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PIGMENTATION OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei,* BOONE 1931) WITH ESTERIFIED AND SAPONIFIED CAROTENOIDS FROM RED CHILI *Capsicum annuum*) IN COMPARISON TO ASTAXANTHIN

PIGMENTACION DEL CAMARON BLANCO DEL PACIFICO (*Litopenaeus vannamei,* BOONE 1931) CON CAROTENOIDES DE CHILE (*Capsicum annuum*), ESTERIFICADOS Y SAPONIFICADOS, EN COMPARACION CON LA ASTAXANTINA

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Abstract

Pacific white shrimp were fed diets containing esterified and saponified carotenoids obtained from red chili extracts. The pigmenting effect of these carotenoids was compared with a non-pigment supplemented control diet and synthetic astaxanthin (Carophyll Pink) supplemented diet. After 14 days the shrimp showed that the diet containing 250 mg/kg of esterified carotenoids produced a better pigmentation effect in the exoskeleton and a slightly lower pigmentation effect in the abdomen than astaxanthin. These results suggest that capsanthin, which accounts for around 40% of the total carotenoids in red chili extracts, is metabolized and deposited as astaxanthin in the abdomen and exoskeleton of Pacific white shrimp.

Keywords: red chili extracts, astaxanthin, Pacific white shrimp, pigmentation.

Resumen

Camarones blancos del pacífico fueron alimentados con dietas que incluían carotenoides esterificados y saponificados provenientes de extractos de chile rojo. El efecto pigmentante de estos carotenoides se comparó con el de una dieta control sin pigmentos añadidos y con una dieta que incluía astaxantina sintética (Carophyll Pink). Los camarones alimentados con la dieta conteniendo 250 mg/kg de carotenoides esterificados mostraron una mayor pigmentación en el exoesqueleto y una pigmentación ligeramente menor en el abdomen que la inducida por la astaxantina después de 14 días. Estos resultados sugieren que la capsantina, que representa alrededor del 40% de los carotenoides totales en los extractos de chile rojo, es metabolizada y depositada como astaxantina en el abdomen y exoesqueleto del camarón blanco del Pacífico.

Palabras clave: extractos de chile rojo, astaxantina, camarón blanco del Pacífico, pigmentación.

1. Introduction

Carotenoids are the main pigments of many aquatic animals (Meyers, 1994). In aquaculture, astaxanthin and canthaxanthin are commonly used as pigment sources in fish and shrimp. Carotenoids in the Penaeidae include astaxanthin and its esters, carotenes and xantophylls (Fisher *et al*., 1957). The following carotenoid pigments were found in shrimp caparace (Decapoda: Penaeidae): lutein, tunaxanthin, astaxanthin diester, astaxanthin monoester and free astaxanthin (Négre-Sadargues *et al.*, 1993). The main pigment that colors shrimp muscle is Astaxanthin (3,3 dihydroxy-β-carotene 4,4-dione), and when present in the diet it can be deposited directly in the tissue as an ester of astaxanthin (Tanaka *et al*., 1976; Yamada *et al.*, 1990).

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Astaxanthin in its various forms was found to be most important pigment both in studies in shrimp using astaxanthin from marine sources have been described by various authors (Tanaka *et al*., 1976; Latscha, 1990; Chien and Jeng, 1992). Liao *et al*. (1993), Vernon-Carter *et al*. (1996) and Arredondo *et al*. (1999) have also examined shrimp pigmentation by plant carotenoids. Synthetic carotenoids have received considerable interest (Spinelli and Mahnken, 1974; Tanaka *et al*., 1976; Otazu and Ceccaldi, 1984; Yamada *et al*., 1990; Negre-Sadargues *et al*., 1993). It has been shown that the inclusion of pure carotenoids or exoskeleton and in abdomen (Carreto and Carignan, 1984). Pigmentation crude carotenoids in diets improves pigmentation in crustaceans (Katayama *et al*., 1972 a, b; Chien and Jeng, 1992; Okada *et al*., 1994). Thus, the main purpose of this work was to determine if the esterified and saponified carotenoids obtained from red chili pepper (*Capsicum annuum*) are bioavailable and deposited in the tissues of the Pacific white shrimp (*Litopenaeus vannamei,* Boone 1931).

Table 1. Composition (g/100 g dry diet) of the experimental diets for shrimp pigmentation.

Ingredients	CD	R200	R250	B200	CP
White fish meal	27.00	27.00	27.00	27.00	27.00
Soybean meal (defatted)	14.00	14.00	14.00	14.00	14.00
Shrimp meal	17.00	17.00	17.00	17.00	17.00
Yeast	15.00	15.00	15.00	15.00	15.00
Wheat starch	20.00	19.66	19.59	17.96	19.87
Cholesterol	0.50	0.50	0.50	0.50	0.50
Cod liver oil	2.00	2.00	2.00	2.00	2.00
Soybean lecithin	1.00	1.00	1.00	1.00	1.00
Basfind™ (binder)	0.50	0.50	0.50	0.50	0.50
Ascorbic acid	0.50	0.50	0.50	0.50	0.50
Mineral premix [2]	1.50	1.50	1.50	1.50	1.50
Pigment source	-	0.34	0.41	2.04	0.13
Crude protein	43.29	41.70	41.45	42.81	43.35
Crude fat	6.83	6.19	6.79	6.12	6.95
Ash	8.25	8.13	7.35	8.24	7.02
Nitrogen free extract	37.63	39.93	40.03	38.32	38.39
Fiber	4.00	4.05	4.38	3.97	4.29
Calculated digestible energy (MJ/kg) [3]	18.0	17.7	17.9	17.7	19.0
Determined total carotenoids concentration (mg/kg)	8.02	197.9	253.6	199.4	98.8

[1] Vitamin mix (mg/100 g dry diet): para-aminobenzoic acid, 5.55; biotin, 0.22; inositol, 222.06; nicotinic acid, 22.21; Ca-pantothenate, 33.31; pyridoxine-HCl, 6.66; riboflavin, 4.44; thiamine-HCl, 2.22; menadione, 2.22; beta-carotene, 5.55; tocopherol, 11.10; calcipherol, 6.66; cyanocobalamine, 0.555; folic acid, 4.44; choline chloride, 66.6; Na-ascorbate, 1110.3.

[2] Mineral mix (g/kg dry diet): K_2HPO_4, 1.008; $Na_2HPO_4.7H_2O$, 2.167; $Ca(H_2PO_4)$. H_2O, 2.671; $CaCO_3$, 0.978; Ca-lactate, 1.663; KCl, 0.282; $MgSO_4.7H_2O$, 1.008; Fe-citrate, 0.121; $AlCl_3.6\ H_2O$, 0.0024; $ZnSO_4.7H_2O$, 0.048; $MnSO_4.4\text{-}6\ H_2O$,0.0108; $CuCl_2$, 0.0015; KI, 0.0023; $CoCl_2.6\ H_2O$, 0.0141; celufill, 0.0216.

[3] Calculated Digestible Energy (kJ/100 g diet) (Gaxiola, 1991).

2. Materials and methods

2.1 Diet preparation

A control diet (CD) was formulated based on that reported by Gaxiola (1991) for *Penaeus schmitti* (Table 1). Three experimental diets were prepared by adding the requisite amount of the different pigment sources in partial substitution of wheat starch in the CD.

Concentration of each pigment source in the diets was selected based on a study in which it was found that similar coloration was achieved with *Penaeus monodon* when feed 50 mg/kg astaxanthin than 125 mg/kg beta-carotene from *Dunaliella salina* during 7-8 weeks (Boonyaratpalin *et al*., 2001). The pigment sources used were: Carophyll Pink (8% synthetic astaxanthin, Hoffman-La Roche, Basel, Switzerland) in a theoretical concentration of 100 mg kg^{-1} (reference diet CP); Rodofila (6.5% total carotenoids from red chili oleoresin, Bioquimex-Reka, S.A. de C.V., Queretaro, Mexico) at theoretical concentrations of 200 (diet R200) and 250 mg/kg (diet R250); and Biored-L (1.029% total carotenoids from saponified red chili oleoresin, Bioquimex-Reka, S.A. de C.V., Queretaro, Mexico) at a theoretical concentration of 200 mg/kg (diet B200). The manufacturers supplied carotenoids concentration of pigments. A separate experiment showed that by adding a 5% excess of the pigments of that required to obtain the desired theoretical total carotenoids concentration in the experimental diets, made up losses incurred in the pigment incorporation process to the CD.

The shrimp larvae dry ingredients were finely ground in a SAMAP Electric Cereal Mill with adjustable millstones model F100 (Andolsheim, France). The resulting flours were mixed for 10 min in a Hobart mixer N-50 (Troy, OH), after which the pigments (Carophyll Pink in its microcapsulated presentation; Biored-L in its aqueous solution presentation; and the oil soluble Rodofila was previously dispersed with the oil ingredients of the diet) were added with a further 10 min mixing. The ensuing masses were extruded through a manual meat grinder (Sanson, Mexico City, Mexico) dried in a vacuum oven (Cole Parmer, Chicago, ILL.) at 60 °C for 2.5 h. The dry collets were broken to a length of about 0.5 cm, placed in plastic containers and stored at 4 °C until required.
Moisture content, crude protein, crude fat, ash, and carbohydrate contents were determined based on the official methods of analysis of the AOAC (1995).

2.2 Feeding trials

The shrimp used in this assay were of the species *Litopenaeus vannamei* (Boone, 1931) commonly known as the Pacific white shrimp. 120 organisms with a length of 6-8 cm and average weight of 6.0 ± 0.14 g were allotted randomly to 10 tanks (40 cm wide, 50 cm long and 30 cm deep), having a density of 60 shrimp/m^2, and fed in duplicate groups. Seawater was filtered through 5 μm cartridge filter, and was maintained with a salinity of 35.0 ± 0.5 g/L, an average temperature of 28.0 ± 0.4°C, pH of 7.38 ± 0.21 and dissolved oxygen of 6.5 mg/L. Seawater was changed three times a week.

The shrimp were fed twice daily at eight % of body weight per day at 0900 h and 1800 h, during 28 days. The amount of feed fed daily was decreased accordingly to shrimp sampled. Shrimp mortality was recorded daily and upon the termination of the experiment the shrimp were weighed individually. Percent weight increase per shrimp was calculated by dividing the mean percent increase in weight by the number of shrimps per tank.

2.3 Total carotenoids extraction and quantification

2.3.1 In feed

Ten grams of feed were grinded to pass No. 40 sieve and put into a 100 mL volumetric flask. Pipette 50 mL acetone into flask, stopper, and shake in water bath at 56 ± 1 °C during 10 min. Cool flask with iced water. Dilute the volume with acetone, stopper and swirl for two min. Let flask to rest for 15 min. Pipette 5 mL from each flask into a 50 mL volumetric flask to which 20 mL of acetone had been previously added. Dilute volume with acetone. The extracts were measured in a spectrophotometer against acetone. The total carotenoids concentration (TCC) was calculated from the peak absorbance in acetone at 460 nm with the following equation (Bioquimex-Reka, 1998):TCC (g/kg) = (A_{460} x 0.164 x DF)/(40 x W), where:

A_{460} = absorbance at 460 nm
W = weight of sample in grams
DF = dilution factor = (100 x 50)/5
0.164 = ASTA (1986) conversion factor for color units
40 = ASTA (1986) conversion factor to g/kg

2.3.2 In shrimp

The determination of pigmentation was carried out at the beginning by randomly selecting two organisms from each tank. At day 14 and 28 four shrimp were sampled each time at random from each tank, and sacrificed. Shrimp from each tank were pooled as one sample in order to isolate sufficient pigment. The viscera (protoventriculus, hepatopancreas and intestine) were removed from the shrimp, whilst the exoskeleton and abdominal muscle were immediately homogenized with an Ultra-Turrax homogenizer for 1 min, and 10 g samples were extracted with acetone and analyzed for TCC as indicated above.

2.4 Statistical analyisis

Treatment effects were identified using one-way analysis of variance (ANOVA) with the five diets as independent variables, prior to *ad hoc* transformation (α=0.05). Tukey's test was conducted to determine the effects among the diets (Montgomery, 1984).

Table 2. Total carotenoids concentration (g/kg of body weight) in the shrimp fed the experimental diets.

Diet	CD	R200	R250	B200	CP
Abdomen muscle					
0 days	8.2				
14 days	9.3	11.0 ± 0.15^{b}	15.5 ± 0.18^{c}	14.5 ± 0.18^{c}	17.0 ± 0.26^{d}
28 days	8.6	10.0 ± 0.14^{b}	14.7 ± 0.09^{c}	9.0 ± 0.11^{a}	16.2 ± 0.15^{d}
Exoskeleton					
0 days	41.0				
14 days	42.7^{a}	77.0 ± 0.29^{b}	100.0 ± 0.29^{d}	79.0 ± 0.38^{b}	89.0 ± 0.30^{c}
28 days	41.8^{a}	75.0 ± 0.61^{b}	108.0 ± 0.40^{c}	78.0 ± 0.46^{b}	100.0 ± 0.32^{c}

Values are means ± SEM
Means in the same row with different superscripts are significantly different at $p<0.05$
Where: CD=Control diet; R200= 200 mg/kg esterified carotenoids; R250=250 mg/.kg esterified carotenoids; B200=200 mg/kg saponified carotenoids; CP=100 mg/kg synthetic astaxanthin (Carophyll Pink).

3. Results and discussion

Proximate analysis indicates that the experimental diets were isocaloric, isoproteic and isolipidic, and that inclusion of the different pigment sources did not alter the available digestible energy. Furthermore, by adding an extra 5 % of the pigments amount to the experimental diets the actual TCC concentration almost matches the theoretical TCC (Table 1).

The total carotenoids concentration analysis in the exoskeleton and muscle of the shrimps (Table 2) indicates that at any time period and irrespective of the diet fed to shrimp the pigment content in the exoskeleton is considerably higher than on the abdominal muscle, the former being approximately 4.4-8.6 times higher than in the latter. The non-pigmented control diet showed the smallest increases in pigmentation in both exoskeleton and muscle after 14 and 28 days trial, whereas diet R250 showed the highest increase in exoskeleton pigmentation and diet CP in muscle pigmentation after 14 and 28 days. While exoskeleton pigmentation remained more or less constant for diets CD, R200 and B200, but increased for diets R250 and CP, muscle pigmentation decreased with all diets from day 14 to day 28. These results tend to suggest that tissue saturation of the muscle by the dietary carotenoids takes place, followed by depletion. Vernon-Carter *et al*. (1996) and Yamada (1990) reported a similar behavior, but it took place not only on muscle but also in the exoskeleton, whereas Arredondo-Figueroa *et al*. (1999) found that a decrease in muscle pigmentation, but an increase in exoskeleton pigmentation occurred with increasing feeding time. However, the pigment sources used by these authors were different from those used here, as well as the experimental trial length in the latter study. Arredondo-Figueroa *et al*. (1999) argue that as muscle pigmentation is the main sensory characteristic that the consumer perceives, it is very important to establish the adequate trial length time required for obtaining maximum degree of pigmentation. Also, as the feeding trial is extended, the degree of pigmentation between the exoskeleton and muscle is increased, so that the perception of muscle pigmentation is further minimized.

3.1 Abdomen pigmentation

Table 2 shows TCC in the abdominal muscle of the shrimp for the different treatments at days 14 and 28. At day 14, the data show that the highest pigment assimilation occurred for diet CP followed by R250, B200, R200 and CD, respectively. All the experimental diets were significantly different among themselves, excepting diets R250 and B200, which were non-significantly different ($p>0.05$). These results suggest that diet B200 is as effective as diet R250 regarding abdominal muscle pigmentation.

As mentioned earlier, after 28 days pigment concentration in the abdominal muscle dropped from the values exhibited at day 14, with all the diets still being significantly different among themselves with the exception of diet CD and B200, were non significantly different ($p>0.05$). As at day 14, diet CP showed the highest pigment concentration, followed by diet R250, R200, B200 and CD, respectively. These results suggest that: (1) the depletion mechanism of the carotenoids from saponified red chili oleoresin are much more pronounced than that of the carotenoids from the unsaponified red chili oleoresin, and (2) that despite the relatively higher concentrations of the red chili pigments used the assimilation of astaxanthin is more efficient. The first point suggests that oil soluble carotenoids are more efficiently absorbed to tissue (Latscha, 1990) than water soluble carotenoids after tissue saturation point is reached. The second point suggests that a selective absorption of the

dietary carotenoids takes place that is probably related to the dominant carotenoid in the chili oleoresin extracts. For example, the data provided by the manufacturers for the red chili oleoresin (Rodofila) shows that trans-capsanthin makes up 42.41 % of the total carotenoids, whereas in the saponified red chili oleoresin (Biored-L) it represents 40.18 % of the total carotenoids.

3.2 Exoskeleton pigmentation

Diets R250 and CP showed a non-significant ($p>0.05$) decrease in their hypodermis pigmentation during the feeding trial duration. All experimental diets showed a significant pigmentation difference ($p<0.05$) among themselves after 14 days, excepting diets R200 and B200. The highest pigmentation was exhibited by diet R250, followed by diets CP, B200, R200 and CD, respectively. As the feeding trial continued to 28 days, diets R250 and CP showed non-significant ($p>0.05$) differences between them, but were significantly different ($p<0.05$) from the rest of the diets. Also, these two diets showed an increase in pigmentation from day 14 to day 28, whereas the rest of the diets showed a very slight decrease in pigmentation. These results indicate that selective absorption of capsanthin is as effective as astaxanthin in the exoskeleton.

3.3 Biological evaluation

Table 3 shows the weight gain per day and the final weight of the shrimp after 28 days. Diets R250, R200 and B200 were non-significantly different ($p>0.05$) among them regarding weight gain, but were significantly different ($p<0.05$) from diets CP and CD, and these two last ones between themselves. Also, all the pigmented diets showed non-significant differences ($p>0.05$) in survival percentage, but were significantly different ($p<0.05$) from the CD diet. These results suggest that the inclusion of carotenoids in the diet, whichever their source or whether oil or water soluble, influence the physiological function of shrimp, in this case enhancing nutrient assimilation and shrimp health.

Table 3. Mean weight, weight gain and survival of *Litopenaeus vannamei* fed the experimental diets after 28 days.

Diet	CD	R200	R250	B200	CP
Initial weight (g)	5.9 ± 0.5^{a}	5.7 ± 0.4^{a}	6.2 ± 0.4^{a}	5.8 ± 0.4^{a}	5.9 ± 0.5^{a}
Final weight (g)	7.9 ± 0.6^{a}	8.1 ± 0.6^{a}	8.8 ± 0.7^{a}	8.2 ± 0.6^{a}	8.0 ± 0.7^{a}
Weight gain (mg/d)	121.1 ± 0.1^{a}	150.4 ± 0.2^{b}	149.7 ± 0.1^{b}	147.7 ± 0.3^{c}	119.8 ± 0.3^{d}
Survival (%)	80.5 ± 0.7^{a}	92.3 ± 0.8^{b}	94.0 ± 0.9^{b}	93.3 ± 0.7^{b}	92.1 ± 0.9^{b}

Values are means ± SEM
Means in the same row with different superscripts are significantly different at $p<0.05$
Where: CD=Control diet; R200= 200 mg/kg esterified carotenoids; R250=250 mg/.kg esterified carotenoids; B200=200 mg/kg saponified carotenoids; CP=100 mg/kg synthetic astaxanthin (Carophyll Pink).

Conclusions

The Pacific white shrimp muscle and exoskeleton can be suitable pigmented with carotenoids from red chili extracts when the concentration of the main carotenoid (capsanthin) is equivalent to that of astaxanthin. It would seem that a greater benefit could be achieved if dietary esterified carotenoids are employed rather than saponified carotenoids. Feeding trial lengths should be carefully established, as a mechanism of pigment assimilation, saturation and depletion seems to be involved. Depletion of water soluble carotenoids is much more pronounced than that of oil soluble carotenoids in the abdominal muscle.

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