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CAROTENOIDS FROM PLANTS USED IN DIETS FOR THE CULTURE OF THE PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

CAROTENOIDES DE PLANTAS INCLUIDOS EN DIETAS PARA EL CULTIVO DEL CAMARON BLANCO DEL PACIFICO (*Litopenaeus vannamei*)

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Abstract

The use of carotenoids as pigments in aquaculture diets is well documented. These pigments seem to have many physiological functions that include a role as antioxidant and provitamin "A". A common characteristic of shrimps is their pink flesh when cooked. Dietary carotenoids, among them astaxanthin, are the responsible for the ability of captured shrimps for developing their characteristic color when cooked. These carotenoids are sourced mainly from krill and phytoplankton. However, for cultured shrimps carotenoids are not available unless included in their feeds. The current limited production of astaxanthin cannot meet the growing demand for this pigment, so that scientists have strived to find new alternative sources for this pigment. Carotenoids from plant sources seem to provide an interesting option to this problem. The present study is aimed at showing the state of the art in the research on the use of pigments from plant extracts and their potential for their incorporation in the feed of shrimps (*Litopenaeus vannamei*). Some promising alternative plant sources for the pigment astaxanthin are the carotenoids from the yeast *Phaffia rhodozyma*, the microalgae *Haematococcus pluvialis*, *Dunaliella salina* and *Spirulina* , the petals from *Adonis aestivalis* and *Tagetes erecta*, the red chili bells from *Capsicum annuum* and the leguminous *Leucaena leucocephala*. Many of these carotenoids sources have been used at concentrations of 100 to 450 mg/kg in the diet of shrimps, and in particular for the white shrimp (*L. vannamei*), a marked increase of carotenoid content in the exoskeleton and abdomen has been observed. This suggests that the different carotenoids contained in plant extracts such as zeaxanthin, lutein and capsanthin are converted into astaxanthin.

Keywords: plant pigments, carotenoids, pacific white shrimp, diets.

Resumen

El uso de pigmentos carotenoides como aditivos en la acuicultura está bien documentado, y aparentemente su función principal en el organismo es actuar como un antioxidante y promotor de la producción de vitamina "A". Una característica común de los camarones es su color rosa cuando se cuecen, el cual se obtiene del consumo de pigmentos carotenoides, y principalmente de la astaxantina, provenientes de su dieta de fitoplancton y krill. La producción actual de la astaxantina no cubre las demandas de los productores, por lo cual los científicos están buscando fuentes alternativas de pigmentos, principalmente de origen vegetal, que tengan un bajo costo, una alta concentración de pigmentos y una calidad constante del producto. El presente estudio tiene como finalidad mostrar el estado de avance en la investigación de la utilización de pigmentos extraídos de plantas y su potencial en la incorporación en la alimentación del camarón (*Litopenaeus vannamei)*. Algunas promisorias fuentes carotenoides sustitutos de la astaxantina son la levadura *Phaffia rhodozyma*; las microalgas *Haematococcus pluvialis*, *Dunaliella salina* y *Spirulina spp*.; pétalos de *Adonis aestivalis* y *Tagetes erecta*; el chile rojo *Capsicum annuum*; y la leguminosa *Leucaena leucocephala*. Los carotenoides provenientes de estas fuentes han sido incluidas en concentraciones que van de 100 a 450 mg/kg en la dieta para camarones. En el caso particular del camarón blanco (*L. vannamei*) se ha notado un incremento marcado de carotenoides en el exoesqueleto y el abdomen cuando se le alimenta con dietas suplementadas con extractos de las plantas mencionadas. Esto sugiere que la los carotenoides como la zeaxantina, la luteína y la capsantina están siendo convertidos a astaxantina.

Palabras clave: pigmentos de plantas, carotenoides, camarón blanco del pacífico, dietas.

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1. Introduction

Aquaculture as a means of plant and animal production is growing rapidly in the world. In turn, the commercial culture of various shrimps and prawns species for food is one of the fastest growing areas of aquaculture (Rosenberry, 2005). A common characteristic of shrimps is their pink flesh, the color coming from carotenoid pigments, primarily astaxanthin. The use of carotenoids as pigments in aquaculture species is well documented, and apparently their broader functions include role as antioxidant and provitamin “A” activity inducer, as well as enhancing immune response, reproduction, growth, maturation, and photoprotection (Howell and Matthews, 1991). They improve the tolerance in environments with high ammonia levels and low oxygen levels (Meyers, 1994).

An extensive body of data evidences the vital role of carotenoids in the physiology and overall health of aquatic animals and suggests that carotenoids are essential nutrients that should be included in all aquatic diets (Grung *et al.,* 1993). Most crustaceans possess a mixture of carotenoids in the carapace, blood, eyes, midgut gland, and ovary. Carotenoids are a family of over 600 natural lipid-soluble pigments that are produced by microalgae, phytoplankton, and higher plants (Britton *et al., 1*981).

The effect of diet supplementation with carotenoids has been studied in three main research lines: a) knowledge of the factors that influence pigmentation (Storebakken and No, 1992); b) mechanisms of absorption, deposition, metabolism, and biological activity of the pigments in the diverse species (Matsuno and Hirao, 1989); c) search for alternative sources of pigments for supplementing in aquaculture diets for achieving and improved (Tanaka *et al.,* 1976; Chien and Jeng, 1992; Liao *et al.,* 1993), or corrected (Menasveta *et al.,* 1993) the color of aquaculture species, mainly cultured penaeids (Lorenz, 1998), in order to achieve a better market price (Liao and Chien, 1994).

The present study was aimed at showing the state of art in the research of using pigments from plants extracts and their potential for their incorporation in the feed of culturd shrimps (*Litopenaeus vannamei*).

2. Carotenoid plant sources

The scientific literature, indicate that the main sources of pigment plants carotenoid are the yeast *Phaffia rhodozyma* and petal meal *Adonis aestivalis* that contain an important amount of astaxanthin; *Spirulina* with high amounts of lutein; the microalgae *Dunaliella salina* and Aztec marigold petal meal *Tagetes erecta* (toreador and sovereign strain) with zeaxanthin; red chili or paprika *Capsicum annuum* that include capsanthin and zeaxanthin, and the microalgae *Haematococcus pluvialis* with astaxanthin and cantaxanthin. An important source of carotenoids, like lutein, β-carotene, flavoxanthin, violaxanthin, lycopene and astaxanthin diesters, are present in *Adonis annua*, *A. aestivalis*, *A. flammeus*, *A. turkes tanika*, *A. dentata*, *A. vernalis* species, known as red manzanillas and Viola tricolor (Markovits, 1992).

Astaxanthin content in *A. annua* is of 300 to 500 mg/kg of flower, whereas in selected plants, under adequate irradiation conditions, flowers have been obtained with contents as high as 10,000 ppm of astaxanthin in their petals. This source of carotenoids has been used in the rainbow trout for pigmentation (Markovits, 1992).

The esterified and non-esterified oleoresins of the “cempasúchil” (*Tagetes erecta*) flower contain the pigments lutein and zeaxanthin. This source of carotenoids has been used to provide pigmentation in the Pacific white shrimp (*L. vannamei*) (Vernon-Carter *et al.,* 1996; Arredondo-Figueroa *et al.,* 1999). Paprika contains the xanthophylls ß-carotene, ß-cryptoxanthin, capsanthin, and capsorubin, some of which can apparently be slowly converted to astaxanthin after some time (Latscha,1991; Arredondo *et al.,* 2004).

Table 1. Plant pigment sources including in dietary feed in shrimp.

Aquaculture species	Pigment source	Carotenoids	Dietary concentration (mg/kg)	Reference
P. japonicus	*Carophyll Pink*	Astaxanthin, β-caroteno, canthaxanthin	200	Yamada et al., 1990.
P. japonicus	*Carophyll Pink* *Dunaliella salina*	Astaxanthin β-carotene	500 100	Chien and Jeng., 1992.
P. japonicus	*Carophyll Pink*	Astaxanthin	100	Negre-Sadargues et al., 1993.
P. japonicus	*Carophyll Pink*	Astaxanthin	60	Petit et al., 1998.
Penaeus brasiliensis and Penaeus dorarum	*Leucaena leucocephala*	--	450	Araneda, 1990.
Penaeus monodon	*Carophyll Pink*	Astaxanthin	50	Menasveta et al., 1993.
Penaeus monodon	*Spirulina* *Phaffia rhodozyma*	Zeaxanthin Astaxanthin	300	Okada et al., 1994.
Penaeus monodon	*Carophyll Pink*	Astaxanthin	80	Pan et al., 2001.
Penaeus (L.) vannamei	*Tagetes erecta*	Lutein Zeaxanthin	230-350	Vernon-Carter et al., 1996.
Litopenaeus vannamei	*Tagetes erecta*	Lutein Zeaxanthin	50-350	Arredondo-Figueroa et al., 1999.
Litopenaeus vannamei	*Capsicum annuum*	Capsanthin	200-250	Arredondo et al., 2004.

The astaxanthin diester from *A. annua* is hydrolyzed in the intestinal tract and transferred to the skin where it is re-esterified with fatty acids (Markovits, 1992).

3. Absorption and storage

Carotenoids from caparace and internal organs of shrimp have been isolated and identified. Astaxanthin was found to be the most important pigment both in the exoskeleton (95%) and muscles (Carreto and Carignan, 1984) and has been shown to be responsible for the desirable body color of prawns upon cooking (Okada *et al.,* 1994). Crustaceans and other aquatic animals are unable to produce astaxanthin *de novo*, only plants and protists (bacteria, algae, fungi) are capable of synthesizing carotenoids. Shrimp receiving natural astaxanthin from microalgae and microcrustaceans take up the corresponding (3S, 3'S) and (3R, 3'R) isomers (Whyte *et al.,* 1998). Most of astaxanthin within the epidermal tissue is in the mono-esterified form, meaning that one of the hydroxyl groups is esterified to a fatty acid; whereas, carotenoid and protein complexes, called carotenoproteins and carotenolipoproteins, predominate in the exoskeleton (Howell and Matthews, 1991). Carotenoids are synthesized through the isoprenoid pathway which also produces such diverse compounds as essential fatty acids, steroids, sterols, vitamins A, D, E, and K. Within the various classes of natural pigments, the carotenoids are the most widespread and structurally diverse pigmenting agents. They are responsible, in combination with proteins, for many of the brilliant yellow to red colors in plants and the wide range of blue, green, purple, brown, and reddish colors of fish and crustaceans. The general distribution and metabolic pathways of carotenoids has been extensively detailed previously (Matsuno and Hirao, 1989) (Fig. 1).

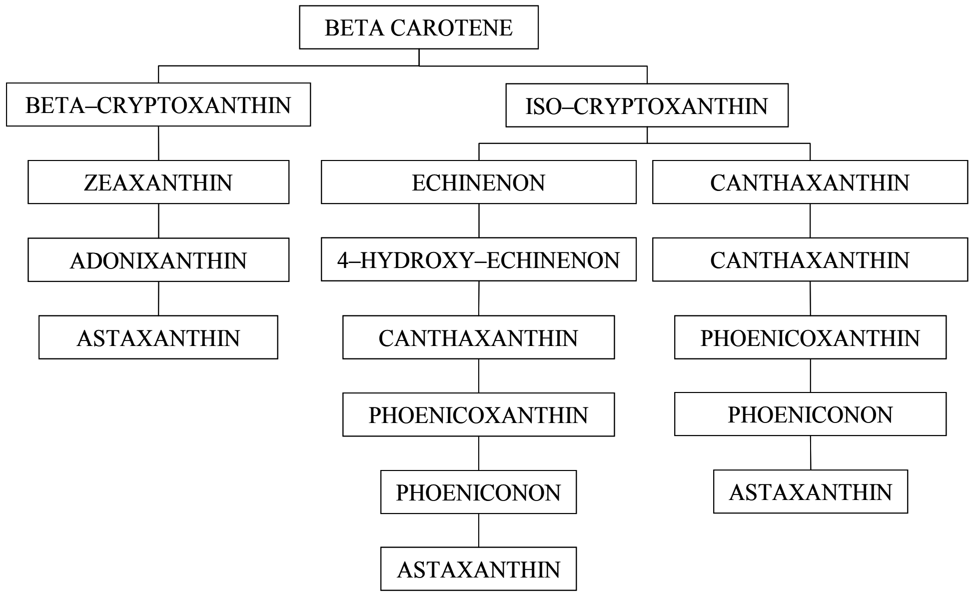

Fig. 1. Metabolic pathway of carotenoids in shrimp (Latscha, 1991).

Analysis of the tissues from experimental groups revealed that the astaxanthin-fed group increased 318% in carotenoids, and had a normal appearance. Those fed the commercial diet without astaxanthin had a carotenoid increase of only 14% and had a blue hue (Menasveta et al. 1993). Carotenoids and carotenoproteins content in the exoskeleton and the muscular epidermis of cultured shrimps, *P. monodon* and *L. vannamei,* has been analyzed (Okada et al., 1994; Vernon-Carter *et al.,* 1996). The main carotenoid, astaxanthin, was more abundant in the muscular epidermis than in the exoskeleton. Only blue carotenoproteins having free astaxanthin as the prosthetic group were found. The content of astaxanthin, in the form of carotenoproteins, in the muscular epidermis was almost constant irrespective of the body color. The content of astaxanthin esters, especially monoester in the muscular epidermis, was higher in well-pigmented prawns than in pale ones. As a result the color of the white shrimp is expressed by the interaction between carotenoproteins and red astaxanthin esters (Howell and Matthews, 1991).

Dietary astaxanthin, beta-carotene, and canthaxanthin are deposited in prawns' body tissue mainly as astaxanthin esters (Yamada *et al.,* 1990). A marked increase of carotenoid content in the exoskeleton was observed when organisms were fed with *Spirulina*-supplemented diets. This suggests that zeaxanthin, one of the major carotenoids in *Spirulina*, was rapidly converted to astaxanthin (Nakagawa and Gomez-Diaz, 1975; Liao *et al*., 1993; Lorenz, 1998). Most carotenoids present in the wild *P. monodon* were astaxanthin, astaxanthin esters, and small amounts of β-carotene. A total carotenoids concentration of 26.3 ppm has been isolated from the exoskeleton of wild shrimp, as well as from farmed shrimp supplemented with sufficient astaxanthin. Whereas, specimens displaying Blue Disease had total carotenoids concentrations of only 4 to 7 ppm in the exoskeleton. Approximately 85% of the carotenoids fraction corresponded to astaxanthin esters, the remaining 15% consisted of free astaxanthin, lutein, and β-carotene, which were also beneficial as pigments and as inducers of provitamin "A" activity.

The red color of cooked crustaceans is produced by the release of the individual carotenoid prosthetic group (astaxanthin) from the carotenoproteins when denatured by the cooking heat. The final color and hue saturation are dependent on the amount of deposited astaxanthin.

4. Growth and survival

Linear and ponderal growth rates are lower in shrimp fed a carotenoid-free diet as compared to groups fed astaxanthin-supplemented diets. Additionally, supplementation of the diet with astaxanthin decreases the postlarvae development period by inducing quantitative variations of molting hormones (Petit *et al.,* 1997). It has been demonstrated that there is a significant decrease in mortality of adult shrimp fed a carotenoid-enriched diets in comparison with individuals receiving carotenoid-free diets. A survival rate of 91% was observed with individuals fed a diet supplemented with 100 ppm astaxanthin compared to 57% in the control group without astaxanthin after 4 to 8 weeks of growth (Yamada et al., 1990). Survival was higher in prawns (*P. japonicus*) fed an astaxanthin diet, and a positive correlation between survival and pigment concentration of tissues suggested that the carotenoids functioned as an intracellular oxygen reserve (Chien and Jeng, 1992). This permitted the crustaceans to survive under hypoxic conditions common in pond cultures. Shrimp fed astaxanthin at 100 mg/kg diet had an average survival rate of 77% in contrast to shrimp supplemented with β-carotene which averaged 40% (Chien 1996). A survival rate of 88.2% in *L. vannamei* was observed with individuals fed a diet supplemented with 350 ppm carotenoids (marigold petals, *Tagetes erecta*) compared to 76.5% in the control group without carotenoids after 5 weeks of growth (Arredondo *et al.,* 1999). Survival (100%) was higher in shrimp (*L. vannamei*) fed paprika (*Capsicum annuum*) than in those fed basal diets (80.5%) (Arredondo-Figueroa *et al.*, 2004). In general terms, shrimps fed with diets containing high carotenoids concentrations exhibited a larger weight gain and a higher survival rate.

5. Maturation

High quality broodstock maturation diets are an essential key for successful and sustained production of nauplii. Most maturation diets depend on fresh or frozen natural feeds such as squid, krill, mussels, and polychaete worms. However, under sustained conditions, a general decline of nauplii quality and larval performance is observed. The degradation is associated with a loss of pigmentation and bleaching of the ovaries of mature females and larval egg yolks. Consequently, there are low larval feeding rates in Z_1, high levels of larval Z_1 deformities, and very low survival to larval stage zoea II. This condition has been termed "Pigment Deficiency Syndrome" (PDS). Paprika has been found to be somewhat effective as a pigmentation source for American lobsters, and has been successfully used as a carotenoid source to reverse the deleterious effects of PDS in High Health broodstock (Wyban *et al.,* 1997). In one experiment, a group of broodstock afflicted with PDS was used to test the inclusion of paprika carotenoids in the diets. After four weeks under simulated commercial conditions, nauplii quality improved dramatically with the mean ZII survival rate increasing from 25% to 83% ($p<0.01$). The percentage of larvae with full guts increased from 49% before paprika to 96% ($p<0.01$), and percentage of deformed larvae decreased from 21% to 4% after four weeks of carotenoid supplementation. There was no significant difference between ablated and unablated females within the treatment groups (Wyban *et al.,* 1997).

6. Diet supplementation

Carotenoids supply to the diet of shrimps has been studied in *P. japonicus*, *P. monodon,* and *L. vannamei*. The ingredients of vegetable origin that have been used in the diet are red yeast (*Phaffia rhodozyma*); microalgae, *Dunaliella salina* (Chien and Jeng, 1992), *Chnoospora minima* (Menasveta et al., 1993), *Spirulina sp.* and *S. pacifica* (Liao et al., 1993; Chien and Shiau, 1998), *Haematococcus pluvialis* (Chien and Shiau, 1998), and *Isochrysis galbana* (Pan et al., 2001); wheat, corn and alfalfa (Meyers and Latscha, 1997); marigold *Tagetes erecta* (Vernon-Carter et al., 1996; Arredondo-Figueroa *et al.,* 1999); paprika *Capsicum annuum* (Vernon-Carter et al., 1996; Arredondo-Figueroa et al., 2004). A marked increase of carotenoid content in the carapace has been observed when organisms were fed with plant pigment source diets.

In recent years, a line of synthetic carotenoids has been widely added to shrimp feeds to promote or augment flesh pigmentation in cultured shrimp, especially during the final stage (e.g., 8 to 16 weeks) of production. Regardless of source, currently available natural feed ingredients high in carotenoid pigments are expensive and add significantly to the cost of shrimp diets.

The exclusive use of pelleted feeds has led to progressive loss of exoskeleton pigmentation through successive molt cycles of crustacean decapods (Sommers et al., 1991). Loss of shell color detracts from acceptance of decapods by commercial markets. This change suggests a dietary carotenoid deficiency. Supply of plant material maintains crustacean shell pigmentation and may correct other nutritional deficiencies due to the use of artificial diets, thereby improving growth rates. Use of a specific refined source of carotenoids is desirable for incorporation into pelleted diets for nutritional research. However, recent progress in improving growth performance on artificial diets has been made by including animal and plant extracts that contain carotenoids and essential growth factors, such as polyunsaturated fatty acids, and to reduce loss of water soluble vitamins and amino acids. Unfortunately these crustacean products are expensive and commercially unavailable in Latin America.

Arredondo-Figueroa *et al.* (1999) studied the effects of various dietary unesterified Aztec marigold carotenoid concentrations on the pigmentation of the Pacific white shrimp. Four pigmented diets containing the unesterified marigold extracts at concentrations of 50, 100, 200, and 350 ppm, a reference diet containing 200 ppm Carophyll pink, and a non-pigmented control diet were fed to the shrimp during 35 days. Abdominal muscle and exoskeleton pigmentation was influenced by the diet pigment concentration and by the duration of the feeding test. The degree of abdomen pigmentation achieved by the 200 ppm Carophyll diet was equaled by the 200 ppm unesterified marigold diet, whereas the exoskeleton pigmentation was superior with the Carophyll-containing diet than with any diet containing up to 350 ppm unesterified marigold pigment.

Pacific white shrimp were fed diets containing natural pigments from chilli peppers (*Capsicum annuum*) extracts, esterified and saponified (Arredondo et al., 2004). The pigmenting effect of theses carotenoids was compared with a carotenoid-free control diet and a synthetic astaxanthin (Roche Carophyll Pink) supplemented diet. It was found that after 15 days of feeding, the shrimp showed that the saponified chilli extract and the high concentration esterified chilli extract supplemented diets produced a better pigmentation effect (between 7.5 to 11%) in the exoskeleton than astaxanthin. During the same 15 days period, non-significantly different results were observed in the carotenoid content of the shrimp abdominal muscle when fed with all diets except the low concentration esterified chilli extract diet, which showed a significantly lower value. Theses results indicated that the main carotenoid source in *Capsicum annuum*, namely capsanthin, was possibly metabolized into astaxanthin and deposited by the Pacific white shrimp.

When plants are given in the diet of shrimps, it is important to determine the presence of antinutrients, such as the toxic glycosides in *A. annua* (Markovits, 1992) and in leguminous seed meal (Cabanillas et al., 2002).

Conclusions

A series of studies on the effects of pigment supplementation to the diet of the shrimp (*Litopenaeus vannamei*) reveal that this improves growth, reduces the mortality rate, and enhances the general performance of the animal. In addition, a large amount of empirical data suggests that sufficient carotenoids supply is essential for the well-being of the animal. Astaxanthin or canthaxanthin should be regarded as a vitamin for shrimp and added to all shrimp diets at a level above 10 mg/kg dry diet.

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