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The role of astaxanthin in shrimp pigmentation

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Abstract. — *Carotenoids are exogenously derived isoprenoid compounds which are responsible for pigmentation in crustaceans. While implants and bacteria are able to synthesize these carotenoids de novo, shrimps like other animals entirely depend on their dietary supply. The major carotenoid found in most crustacean tissues and responsible, e.g. for the typical colour of penaeids is astaxanthin (3,3'-dihydroxy-i, i-carotene-4,4'-dione). With increasing industrialization in shrimp farming there is a growing demand for synthetic, nature-identical carotenoids, not only for pigmentation, but also for the maintenance of growth and fertility.*

Following some general considerations on the importance of colour, pigmentation and carotenoid pigments some preliminary results from recent investigations on the improvement of pigmentation and the so-called blue shrimp syndrome will be presented.

INTRODUCTION

The cultivation of prawns and shrimps in brackishwater coastal ponds has been practised in different parts of the world for decades. Traditionally, however, extensive culture methods have been employed with minimal inputs of seedstock, fertilizer and feed where farmers raised incidental crops of wild shrimps in tidal fish ponds. In such extensive cultures, where a large amount of land is involved to the number of animals reared, shrimps feed on natural food in the pond and traditionally reached marketable size after a period of 6-12 months. As a consequence, yields per unit of land were typically low.

During the past ten years, however, there have been major technological advances leading to significant improvement of culture methods and shrimp production. Since then shrimp industry experienced an incredible boom. Shrimp farms have been established in over 40 countries increasing their contribution to the world shrimp production from 2.1 % in 1981 to more than 22 % in 1988. The bulk of farmed penaeid species consisting mainly of 3 species (Fig. 1), namely the giant tiger shrimp

(*Penaeus monodon*) contributing 33 %, the chinese white shrimp (*P. chinensis*) 22 % and the western white shrimp (*P. vannamei*) 18 % to the world production of penaeid shrimps. Though some first tremors have been experienced by the shrimp industry during 1988, at least partly caused by a significant increase in competition, diseases and quality requirements, shrimp farming is still expanding at such a pace that no one can keep up with the numbers.

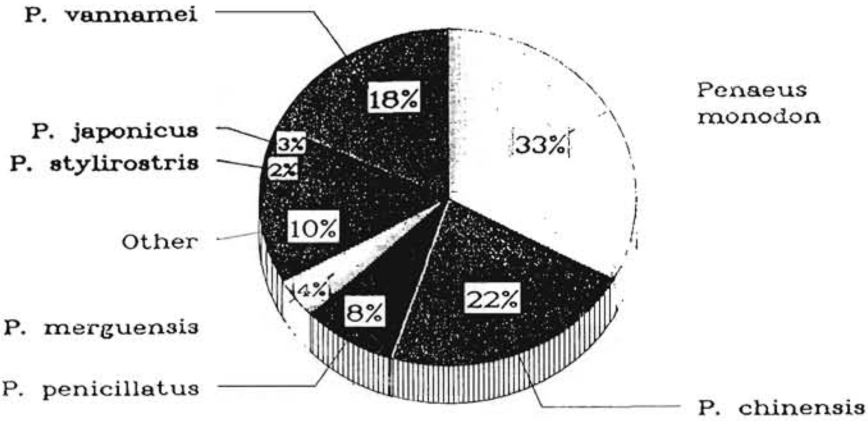

Fig. 1. — Production by species.

However, future outcomes in terms of profitability will largely depend on the productivity and the quality of the shrimp produced. Since the shrimps in close proximity, as e.g. in intensive culture systems (present trend in shrimp farming is still toward intensive and even super-intensive culture methods) compete more aggressively for the same resources in the given space and since the high density in the ponds practically eliminates the growth of natural food this outcome, as a consequence, will largely depend on the quality of feed used during the whole farming cycle involving maturation hatchery nursery and grow-out phases.

SHRIMP QUALITY CRITERIA

According to most Southeast Asian shrimp processors and importers, colour besides others (Table 1) is one of the most important, but widely neglected quality criteria for penaeid shrimps. In fact, today poor general pigmentation as well as a sort of blue discolouration also known as the so-called « blue shrimp syndrome » is one of the most alarming problems plaguing the shrimp industry in that region.

IMPORTANCE OF COLOURS AND PIGMENTATION

Colours are hardly just waste or luxury products of a profligate nature, but rather significant in supporting a multiplicity of biological functions. Pigments, and their respective colours often trigger various physiological processes and widely differing complex behaviour patterns which are essential to e.g. inter- and intraspecific communication, species recognition, courtship, reproduction and brooding as well as to the survival of the individual acting for example as lure, warming, camouflage and protection. One might, however, object that — in captivity — the mechanisms of protection and defense are no longer necessary. Sex attraction by colours in the mating season is also superfluous because of artificial insemination. Nevertheless, colours (pigments) as noted also influence many physiological processes and last but not least have a significant impact on man's choice of food.

Visual appearance, especially colour is one of the most important characteristics of foods in determining their selection prior to actual consumption.

As man in earliest times, the consumer, often unconciously relates the appearance of a natural colouration with e.g. the ripeness, freshness, taste and healthiness or simply with the quality of a product. Conversely, if colouring or pigmentation of a product is inappropriate or off-putting the consumer usually considers the product to be improperly processed, spoiled or of low quality.

Tab. 1. — Major Shrimp quality Criteria.

Size
Shell
Colour
General Appearance
Uniformity
Texture
Flavour/Odour

Tab. 2. — The Major types of Pigments found in Crustaceans.

Hemoglobins
Hemocyanins
Flavins
Cytochromes
Carotenoids
Melanins
Ommochromes
Pterins
Rhodopsin

THE ROLE OF ASTAXANTHIN IN SHRIMP PIGMENTATION

In crustaceans the basic nature of body colouration relies on specific pigments present in the subepidermal chromatophores and/or in the principal layer of the animals exoskeleton. Among the different types of pigments found in crustaceans (Table 2), the carotenoids are by far the most significant ones in determining their pigmentation (Goodwin, 1954; Schiedt, 1987).

Carotenoids are exogenously derived isoprenoid compounds representing not only one of the most wide-spread groups of natural pigments, but probably also have the most varied structures and functions (see Latscha, 1989, for references). Regarding the structures, formally, all carotenoids can be derived from the acyclic $C_{40}H_{56}$ polyene lycopene by reactions involving e.g. hydrogenation, dehydrogenation, cyclization, chain elongation and oxygen insertion. Their wide range of colours from almost colourless to yellow and dark red is due to the varying chromophore in different groups of carotenoids and consisting in different groups of a varying conjugated polyene system in the molecule.

Carotenoids are of almost universal distribution in living matter occuring from the most primitive bacteria (*Archebacteria*) and procaryotic blue-green algae (Schizophycaea) to the highly developed flowering plants (Angrospermae) and from the protozoans right up to the mammals. It is estimated that, in nature, more than 100 million tonnes are produced annually, which represents an output of over 3 tonnes per second ! Since the earliest scientific studies on these lipophilic pigments about 600 naturally occuring carotenoids have been isolated and identified in the plant and animal Kingdom (Pfander, 1987).

Despite their wide distribution, however, the *de novo* synthesis of carotenoids is confined to certain microorganisms, fungi, algae and the higher plants. Animals, in contrast, unable to synthesize these pigments *de novo* fully depend on a dietary supply. In animals even a species-specific absorption of various carotenoids must be taken into account. In crustaceans, however, this is less pronounced since they apparently absorb e.g. i-carotene and astaxanthin as well as some other carotenoids. Once absorbed, the ingested carotenoids are either deposited as such or converted into species-specific compounds.

In crustaceans including the commercially most important penaeids, the most prevalent carotenoid found in the integuments is astaxanthin, 3,3'-dihydroxy-i,i-carotene-4,4'-dione (Table 3, Schiedt, 1987) representing about 65-98 % of the total carotenoids present and consisting of 3 sterioisomers (3S,3'S; 3S,3'R; 3R,3'R) as shown in Table 4.

As depicted in Table 3 three different forms of this particular pigment are recognized in crustaceans, namely diesters, monoesters and the free form. The esters generally representing the bulk of astaxanthin in the integumental tissues. Pigmentations in crustaceans or shrimps respectively is further complicated by the wide occurence of this pigment in carotenoid-protein complexes commonly termed carotenoproteins or chromoproteins which due to usually marked bathochromic shift in the light absorption maximum caused by characteristics of the pigment-protein bonding exhibit colours (e.g. green to purple) which largely differ from the colour of the pigment itself (Chersman et *al.*, 1967). It is obvious from these, that pigmentation in general, but particularly that of crustaceans is rather complex and largely influenced by a multiplicity of pigment-, feed-, animal-, and disease related as well as environmental factors of which the most important ones are summarized in Table 5, and giving rise to

Tab. 3. — Carotenoid Content and composition in Various Wild Species of Penaeidae.

Carotenoids	P. vannamei	P. monodon	P. japonicus	Metapenaeus monoceros
Content mcg/g tissue	56	52	38	44
Composition	%	%	%	%
β, β-Carotene	0.5	0.2	0.0	3.0
Yellow xanthophylls*	30.0	0.4	19,0	23.0
7,8-Didehydroastaxanthin	5.0	1.0	2.0	2.0
Astaxanthin total	65.0	98.0	79.0	72.0
Diesters	50.8	27.5	46.8	30.5
Monoesters	44.6	58.2	36.7	52.8
Free form	4.6	14.3	16.5	16.7

* Ester of fatty acids, no lutein, zeaxanthin, presumably three hydroxy groups.

Tab. 4. — Composition of Optical Isomers of Astaxanthin in Various Wild Species of Penaeidae.

Optical isomers	P. vannamei	P. monodon	P. japonicus	Metapenaeus monoceros
(3R, 3'R)	23*	19*	15*	20*
(3R, 3'S; meso)	44	45	40	42
(3S, 3'S)	32	36	45	38

* % of total astaxanthin.

significant variations e.g. between wild-caught and farmed animals (Table 6).

However, pigmentation of shrimps may be influenced by several factors, the achievement of an optimal and consistent pigmentation nevertheless is primarily a question of the amount and type of available carotenoids in the feed. In industrialized shrimp farming, the animals are deprived of their natural feed sources. If, therefore, the respective carotenoids normally present in the diet, or possibly a substitute, are not included in the feed, the carotenoid content will decrease and the integuments will fade as depicted in Fig. 2 (Latscha et *al.*, unpublished). Though the ultimate cause of mentioned « blue shrimp syndrome » is not fully understood at present, it is yet widely attributed to a most probable dietary lack of carotenoids like astaxanthin or its precursors. In fact recent investigations of blue shrimps have consistently revealed a significantly lowered total carotenoid content (Table 7) due mainly to a deficiency of astaxanthin in these animals. The inclusion of 50 ppm astaxanthin (CAROPHYLL Pink (R)) into the commercial diets in field trials performed in consequence resulted in the accumulation of exoskeleton pigments and the successful conversion of abnormally blue pigmented individuals of *P. monodon* into normal pigmented ones.

Tab. 5. — Factors influencing Pigmentation.

Pigment-related factors	type amount form stability
Feed-related factors	manufacturing composition intake/FCR biovailability administration period
Animal-related factors	species age/stage sex/maturation genetics tissue molting
Environmental factors	culture method soil condition water quality light intensity
Disease-related factors	eg. vibriosis
Consumer-related factors	

Tab. 6. — Astaxanthin Content of Wild and farmed Penaeus monodon.

P. monodon	Total Astaxanthin content (mg/kg)	
origin	mean	range
Wild catch	54.1	40.16-61.92
Farmed	18.7	9.96-20.90

Tab. 7. — Mean Carotenoid Content and Distribution in « Blue » and « normal » individuals of Penaeus monodon.

P. monodon	Carotenoids	% Distribution in	
individuals	mg/kg	Shell	Flesh
Normal	78.37	84.46	15.54
Blue	7.63	85.15	14.85

Mean body weight : normal : 37.64 g, blue = 40.64 g.

The provision of an accurate carotenoid source in the field, therefore, is important in yielding a natural pigmentation acceptable to the consumer as well as to improve the animals general performance.

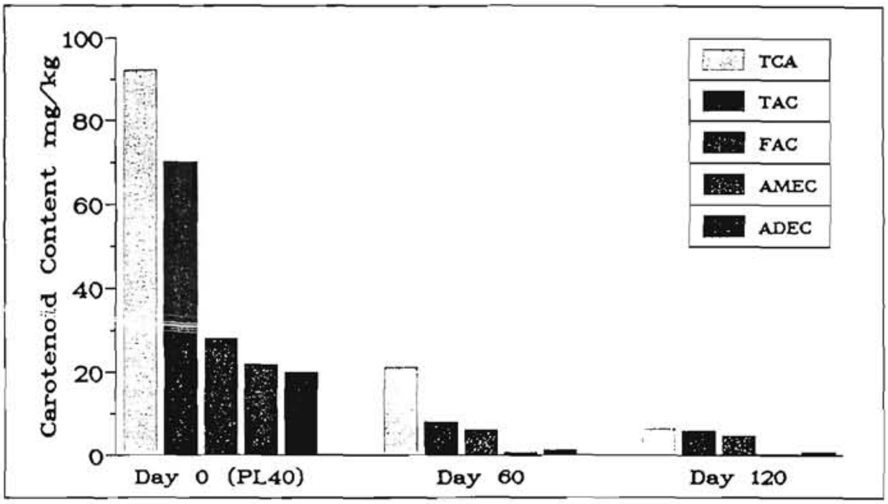

Fig. 2. — Decrease in the carotenoid content of *Penaeus monodon* fed a carotenoid deficient diet (Blue Shrimp Induction).

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