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CRITICAL REVIEW: CHOLESTEROL AND FAT-SOLUBLE VITAMINS ON SHRIMP FEEDING

Rodrigo Barbosa Lima*[1], Denise Fontana Figueiredo-Lima[1]

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ABSTRACT - Lipid quality on shrimp nutrition is very important, especially regarding to its content of essential substances. Nutrient intake is instinctively adjusted when animal selects its feeds in the wild. In captivity, it is possible to observe the cholesterol requirement, as in shrimps, as in other crustaceans, since they do not possess the ability to synthesize this indispensable nutrient for animal survival and development. Cholesterol is a relatively small part of the essential lipids for shrimp, and cannot be replaced by phytosteroids. Since cholesterol is the precursor of the ecdisone hormone (which controls molts), it is particularly more necessary during larvae stages. Also, fat-soluble vitamins are vital for shrimp growth. However, vitamins D and K functions are still partially known, because the premises of their functions in vertebrates have no equivalent in crustaceans. In this sense, only the relevance of vitamin E to protect PUFA has an explanation so far. On the other hand, the carotenoid astaxanthin appears to be as effective as an antioxidant that is difficult to justify the need for tocopherol. Dietetic imbalance in essential lipids turns shrimps susceptible to opportunistic infections. This review describes the cholesterol and fat-soluble vitamins importance in shrimp feeding.
Key words: carotenoids, cholesterol, fat-soluble vitamins, nutritional requirement, phospholipids, syndromes.

REVISÃO CRÍTICA: COLESTEROL E VITAMINAS LIPOSSOLUVEIS NA ALIMENTAÇÃO DO CAMARÃO

RESUMO - A qualidade dos lipídios na nutrição do camarão é muito importante, especialmente em relação ao seu teor em substâncias essenciais que é instintivamente ajustado quando animal seleciona seus alimentos em estado selvagem. Em cativeiro pode-se observar em camarão, assim como em outros crustáceos, há exigência dietética de colesterol uma vez que eles não possuem a capacidade de sintetizar este nutriente indispensável para a sobrevivência e desenvolvimento do animal. O colesterol é uma parte relativamente pequena dos lipídios essenciais para o camarão, e não pode ser substituído por fitoesteróis. Uma vez que o colesterol é o precursor do hormônio ecdisona (que controla as mudas), é particularmente mais necessário durante as fases de larvas. As vitaminas lipossolúveis também são vitais para o crescimento do camarão, no entanto, as funções de vitaminas D e K, ainda são parcialmente conhecidas, porque as premissas de suas funções em vertebrados não têm equivalente em crustáceos. Neste sentido, apenas a relevância da vitamina E para proteger os PUFA tem uma explicação, e ainda o carotenoide, astaxantina, parece ser tão eficaz como antioxidante que é difícil justificar a necessidade de tocoferol. O desequilíbrio dietético em lipídeos essenciais torna o camarão susceptível a ocorrência de infecções oportunistas. Esta revisão descreve a importância do colesterol e vitaminas lipossolúveis na alimentação do camarão.
Palavras-chave: carotenóides, colesterol, vitaminas lipossolúveis, exigência nutricional, fosfolipídios, síndromes.

INTRODUCTION

The cultivation of aquatic organisms represents a remarkable survival source since a long time (ZHIWEN, 1999) and, lately, a profit source. Encouraged by commercial interests, shrimp cultivation under intensive conditions, frequently disregards the relevance of natural food availability as a source of essential nutrients, which demand increases once fish industry by-products are replaced in the diet.

Former studies approaching lipid essentiality for crustaceans demonstrates that, for these animals, the lack of their proper mixture in the diet, besides affecting growth, reproduction and survival, also causes metabolic disturbances, affects inflammatory responses and certain immune responses (CASTELL et al., 1975; KANAZAWA et al., 1978, 1979; SHEEN et al., 1994; DUERR; WALSH, 1996; COUTTEAU et al., 1997; SAMUEL et al., 1997; THONGROD; BOONYARATPALIN, 1998; REDDY et al., 1999; HOLMBLAD; SÖDERHÄLL, 1999; SHEEN, 2000; TAHARA; YANO 2003). In this context, nowadays, the emergence of pathologies named "syndromes" such as Acute Hepatopancreatic Necrosis Syndrome and Early Mortality Syndrome, are very alike to those cases of dietetic lipid deficiency (BOWSER; ROSEMARK, 1981; MATTHEWS, 1991; HE et al., 1992; HOWELL; OKADA et al., 1995; PAIBULKICHAKUL et al., 1998).

[1]Federal Rural University of Pernambuco, Av. Bom Pastor s/n, Boa Vista, CEP 55096-901, Garanhuns, Pernambuco, Brazil. E-mail: rodrigo_zoo@yahoo.com.br. *Autor para correspondência

Symptomatology of “Acute Hepatopancreatic Necrosis Syndrome (AHPNS)” observed in shrimp nurseries (NACA, 2012), involves, almost absolutely, anomalies in the hepatopancreas, a preeminent organ for metabolic lipid conversions. When considering this scenario, infections are perceived as opportunistic. Such possibility, would explain, the reason why, initially, agents considered as responsible for syndromes were not identified (NACA, 2012). On the other hand, the mere quantification of nutrients in the animal’s organism would validate the hypothesis (MONTAÑO; NAVARRO, 1996) revealed either from the diagnosis, or from the lack of symptoms, as soon as the animals received nutrients in their diet.

Aside from the topicality of the approach discussed above, disease treatment has not been the focus of this review; nevertheless, it serves to contextualize the relevance of the subject “lipids in the diet” in preserving shrimp health. Experience has shown that nutritional disturbances are caused by demand and, in some cases, the excess (SHEEN et al., 1994, DUERR; WALSH, 1996, SAMUEL et al., 1997; THENGROD; BOONYARATPALIN, 2000) of dietetic lipids (BARBOSA LIMA; FIGUEIREDO-LIMA, 2016). Therefore, a critical analysis of pioneer studies that elucidate biochemical mechanisms and the importance of lipids to shrimps, notably cholesterol and fat soluble vitamins was performed. The justification of this review is based on the premise of searching solutions for new challenges coming from the reflexive analysis of past events.

Cholesterol on shrimp feeding

Cholesterol functions

Cholesterol is an indispensable component of plasmatic membrane of animal cells, since it is necessary to build the lipid rafts and caveolae which control many functions related to chemical signal transmission and execution (SIMONS; IKONEN, 1997; BROWN; LONDON, 1998). Besides, cholesterol oxidative catabolism produces several important hormones (STRYER et al., 2002). In invertebrates, cholesterol is the precursor of the hormone named ecdysone, which controls part of the molt cycle in immature form of animals, nowadays classified as ecdysozoa (AGUINALDO et al., 1997). On the other hand, a direct cholesterol precursor, 7-dehydrocholesterol, forms the seco-steroids, which the most known agent is the vitamin D (“calciferol”). From vitamin D derives, in vertebrates, the anti-rickety hormone, 1.25-dihydroxi-colecalciferol (“calcitriol”) that acts as ecdysone and steroids hormones in general, in gene transcription through a nuclear receptor (STEINMETZ et al., 2001; STRYER et al., 2002).

Crustaceans are not able to synthesize steroids and require these nutrients from dietetic sources for growth, development and survival. Cholesterol is a sterol source of high nutritive value for shrimps.

Nowadays, several shrimp species are, undoubtedly, the aquatic invertebrates more largely cultivated, due to the profitability they provide, and the shrimp *Litopenaeus vannamei*, occupies one of the top positions in aquaculture, in terms of production (MONTAÑO; NAVARRO, 1996; ROSENBERRY, 1998).

Cholesterol essentiality in crustaceans

In a pioneer study, Teshima and Kanazawa (1971) demonstrated that three agents of decapods crustaceans, among them the shrimp *Penaeus japonicus*, were not able to synthesize cholesterol from acetate, regular precursor of steroids in vertebrates. In assays on nutritive requirements of *P. japonicus,* Kanazawa et al. (1971) proved, at the same time, that this species is not able to grow and survive when kept under a diet without cholesterol. Lately, Japanese researches results were confirmed not only in the named species (DESHIMARU; KUROKI, 1974), but also in other crustaceans lines as a result of studies with *Homarus americanus* (CASTELL et al., 1975; D’ABRAMO et al., 1984), *Pacifastacus leniusculus* (D’ABRAMO et al., 1985b), *Cardisoma guanhumi* (DOUGLASS et al., 1981), *Macrobrachium rosenbergii* (BRIGGS et al., 1988) and *L. vannamei* (AKIYAMA et al., 1992). In a further research, Teshima et al. (1997) asserted having observed in *M. rosenbergii* non dietetic cholesterol accumulation, which they consider to be the evidence of cholesterol biosynthesis as feasible in the giant Malaysian shrimp. However, no data from other researchers were published in order to confirm this result, which is not even mentioned in papers further published on the subject (GONG et al., 2000a; SMITH et al., 2001; WOUTERS et al., 2001b).

Cholesterol quantitative requirement

Since the former quantitative results on cholesterol requirements in crustaceans, there were some results divergences, including when considering the same species. In Penaeus japonicus, Kanazawa et al. (1971) indicated a value of 0.5% of cholesterol in ration as suitable, while Shudo et al. (1971) found that 0.2% was enough and Deshimaru and Kuroki (1974) recommended 2.1%. In *Homarus americanus*, Castell et al. (1975) reported 0.5% as the optimum level, D’Abramo et al. (1984) did not find differences of ration effect between 0.19 and 0.59% and Kean et al. (1985) recommended a cholesterol level of 0.2 to 0.5%. It is undeniable that a great part of this confusion might be explained due to methodology variation, not only in ration preparation, but mainly, due to type and previous animals conditioning. As for minimum necessary cholesterol concentration, the previous recommendation of 0.5% or even more of cholesterol probably may be corrected for less, since the cholesterol is not contaminated with phytosteroids (KANAZAWA et al., 1971) and shrimps have already surpassed their first larvae stages. In performed assays, Sheen et al. (1994) proved in *Penaeus monodon* that 0.2% of cholesterol was enough for juveniles that grew from 250 mg to approximately 1 g. Similarly, Gong et al. (2000a) did not find differences between 0.2% and 0.5% of included cholesterol on diet of *L. vannamei* which elevated its weight from 0.75 g to 6 g, approximately. Regarding to *P. monodon*, Smith et al. (2001) detail conditions in which that under adults (around 3 g) consume very little cholesterol (0.17%) to reach its needs (75 mg of cholesterol $\cdot kg^{-1}$ d^{-1}) in such manner that researchers conclude that it is unnecessary to add this nutrient, which normally comes with protein in rations. However, it is worth to observe that in the same *P. monodon* studied by Sheen et al. (1994) and Smith et al. (2001), zoea and mysis stages might need, perhaps, more than 1% of cholesterol in feeding (PAIBULKICHAKUL et al., 1998), effect attributed by the authors to the cholesterol function as ecdysone precursor, important to avoid mortality due to incomplete ecdysis (BOWSER; ROSEMARK, 1981).

Interaction between cholesterol and phospholipids

In preparing an experimental diet, not always is possible to dose each part independently from other components. Regarding to lipids, problem is aggravated because every fat is insoluble in water, but they mixture freely among themselves. Consequently, high cholesterol nutritional requirements were found in former assays with crustaceans, simply because shrimps do not absorb cholesterol efficiently in a ration without other lipids, and excrete most of the cholesterol without using it (TESHIMA; KANAZAWA, 1983; D'ABRAMO et al., 1985a). In later years, the idea that cholesterol essentiality should be investigated with the compounds that chaperone it, phospholipids, in biologic membranes, has risen. Studies of D'Abramo et al. (1981, 1982) on *Homarus* sp. evidenciated the lecithin utility to increase cholesterol action, and indicated phosphatidilcholine as responsible for this effect. In studies on *P. japonicus*, Kanazawa et al. (1985) recommended phosphatidylcholine from soybean and Bonito's (*Sarda sarda*) egg as particularly efficient. Chen and Jenn (1991) and Chen (1993) used a purified phosphatidylcholine and verified that this product has more than twice the crude lecithin activity to increase shrimp weight of the named species *P. penicillatus* and *P. monodon*, respectively. Next, Thongrod and Boonyaratpalin (1998), studying *P. merguiensis*, proved the advantage to add 1% to 2 % of lecithin to the diet and did not observe any benefit in adding cholesterol, once the basal ration contained 0.6% of cholesterol esters. Finally, in a research aiming to investigate lecithin interaction with cholesterol essentiality, Gong et al. (2000a) concluded that cholesterol in a 0.11% level on ration is enough for *L. vannamei* juveniles, since lecithin concentration in the same feeding reaches 5%, whilst in a lecithin free diet, requirement attains up to 0.41% of cholesterol. In a recent paper, these values are confirmed with three purity lecithin degrees, with the very same result (GONG et al., 2001). With this knowledge, one may argue which would be the most interesting alternative on a commercial ration formulation. Nevertheless, there is no doubt that cholesterol requirement can not be seen isolated and regardless polar lipids content. In another study, Gong et al. (2000b) presented evidences to qualify phosphatidylinositol, maybe, as even more relevant than phosphatidylcholine itself. Up to which point these phospholipids, their components, choline and inositol, may be considered as essential factors in nutrition, depends, particularly, on the experimental material. Gouillou Coustans and Guillaume (2001) raised the same question and named them of "quasi-vitamins". Probably it is certain to affirm that in larvae and juvenile fast growth of any shrimp, inclusion of ingredients as lecithin becomes them essential. (COUTTEAU et al., 1997), although their biosynthesis is completely feasible in crustaceans (SHIEH, 1969; TESHIMA et al., 1986c; KANAZAWA; KOSHIO, 1994). Despite the positive effects on developing shrimps known for decades, the real causes of nutrition relation and/or interaction between cholesterol and phospholipids were really difficult to explain, and therefore, their mechanisms are not completely understood up to date. It is certain that phospholipids act as emulsifiers over neutral lipids (as it is cholesterol) and, therefore, should facilitate digestion and/or absorption of such nutrients. On the other hand, doubts remain on phospholipids interaction with detergents that are physiologically in charge of the lipid digestive metabolism, as N-(N-dodecasarcosil) taurine for crustaceans (LESTER et al., 1975). In crustaceans' hemolymph, lipids are transported through a high density lipoprotein (HDL), also described for the first time by Teshima and Kanazawa (1979, 1980). It was proved that cholesterol and/or lecithin increase this lipoprotein concentration (D'ABRAMO et al., 1982, 1985a; TESHIMA et al., 1986a, 1986b; BAUM et al., 1990); and for that reason, several researchers were not concerned with deeper explanations (TESHIMA et al., 1986c; COUTTEAU et al., 1997; GONG et al., 2000a, 2000b). However, in this simple view, the whole part between phospholipids absorption and HDL production is lacking. In the latest years, several transporters ABC type that shift cholesterol from one side to another in the membrane and function as much in intestinal absorption as in discoid HDL production, in vertebrates, were unveiled. However, they are specific only for steroids and are not influenced by the presence of phospholipids in the digestive tube (SIMONS; IKONEN, 2000).

Cholesterol adverse effects

Cholesterol ingested in high doses is inadvisable in humans and in crustaceans. Several researchers who tried to find the minimum cholesterol concentration necessary to sustain juvenile crustaceans' growth confronted themselves with the observation that cholesterol excess inhibits growth and decrease animal survival (CASTELL et al., 1975; SHEEN et al., 1994; DUERR; WALSH, 1996; SAMUEL et al., 1997; THONGROD; BOONYARATPALIN, 1998; SHEEN, 2000). In most of the cited papers, lacks any evidence - besides demographic data - to suggest a biochemical mechanism of this apparent cholesterol toxicity. However, other researches described that cholesterol lead to an increase of triglycerides concentration in the hepatopancreas and of phospholipids in abdominal muscle (CHEN; JENN, 1991; CHEN, 1993; GONG et al., 2000a). The mentioned authors, unfortunately, did not present histological or ultra-structural images of the affected tissues, but one may suppose that the high cholesterol chemical resistance, that causes macrophage death and arteriosclerotic plaques in humans, would be capable of producing permanent stocks and irreparable damages also, in invertebrates vital organs. Even so intriguing to physiologists, cholesterol toxic effect logically has no practical relevance for aquiculture, once high prices of this input does not stimulate mistakes by excess.

Ecdysone and molts

Arthropods and nematodes accuse a peculiar form of growth due to their exoskeleton that needs to be changed in certain intervals in a sophisticated process, named molt (CHANG, 1995). Hormone that stimulates molts in every cited animal is ecdysone, a steroid produced by cholesterol oxidation. Ecdysone active in shrimps, as in many insects, is 20-hydroxy-ecdysone (WILDER; AIDA, 1995). However, this active form is generated only in peripheral tissues; the Y organ, endocrine gland supposedly responsible for biosynthesis, produces an inactive precursor, the 3-dehydro-ecdysone (RUDOLPH et al., 1992; RUDOLPH; SPAZIANI, 1992; BOECKING et al., 1993). Biosynthetic sequences of steroid hormones, divided among several organs, are also known, in vertebrates, for instance, in testosterone and calcitriol (STRYER et al., 2002).

After egg hatching, crustacean larvae go through several molts in a little time and therefore, are particularly dependent upon essential material. Up to date, larviculture depends, mainly, on live organisms as microalgae, rotifers, copepods and nauplius of *Artemia* sp. and only lately researches have risen aiming an artificial feeding. With this goal, Paibulkichakul et al. (1998) investigated the requirements of three larval stages of *P. monodon* for soybean lecithin and cholesterol. It was previously mentioned that larvae need much more cholesterol (1%) than juveniles (< 0.5%), but the mentioned paper data suggest that results could be better if using cholesterol concentration even higher. The 45% of larvae lost in Paibulkichakul et al. (1998) assays between zoea and mysis stages, respectively, presented clear evidences of a “lethal molt syndrome” attributed to cholesterol lack and, therefore, of ecdysone (BOWSER; ROSEMARK, 1981; ROTHLISBERG, 1998). Nevertheless, not only steroids, but also other lipids, classically essentials, named poly unsaturated fatty acids (“PUFA”, “HUFA” or “EFA”) are possibly responsible for this problem.

Fat-soluble vitamins

Vitamin A and Carotenoids

The vitamin A has a long history and has been recognized by the classical works of WALD as a precursor of retinal, visual pigment in vertebrates and invertebrates (STRYER et al., 2002). Said vitamin along with other retinoids can be generated from various carotenes (OLSON, 1989), and all these compounds serve as excellent antioxidants which combat free radical generation, lipid peroxidation and cell death caused by oxidative stress (HIRAMATSU; PACKER, 1990; TSUCHIYA et al, 1992; OLSON, 1993; MIKI et al, 1994; LINAN-CABELLO et al, 2003). In addition, it was discovered from the 80s that retinoids are hormones that control in animals and the man beyond the metabolism, the cellular differentiation, ie the formation and maintenance of all types of tissue (PETKOVICH, 1992; WEI, 2003). In the crab *Uca pugilator*, was shown (DURICA; HOPKINS, 1996; DURICA et al., 1999) that, the control of the limbs regeneration and molting cycle is done by a retinoid (in addition to ecdysone). In nuclear receptors responsible for these functions, certain retinoid occupies only one of the two sites of the hormone and the other reacts with a different ligand (MANGELSDORF; EVANS, 1995; WEI, 2003). In the case of PPAR receptors was identified two cases, where another hormone is an eicosanoid (FORMAN et al., 1995) and thus could be explained a coincidence of the nutritional requirement for PUFA and vitamin A.

Because of the cited conversion of carotenoids and retinoids, there is research on the requirement of vitamin A need, even in very cultivated species, since many components of a feed containing carotenoids. Even so, the need for vitamin for growth in shrimps can be qualitatively verified with ease. In an interesting experiment, Chen and Li (1994) found with higher requirement *P. chinensis* (18,000 UI/100 g) 1 g shrimp than 7 g shrimp (12,000 UI/100 g); estimates generally recommend about 15,000 IU/100g.

Crustaceans, the carotenes are soon converted into astaxanthin, to the point that Dall (1995) questions the relevance of retinoids in *P. semisulcatus* beyond sight for practically, the author, have not found in other organs, except in the eyes of this shrimp (cultivated). Linan-Cabello et al. (2002), however, they consider possible the conversion of astaxanthin retinoids (less oxidized) and provide evidence in another study in which wild females of *L. vannamei* accumulate more retinal in the ovaries than cultured females. It is known that astaxanthin is an antioxidant 100 times more potent than vitamin E and as such, should strengthen the immune protection of crustaceans. In fact, studies have been published showing that shrimp with more astaxanthin it have perform better in endurance tests and at the same time, lower levels of superoxide dismutase, enzyme which indicates the presence of oxidative stress. Additionally, it was proven that astaxanthin stimulates growth and molt cycle in post-larvae of *P. japonicus*. It was discovered decades ago that the meeting of astaxanthin with crustacianina protein forms matter that becomes red when shellfish are cooked or fried. However, only in the 90s it was explained that the disease that turns dark green carapace of the “black tiger”, (*P. monodon*) in captivity, in blue, is actually a astaxanthin deficiency, lowered up to 15% of the normal value.

Several studies on carotenoids and vitamin A crustaceans, aim females “maturing” for reproduction, because the successive changes are reflected in the intensification of the color of the gonads, by deposition of carotenes. The evidence points to the dependence of fertility carotenes level and/or retinoid in the diet.

The transformations of ingested carotenes result, in shrimp’ hepatopancreas, in an accumulation of astaxanthin which is transported when the ovaries vitelogenesis begins. The deposit of the carotenoid in the gonads appears to be essential to the success of maturation.

However, as in the case of PUFA it is useful to remember that the reproduction only starts with the generation of gametes (which incidentally, almost nothing is known about spermatogenesis, because all researchers prefer to work with females).

Certainly, the astaxanthin, the common retinoids - or unknown retinoids - and probably the two types of poliprenes together, will be required to support at outbreak of eggs and the various seedlings of stage nauplius', all dependent on the material deposited in the egg, while the intake of exogenous resources not yet exist. For this reason, the condition of the matrix is critical not only for the production of eggs but also for the development of the early stages larvários. Also explains why it is has been recently made many studies with wild matrices. Unfortunately, there are serious problems regarding the supply of carotenes for arrays in captivity furthermore may be missing certain mixture of several different compounds that are present in nature.

Unfortunately, there are serious problems regarding the supply of carotenes for arrays in captivity furthermore may be missing certain mixture of several different compounds that are present in nature.

It was adopted in many larviculture institutes a cruel method to overbalance the hormonal system of matrices and force repeated ovulations until the animal exhaustion: cauterization of the glands under eyestalks. With this surgery, the metabolism of the carotenoids undergoes changes that may harm the procedure.

The addition of the carotene to formulas of the fortified diets includes its own application problems because these substances are decomposed, as much by light as by temperature, the oxygen, many enzymes and other ingredients present in a commercial feed. There now lacks the means to create large scale microorganisms and microalgae rich in carotenes (*Phaffia rhodozyma*, *Haematococcus pluvialis*, *H. lacustris*, *Dunaliella salina*, *Spirulina*), there is till transgenic yeast for this purpose. Often, low-tech solutions attract much interest, for example, employ ground chili.

α-Tocopherol (Vitamin E)

Vitamin E is a major antioxidant linked to lipid cell membranes and lipoproteins. It protects the polyunsaturated fatty acid of the peroxidation through the free radicals. The most active isomer of vitamin E is the *α*-tocopherol; so then the two denominations are not discriminated against. Tappel (1968) suggested that vitamin C can regenerate vitamin E partially oxidized by the radicals. Hypothesis was confirmed and then imply that large doses of ascorbic acid in vivo can reduce the requirement tocopherol.

In vertebrates, vitamin E deficiency affects fertility, stability of erythrocytes and seems to have some connection with the running of seleno-cysteine. With crustaceans, knowledge about the action of vitamin E are much more limited than with fish. He et al. (1992) worked with juvenile *L. vannamei* (40 mg) that were studied eight weeks, comparing the deficiencies of vitamins A, D, E and K.

In this material, the lack of vitamin E hampered growth more than any other disability. Moreover, it was the only case in which appeared a darkening of the hepatopancreas in certain analogy to the adipose tissue pathology observed in vertebrates. Alava et al. (1993) observed that *P. japonicus* that the maturation of ovarian depends on the presence of the tocopherol (indicating 100 mg kg^{-1}) and ascorbic acid (indicating 3,000 mg kg^{-1}). Cahu et al. (1995) found the highest concentrations of PUFA, necessary for proper nutrition matrices *P. indicus*. Recorded a hatching in dependence administered tocopherol and recommended a rate of vitamin 300 mg kg^{-1}. In *M. rosenbergii*, Cavalli et al. (2003) submitted arrays to the regimes with different concentrations of vitamins E and C and analyzed the content of vitamins in various organs along with some biometric data, after five months of experience. Moreover, the conditions of the obtained larvae were tested. In this experiment, vitamin E had no influence on important parameters such as survival of adults and larvae and the eggs hatch. Only in dose of 899 to tocopherol, it was incorporationin hepatopancreas and ovary increased. However, the tocopherol in the nutrition array is mirrored in the concentration of vitamin in eggs and also made the larvae a bit more resistant against stress ammonia'.

Vitamin K

The action of vitamin K was explained only in 1978 by Shah and Suttie (1978), more than 50 years after its discovery as anti-hemorrhagic factor in vertebrates. Vitamin K is a cofactor of an carboxylase that transforming the glutamate radicals in various coagulation proteins to turn the carboxyglutamate, Thus creating bridges adhesion for calcium, whose role in the coagulation before it was not understood.

Shiau (1998) studying the requirements the vitamin K in crustaceans found values of 185 mg kg^{-1} for *P. chinensis* and 30-40 mg kg^{-1} for *P. monodon*, with tests based on growth and feed conversion of juveniles.

However, the mechanism of action of vitamin K, discovered by Shah and Suttie (1978), does not work in crustaceans. Even considering a blood clotting in these animals, this phenomenon is based on an entirely different principle of vertebrates, and does not need carboxyglutamate, that it not even exists in crustacean, so that the way how said vitamin influences the growth of crustaceans remains unknown.

Vitamin D

Vitamin D was discovered as the anti-rickety factor in man and had its mechanism of action unraveled in several stages, also long after his discovery. It is known that the compound corresponding to vitamin D3, cholecalciferol, it is not even vitamin, unless as consequence of certain cultural and environmental factors in the development of mankind. Without these restrictive conditions, vitamin D is not essential, because it is produced by sunlight in skin of vertebrates from 7-dehydrocholesterol, a precursor of cholesterol itself.

Cholecalciferol is prohormone of 1.25-dihydroxycholecalciferol substance (calcitriol), which regulates the synthesis of proteins for calcium absorption in the intestine, besides working in very different systems.

Whereas the action of vitamin D in crustaceans, fit the same considerations made concerning vitamin K. Shiau and Hwang (1994) determined in *P. monodon* a requirement of 0.1 mg kg^{-1}. In *L. vannamei*, He et al. (1992) reported a 23% reduction in the growth of juvenile when the vitamin it was removed of the nutrition. However, the authors admitted not knowing its function even if the calcification of the exoskeleton could be a doable target. It is useful to repeat that in vertebrates vitamin as such serves only as a hormone precursor, to add that the hydroxylations necessary for this functional transformation have never been investigated in invertebrates.

CONCLUSIONS

Although lipids form the most important energy stock in animals, they can not surpass a relatively small fraction of shrimp nutrition supplied mainly by proteic catabolism. However, it is even more important the correct composition of ingested lipids, considering their essential components the animal is not able to synthesize.

One of these essential components is cholesterol. Cholesterol is a relatively small part of essential lipids for shrimps, but cannot be replaced by phytosterolds. Since cholesterol is the ecdysone hormone precursor that controls molts, it is particularly necessary in larvae stages.

Among the functions of liposoluble vitamins and provitamins, the function better understood it is the of antioxidant for preservation PUFA provided by vitamin E and astaxanthin. The vitamin A controls development and part of the seedlings beyond vision. It is not known as the vitamins D and K function in invertebrates.

The imbalance of lipids affects growth, reproduction and survival of shrimps and may be related to syndromes occurrence, such as AHPNS or EMS, magnifying the susceptibility of those aquatic organisms to opportunistic infections, masking the real origin of these conditions.

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