20-30%. A good comprehension of CBH metabolism will lead to a greater supply of plant protein sources (soybean meal, pea meal, lupin, canola, wheat gluten, rice bran, distillers) in shrimp feed. A recycling of chitin is done with the re-ingestion of exuviae, which tend to underline the capability for chitin hydrolysis. Can ponds natural productivity help to maintain constant food supply leading to a high glycemia level compared to tank experiments where a zero food supply during 12 hours makes a difference? Keeping that in mind, the formulator will propose diet with as much CBH as possible, taking into account the potential to digest it, whether under native or pre-cooked form, the structure of CBH (ratio amylose / amylopectin), the possibility to stimulate enzymes of intermediary metabolism. To a certain extent it will lead to a great respect of the environment when reducing phosphorous output (wastes). Up to which extent CBH can fit with immune response in regular grower feed for shrimp is worth to be addressed. Complex CBH could represent a potential source of stimulants for immune response.

KEYWORDS: carbohydrates, shrimp, energy

INTRODUCTION

Shrimp and marine shrimp, are described as omnivorous detritivorous, other marine crustaceans are benthophages with a carnivorous tendency; some species such as *Macrobrachium*, crayfish considered herbivorous. The common denominator in these animals is the constant feeding activity, nibbling on the substrate to keep minute amount of organic matter reaching the proventriculus. When stomach contents are examined, it is evidenced pieces of shrimps, polychaetes worms, bivalves, ophiurids, nematodes, plant debris, etc. The products from nibbling activity are difficult to identify (its impact in terms of quantity too even though food full proventriculus occupied 2-3% of total body volume, so require constant activity to obtain good growth rate). Cowey (1992, com.pers.) mentioned that with nature food, about 75% of it would be cleared from the foregut in one hour and Elliot *et al.* (1989) reported 10 minutes transit time in larvae.

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Cousin (1995) pointed out that non parietal carbohydrates from plant represent a small fraction ingested by shrimp, glycogen from animals could be more common polysaccharides in feed ingest but is it without considering a part coming from bacteria. Bacteria can contribute to the bulk of carbohydrates, especially when shrimp are reared in an earthen pond or in super-intensive conditions (Aquacop, 1978). Enzymatic equipment has been examined and led to identification of a alfa-amylase, and alfa -glucosidase, alfa maltase, alfa saccharase, galactosidase, chitinase, chitobiase and cellulase (in *M. rosenbergii*). But basically a system made of alfa -amylase and alfa -glucosidase (maltase) gives the hydrolysis of starch to glucose (Mac Donald, *et al.* 1989). The crustacean peneids adapt quite well to changes in the composition of the diet by induction of digestive enzymes.

However, monosaccharides are absorbed from the digestive tract rapidly and the elevation of blood glucose level could impair the homeostasis, and abnormal mortality rates are recorded under experimental conditions. Polysaccharides such as starch represent today the most common source, but many varieties according to the botanical origin were tested and several degree of gelatinization tested. Hemolymph played an important role in carbohydrate (CBH) metabolism, and internal factors such as molting stage, hormonal control will imply variations due to exogenous supply of CBH.

Glycogen in hepatopancreas is given to play a role as glucose supply. According to Renaud (1949) glucose would combine to ammonia, produced by the catabolism of amino acids, to form glucosamin (N-AG), which would accumulate in hypodermis in preparation to ecdysis. According to recent data (Sedlmeier, 1995), glycogen would be a precursor for chitin. Hemocytes contain glycogen but the concept of "circulating hepatocytes" is today irrelevant because of an absence of G-6P (Glucose 6-Phosphate) to liberate glucose in hemolymph (Loret, 1993). Recently, shrimp were studied in relation between physiology of shrimp and diet manipulation (Rosas *et al.* 2000a) providing ground for better understanding of carbohydrate utilization.

Utilization of CBH by shrimps

Carbohydrates are classified among simple (glucose, trehalose) and complex (starch, glycogen, chitin, cellulose) and the bulk of organic matter in the environment is provided by carbohydrates. With disaccharides, the á 1-4 bond is broken down by amylase, and glycosidic bond is much more resistant. Most animals do not have enzymes for â –1-4 bonds. Within polysaccharides, starch presents a combination of á 1-4 (amylose) and branched chains (á 1-6 amylopectin), glycogen, cellulose and chitin, all are formed from monosaccharide chain units. In carnivorous fish, complex carbohydrates (CBH) are not well digested and digestibility is very low. The digestibility method provided a digestive coefficient (ADC) expressed in percentage, for example a potato starch was found with 26-69% of ADC with CBH ranging from 20-60% in rainbow trout. Shrimp was studied from its ability to digest starch whether native or pre-cooked (Davis and Arnold, 1993, Cousin *et al.* 1996; Cruz-Suarez *et al.* 1994). Among botanical origin we can cite potato, corn (several forms), wheat, cassava, sago palm, rice basically. Digestibility of starch from various botanical origins is given in Table 1. Starch is rich in amylose and is poorly digestible compared to starch rich in amylopectin. Native potato starch is less digestible than pre-cooked one. Wheat native starch is well digestible (92%). Corn starch with 76-99% amylopectin is equally digestible.

Table 1. ADC starch for various botanical origin and different forms (Cousin,*et al.* 1996).

	% ADC
Corn (Standard)	85
Corn (Amylose)	63
Corn (Amylopectin)	85
Corn (pre-cooked)	94
Potato (amylopectin pre-cooked)	96
Potato (standard)	72
Potato (pre-cooked)	93
Wheat (standard)	92

Concerning to the interaction between starch level (%) and digestibility of other nutrient two species were considered: *Litopenaeus stylirostris* and *L. vannamei.* Lipid ADC varied between 82-87% for both species, and protein (mixture of casein and squid meal) between 96-98% showing no interaction of native wheat starch in a range of 10-40% ($P>0.05$). In fish such as carp or trout, poor CBH utilization is related to amount of amylase activity (21 mU/mL in trout and 245 mU/mL in carp) (Walton and Cowey, 1982). In shrimp, the situation is different. A shrimp hepatopancreas (hp) weighing 1 g could present an activity of 1000 mU/mL (Van Wormhoudt, 2000; com. pers.). So, the hydrolysis in midgut of complete CBH is not limited in those two species.

What is the level of glucose in the hemolymph? In fish, blood glucose was not controlled 7 hours later following a 1 g. glucose load. Glucose served as a final for the insulin releasing and may not be the signal (Walton and Cowey, 1982). Amino acids will serve too as a signal for insulin release. Shrimp tend to be potential diabetic-like because control of blood glucose was not found too good (Abdel-Rahman *et al.* 1979). After oral administration of glucose, 24 hours later, shrimp *M. japonicus* still did not returned to the initial level in hemolymph. The sinus gland produced a hyperglycaemic hormone (CHH) which maintain high hemolymph glucose level. An experiment conducted on manual and eyestalk crustaceans (Santos *et al*, 1988) confirmed the hormonal influence on glycemia. In a post-prandial situation, shrimp showed a peak of glycemia. The peak varied according to the nature of starch. In post-prandial studies with starch (Cousin, 1995), the peak of glucose appeared 30 minutes with pre-cooked starch compared to one hour with native wheat starch, and amplitude of variation reached 1.2 g/l. But as it was mentioned by previous authors (Florkin *et al*. 1960, Dean and Wernberg, 1965, Parvathy, 1970, Lynch and Webb, 1973), an efficient mechanism of glucose homeostasis seems to be unnecessary in crustaceans since they can tolerate large variations in blood glucose levels. What utilization is made of CBH by the whole body? Several studies refer to utilization of maltose, glucose, starch, dextrin, sucrose, lactose, fructose and cellulose. (Pascual, 1983, Aquacop, 1976). Cellulose would not be utilized at all, and was added to experimental diets basically as filler. Glucose was found to inhibit growth (Aquacop, 1976, Pascual, 1983, Sick *et al.* 1972). Dextrin, sucrose and starch were quite well used. Starches of different type, exhibited a better growth with *L. stylirostris* under native form (relative growth rate, TCR # 80-86%) than under pre-cooked form (TCR # 63-71%) (Cousin, 1995). This was confirmed in another experiment (206% *Vs* 180%) in spite of a slightly higher intake with pre-cooked starch (1.6 g/g *Vs* 1.4). Even though availability of pre-cooked starch was improved, glucose utilization for growth did not perform as well as with native starch. The reason could be found whether in the apparition of glucose in the blood which was shown earlier with pre-cooked starch compared to native wheat starch, or some metabolic problems related to a reduction of amino acid absorption (Alvarado and Robinson, 1979) or a saturation of hexokinase by substrate.

Utilization of CBH by whole animal

The energy retention in shrimps is more efficient in higher protein diet than low one (because amino acids not used for protein synthesis were more efficiently used as energy source than dietary glucose). In shrimps like *L. stylirostris*, energy retention was less efficient in higher protein diet than low one (Cousin, 1995, Table 2).

Table 2. Energy retention and wheat starch level (Cousin, 1995) for *L. stylirostris* (Cousin, 1995)..

MJ/Kg	Wheat starch %	Protein%	Energy retention %
17	30	35	19
17	25	45	17
17	17	50	15
17	11	55	14

In *L. vannamei*, the trend was the same even though in a range of percentage (10-14) lower than with *L. stylirostris* (14-19).

In fishes, amino acids in high protein diets were being used more efficiently than was glucose as source of energy, so carbon skeleton of amino acids appeared to be a better source of energy; and glucose as energy source was not great. In shrimp, even though a similar trend can be shown, the presence of high carbohydrate level (30%) associated with crude protein (35% CP) tend to improve energy retention (19%). So, even if amino acids are used as a source of energy, starch, native starch, from wheat can spare to a certain extent protein source.

Native starch increases plasma glucose to a high level with a slow return to the initial value due again to the poor regulation in glycemia, and glycogen in hepatopancreas. Glucose from starch can provide glycogen in the hepatopancreas in proportion to the dietary supply (Rosas *et al.* 2000). In gelatinized starch, in spite of the spread out of molecules which allows amylase a better access to glycosyl groups did not procure improve growth rates whether in *L. stylirostris*, or *L. vannamei* (Cousin, 1995). Moreover, an increase in hepatic somatic index (HSI) along with a slight accumulation of glycogen could be observed in *L. vannamei*. Salmon fed with pre-gelatinized starch showed lower glycogen, which tend to increase HSI (4% compared to 1.5% with protein and lipid only). It does not seem to be detrimental to fish to have large liver or shrimp hepatopancreas. An opposite trend was shown in cod, which is carnivorous. When fed with starch (5.7% *vs* 6.2 without starch), even if variation is not as important as in the case of pre-cooked starch fed to RT, it is interesting to see that the same trend was observed with *L. stylirostris* (3.3% *vs* 4.2 without starch). It underlined a point, which will be reviewed later on, the neoglucogenesis pathway.

Hepatopancreatic glycogen in shrimp

In shrimps the turnover of glycogen could be similar to fish and consequently much lower than omnivorous mammals. *Marsupenaeus japonicus* starved for 28 days showed a decreased in HSI (3.3 to 1.8) during the first week (Cuzon *et al.* 1976), which indicated glycogen utilization, but HSI remained constant during the next 3 weeks. In crabs, hepatopancreas glycogen seems to contribute the major source of energy during early stage of starvation.

One of the carbohydrates that shrimp meet in natural environment might be chitin. And the question was raised is the chitin used by shrimp better than other CBH? Chitinase activity was measured in a

number of species and bacteria living in the gut showed chitinolytic activity for a number of species too. Gwinn and Stevenson (1973) have speculated that in *Orconectes limosus*, the major energy source is chitin, because chitin resorbed by the hypodermis before molting provides sufficient material for both new chitin synthesis and energy for molting (1.4 kJ which represents around 25% of energy accumulated in intermolt period).

Chitin

Chitin digestion and assimilation occur and make a significant nutritional contribution to shrimp (Clark *et al*. 1993). Shrimps reared under experimental conditions tend to ingest exuvia of newly molted shrimp with a kind of avidity, which seems to be in relation to the fact that a diet is well-balanced or not. Some diets containing shrimp meal showed poor growth performance and glucosamine could have a beneficial action a new exoskeleton synthesis (Kitabayashi *et al.* 1971) even though the levels tested (0.5-0.8%) represent a small percentage. Dietary supplies of glucosamine could be insignificant compared to the metabolic supply provided that a substantial amount of glucose be available ammonioemia in hemolymph is given to be high (1.6-1.9 mg/ 100 cc in the blood of lobster or crayfish; Florkin, 1966). However, Ross Stevenson (1983) mentioned a pathway for glucosamine involved G6-P and F1-P. And this is contrast with the fact that glucose phosphorylation capacity of shrimp is low (probably less that in fish) and most likely, metabolism of glucose is poor in comparison to mammals. In fishes, level of chitinase in blood and lymphomyeloid tissues is suppressor to the one in gastrointestinal tract, indicating a diffusion role rather than a digestive capability for chitin. In shrimp such a statement do not apply.

Chitin synthesis in shrimp cannot be considered out of the molting cycle, which shows the evolution of organic reserves in the digestion gland. Renaud (1949) clearly demonstrated in crabs the variation in glycogen. Also Cuzon (1976) reported this in *M. japonicus*, and again glycogen in hypodermis, which indicates an intense metabolic activity including important transport of nutrients from an organ to another, starting in stage C of the intermolt period and in preparation to the next molt. Such transfer imply hemolymph which dispatch nutrients to different tissues in which metabolism will be oriented according to a given period of the molt cycle.

Pathways of carbohydrates breakdown

a) The glycogenolysis. In mammals, glycogen is used as a source of glucose within quite strict limit. But in shrimp it is not well controlled and hepatopancreas glycogen is subject to rapid changes. During molt period a progressive accumulation from C to D is noticeable and during a starvation period a drop is observed within a week (Cuzon, *et al.*1976).

Glycogen ----------G-1P
Phosphorylase a

The control of hydrolysis would be made by glucose on the enzymes. Scheer and Scheer (1951) concluded from a 14C study that this sugar is not used primarily as a substrate for glycolysis but as a precursor of chitin.

b) Glycolysis will provide substrates for TCA cycle, which will be a source of energy for muscle. Boulton and Huggins (1970) already investigated the glycolytic enzyme activity and its capacity to support the metabolic rates reported for various Crustacea (Wolvekamp, 1960). Hepatopancreas and

muscle will be the sites of glycolysis. In aerobic cells, glycolysis will provide oxidizable substrates and under anaerobic conditions it will give lactate (in tissues such as muscle, cardiac muscle, hepatopancreas).

Enzymes are active at low concentrations and their level depend upon glucose which is dependent on the nutritional state ranging from very low level in fasting to 20 mM (in fish) after a load of glucose.
Glucose phosphorylating capacity in shrimp hepatopancreas is low. In crab, it increases in stage C (300U/ml) which expresses a storage capacity of the gland (Loret and Devos, 1992).

c) Substrate cycles can give access to a greater metabolic control in glycolitic pathways. It results in an increase in net energy expenditure coming from defined metabolic routes in order to re-synthesize ATP (Newsholme and Crabtree, 1976) suggesting that they can provide a mechanism for catabolism of the excess dietary carbohydrates ingested as alternative to their excretion. The pentose phosphate pathway is considered in fish as a minor pathway. In decapods, the pathway has been considered as a major route during intermolt period, when glycolytic route would be privileged during pre-molt stages (McWhinnie and Kurchenberg, 1962).

In any case, the pentose phosphate pathway provides to the tissues with specific molecule mainly reduced NADPH for the synthesis of unsaturated fatty acids and ribose 5-phosphate for synthesis of nucleotides and nucleic acids. This pathway is under two enzymes (G-6-P dehydrogenase and 6-P glutamate dehydrogenase) which activities can increase 7 fold in carp. Shrimp fed low fat, high cbh diet can be forced to synthesize fat with these two enzymes producing NADPH. It is an explanation for the low tolerance to high fat diet in shrimp during growing period at optimal temperature. This pathway supplies unsaturated fatty acids to the animal. The low glycolytic rate of *P. crassipes* in gills may indicate a relative importance of the pentose cycle.

The glucuronate pathway is an important route for certain polysaccharides, starting from G-1-P. It belongs to some detoxified mechanisms. This is a pathway for vitamin C, except for shrimp (but also fish, guinea pig and primate).

Aerobic breakdown, through the glycolysis provide 38 ATP and the respiratory control within cells may be measured by adenylate energy change (AEC) which is around 1. This indication does not seem to work with shrimp *P. monodon* except in case of emersion transport in sawdust for a few hours generating a severe stress.

Oxygen consumption is limited by ADP level when the ratio of O_2 consumption increase, rephosphorylation ADP-ATP and once ADP is re-phosphorylated, there is a low level of activity of the cycle and O_2 return to resting level. Oxygen consumption and nitrogen excretion provide O: N ratios, which help to identify the type of substrate used for energy. Le Priol (1999) with *L. vannamei* measured and O: N below 8 whatever time after feeding on a low CBH level indicating the use of protein as–energy source. Animals fed on a high CBH level (30%) shifted their energy source from protein tending to the use of lipids (O: N 18) two hours after feeding. Nelson *et al.* (1977), Rosas *et al* (2000b) evidenced the difference of carbohydrate utilization in between *L. vannamei* and *L. setiferus* under laboratory conditions. Whether low or high CBH diets, *L. vannamei* showed O: N below 20 and an indication of a protein energy substrate when *L. setiferus* was shifting to CBH substrate at high level of dietary CBH. This ratio is used by Capuzzo *et al.* (1981) to study metabolism and CBH sources in lobster and evidenced simple sugars presumably leading to synthesis of glycogen reserves which is a long term energy store. Metabolism of starches (corn or wheat) leads to utilization of CBH sub-units

for immediate needs, including a protein sparing effect like in *Carcinus* (Needham, 1957; Toghrol *et al*., unpublished observations).

Carbohydrate synthesis

It will comprise gluconeogenesis, glycogenesis and chitin synthesis. This is the most interesting part of the CBH metabolism in decapods.

1.- Gluconeogenesis. The hepatopancreas of *C. moenas* has been shown to be a gluconeogenic tissue (Toghrol, 1969) containing following enzymes: PK, PC, and PEPCK Glucose is important as a building block for polysaccharides and as a fuel for hemocytes. In case of not dietary supply of glucose, it will be used from other ways and the main one is the reverse pyruvate to glucose. Pyruvate is coming from carbon skeleton of amino acids. Muscle tissue in anaerobic condition for example during a swift swimming for escape, a production of lactate occurs and gluconeogenesis is a route for regenerating lactate to glucose or glycogen.
There is a synthesis of glucose from other forms such as amino acids (carbon residues) which are a necessary supply of glucose.

Thabrew *et al*. (1971) showed that *C. maenas* gill tissue is capable of gluconeogenesis. Crustacean tissues contain free amino acids pool about 10 times the size of that found in mammalian tissues (Huggins and Munday, 1968). The glucose formed in mM/h/g wet weight of digestive gland or hepatocytes provide an idea of the type of substrate (lactate, amino acids) which is favored in a given environmental condition (20-27^{0}C) and an actual feeding status.

The gluconeogenic enzymes will respond to gross change in composition (Table 3). In case of reduction of dietary CBH and increase of protein, glycogenic enzymes are still active but PEPCK (40 DO/min/g protein) is activated significantly ($p<0.05$), compared to PEPCK from hepatopancreas of shrimp receiving 30% starch.

Table 3. Effect of dietary CBH on gluconeogenic enzymes activity (Rosas *et al.* 2000c).

Dietary protein (%)	33	52
Dietary starch (%)	33	1
Protein /energy ME (%)	66	100
Hexokinase (UDO/mg prot./min.)	0.05	0.05
Phosphoenolpyruvate kinase (UDO/mg of prot./min)	2	4[1]

(1) $p<0.05$

2.Glycogenesis

It is an immediate response through uridine diphosphate glucose to form glycogen with the action of glycogen synthetase. It is another uridine, uridine diphosphate acetilglucosamine (UDP) which lead from glucose to chitin (Carey, 1965).

By isotope method, it was shown in fish that glucose instead of being incorporated to glycogen is broken-down to small hexoses and resynthetized. In mammals it occurs and same sort of thing may be happened in shrimp.

3. Chitin Synthesis

This is one of the most debated subjects in CBH synthesis. It is also one essential pathway which will govern the success of ecdysis (diecdysis); it represents each time a real physiological crisis that animal needs to go beyond. Molt will be the period when mortality in tanks or in ponds has the greatest impact because (i) animal will depend on resources made during intermolt period (ii) the absorption of water let shrimp under dependence of the quality of environment surrounding it (temperature, salinity, oxygen, and presence of pathogens).

In the early 50's, the formation of chitin was finding its roots in the reaction between glucose in the hepatopancreas, and $N\text{-}NH_4$, coming from the catabolism of amino acids. Glucosamine was then transported to hypodermis in preparation of molting (Renaud, 1949).

Gwinn and Stevenson (1973) hypothesized a retrieval of chitin before molt at hypodermis level where a chitinase activity exists, approximately half of exoskeleton, which was hydrolyzed in glucosamin to provide building blocks for chitin formation. Such transformation would cover glucosamin needs as well as energy needs during molting process. Bliss (1993) proposed a pathway for chitin synthesis from G-6-P, governed by three enzymes at control points. One of these was the reaction between Fructose-6-P and ammonia catalyzed by glucosamine 6-P "synthetase", and another control point located at the step just before chitin synthesis. Loret (1993) considering that glycogen in hemocytes exceeded largely their energetic demand, proposed precursor role for chitin formation via hemocytes playing the role of osteoblasts in mammals. By adhesion of hemocytes and hypodermis cells and exocytosis of glycogen could happen.

The process of molting is crucial in decapod, and in shrimp, it is quite frequent (every 10 days at 3-4 g average weight). It involves a re-utilization of nutrients during a short sequence (another example of short transport in ovaries, during regression after stress on the breeders) in presence of effective mechanisms.

Glucose utilization

Measurement of glucose turnover rate mM glucose /kg wet tissue is a range of 15-100 in mammals and 2-10 in fish: in crustaceans there is no data showing a specific rate of glycogen breakdown or difficult to measure because of the different routes for metabolism. Glucose utilization in crab by radioisotopes (Hochachka *et al*. 1971) showed the route of glucose utilization in gills, whether glycolysis or shunt of pentose phosphates. The activity of the pentose shunt is high relative to glycolysis. (C_6/C_1 ratio).

Weber and MacDonald (1961) pointed out to nutrition as an essential aspect when metabolic routes are under investigation. Their experiments with mammals indicated that at the enzyme level, the presence or absence of an enzyme or pathway is dependent both upon nutritional state and hormonal balance; in their case, the former was more important. According to Chang and O'Connor (1993) the most interesting question concerning crustacean CBH mechanism no longer concern the existence of a particular pathway but regulation of relative activity of each pathway during the intermolt cycle.

Glucose variation along a molt cycle clearly indicates a higher level (20 mg/100 ml) as soon as stage C-D_0 is reached by the animal, normally fed. But the control of glycemia can be seen in post-prandial situation and in comparison of unablated and ablated animals.

Regulation with crustacean hyperglycemic hormone (CHH) in ablated and unablated crustaceans, was shown by Santos *et al.* (1981). These authors reported a regulatory effect of this hormone on blood glucose level after a meal (earthworms) and an interpretation of the need for high blood glucose in relation to the requirement of carbohydrates for chitin synthesis and 5-HT (serotonin). This last is a neurotransmitter with a minor pathway from tryptophan, a satiety signal in fishes and which is effective on blood glucose (Bauchau, *et al.* 1968) by activation of phosphofructokinase in hepatopancreas (glycolysis). In *C. moenas*, an injection of serotonin, which is secreted by eyestalks leads to an hyperglycaemia measured one hour after injection whether animals were ablated or unablated. Spindler *et al.* (1976) related cyclic nucleotides and crustacean blood glucose levels in crustaceans.

Several hormones or substances can interact on blood glucose, together with insulin: high glycemia in shrimp if given a high CBH not due to impaired binding of insulin in muscle; shrimp muscle, by analogy with fish, (3-10% insulin receptor per mg protein insulin binding) is low. When high CBH fed to shrimp the specific binding is not higher. But, by and large the regulation of glycemia such as in fish is poor and crustaceans tend to cope with high blood level without visible cellular damage. Many more information is needed on this topic: insulin receptor; turnover of glucose, to get a more accurate knowledge on carbohydrate metabolism in shrimp.

Complex CBH and immune response with the emphasis placed on beta-glucans participating in defense mechanisms when a pathogen aggression is happening.

CBH are osmotic regulation with a change in salinity (Rosas *et al*. 2000b), or any other change in abiotic factor placing animals out of their tolerance zone for homeostasis.

Those two last aspects related to CBH utilization increase the interest for plant protein and CBH sources. This can maximize the potentialities of shrimp in an environment where parameters fluctuate in limits sometimes beyond of those acceptable from a physiological point of view by living organisms placed in conditions of semi-intensive production.

CONCLUSIONS

The digestibility of CBH in shrimp varied according to flour type, botanical origin of starch and inclusion level. Native starch was digested as well as pre-cooked starch. Best results were attained with standard wheat starch (ABS, from Roquette Frères, France).

Glucose level in plasma varied according to the botanical origin of starch in the diet (Cousin, 1995). For starch levels with feed up to 45% of available energy, no negative effects on growth was seen. Increasing the amount of starch from 0 to 40% of energy content of starch did not result in decreased feed conversion ratio (FCR). Shrimp can compensate by increasing its food intakes when dietary starch increased, at 22°C, not at 27°C. Low inclusion levels below 3% of the dry weight in the fed were found to promote growth with out special sparing effect the fact that nitrogen excretion was lowered. (Gauquelin, 1996). It is clear that protein retention, PER, growth, etc, depends on an optimal energy balance between protein and fat (Cousin, 1995), keeping the carbohydrates content enough for metabolic need (energy and carapace reformation).

Variation in hepatopancreas glycogen is shown around 13% wet weight were measured with some relation to the nature of starch. At 35% inclusion amylose rich starch provided the lowest glycogen content in hepatopancreas, when pre-cooked starch gave highest hepatopancreatic glycogen values (1%

wet weight). Glycogen concentrations in muscle are very low, probably not affected by starch content in the feed.

Insulin is not measured in shrimp and whether free amino acids were absorbed faster in fish fed without dietary CBH like in cod (Hemre, 1992), could not be verified. During fasting, all the measured plasma metabolites decreased, similar to the regulatory mechanism in cod (Hemre, 1992).
The amount of CBH in the diet prior to experimental infection could be related in *L. stylirostris* with a better resistance with low starch diet (Aquacop, 2000, com pers).

General statements

1.-There are some similarities in terms of CBH utilization between shrimps and carnivorous fishes.
2.- From a metabolic point of view, many work was done in the 60's and 70's in CBH metabolism in decapods (crab mainly) to identify metabolic pathways, but not many data on regulation (insulin receptors), evolution of organic reserves according to molting stage in peneid brought understanding of carbohydrate utilization.

3.- From a practical point of view, comprehension of CBH utilization will help formulate feeds for growing period trying to enlarge its use at expense of protein and take plant protein in replacement of fish meal (which is a largely covered topic in recent years).

4.- Enzymes regulating the metabolism are interesting to be studied (HK, PEPCK, PK FBP) because they help understand the variations to given amount of dietary starch and in the long run with selective program could be leading to identify populations with a potential for maximum CBH utilization.

5.- Digestive enzymes equipment is such that it allows large range of CBH digestion. On the other hand, energy derived from protein sources is utilized by shrimp more than any other source (as fishes). All the difficulty remains to maintain optimal growth when balancing P/E ratio, which would include as many CBH as possible. Many factors are to be taken into account: the botanical origin of CBH sources, processing (pre-cooked versus native), rhythms of feeding.

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