Benefits of a natural astaxanthin from microalgae in shrimp hatchery and nursery stages

These range from dark colouration, a glossy body and hard, slippery shell in broodstock to large dark hepatopancreas and excellent positive rheotaxis in PL42

By Patricio Hidalgo

There is a consensus that microalgae, as part of the natural food chain, are highly beneficial as feed for shrimp health and production, especially in the early stages. This is particularly so for *Haematococcus pluvialis* which like other microalgae, contains proteins, carbohydrates, essential fatty acids, vitamins, nucleotides and minerals. However, *Haematococcus* is also a source of a complex of natural antioxidants, mainly the powerful astaxanthin. Although it is an excellent source of nutrition, a common drawback of microalgae is its higher price than non-natural alternatives.

Today there is a *H. pluvialis* producer in the Atacama Desert in Chile- Atacama Bio Natural Products S.A. This company takes advantage of its exceptional pollution-free surroundings with high light intensity and pure water from the Andes mountains, and has been able to reduce production costs by using a proprietary technology, thus narrowing the price gap and opening the possibility for extensive use. After several years of research, the company has developed a robust, state-of-the-art, culture technology of closed and open photobioreactors.

The benefits of dietary astaxanthin for shrimp range from general health improvement to specific enhancement of functions: metabolism, antioxidation capacity, effects on photo-response, stress alleviation, immune response regulation, source of provitamin A, improved reproduction and brood stock quality, reduced embryonic mortality and enhanced disease resistance. There is evidence proving that astaxanthin supplementation enables efficient defense procedures against unfavourable or stressful situations.

Science-backed assessments

Astaxanthin has a fundamental role in the breeding and farming of aquaculture species and confers a significant improvement on the reproductive performance, egg production and egg quality of aquatic animals (Vassallo-Agius et al., 2001; Ahmadi et al., 2006; Paibulkichakul et al., 2008; Tizkar et al., 2013, 2015; Palma et al., 2016).

Reproductive performance and egg quality

The accumulation of astaxanthin in reproductive tissues via dietary supplementation provides a significant impact on reproductive performance characteristics, which include egg quality and quantity, hatching success and improvement on larvae quality (Pangantihon-Kuhlmann et al., 1998; Paibulkichakul et al., 2008).

Growth performance, survival, stress tolerance and disease resistance

Astaxanthin content has also been directly linked to the ability of eggs to resist extreme environmental conditions (Eisler 1957; Craik 1985; Torrissen 1990). Astaxanthin also affects stress tolerance and disease resistance of different aquatic animals.

Characteristics of natural *H. pluvialis* astaxanthin

There are three unique properties differentiating astaxanthin from this microalgae from synthetic or fermentation-origin astaxanthin. These explain the higher antioxidant power and differences in toxicology, pharmacology and metabolism (Figure 1).

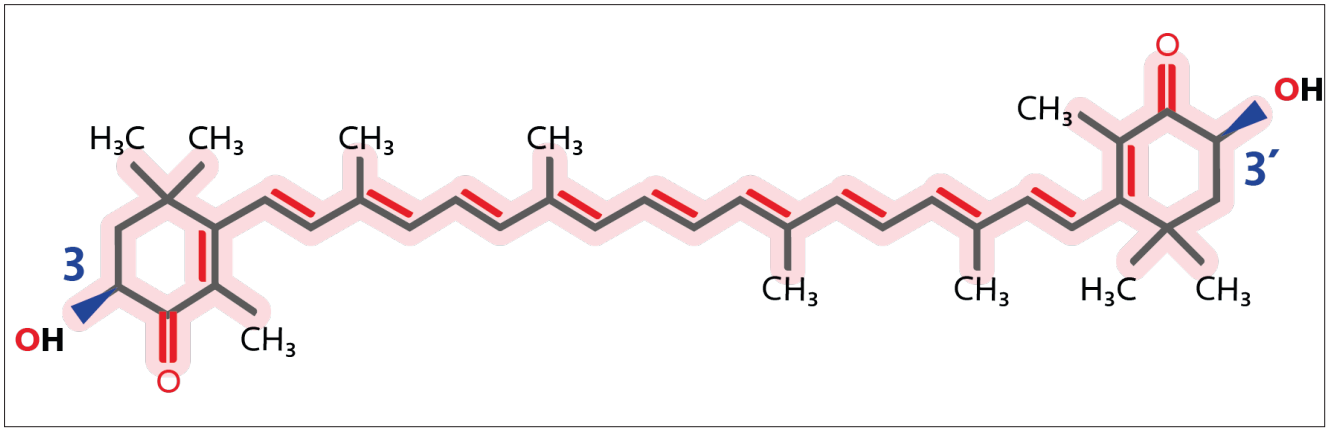

Figure 1. Image of the astaxanthin molecule from *Haematococcus pluvialis* showing its chirality.

The main chemical characteristic that explains most of the different biological responses of *H. pluvialis* astaxanthin is related to its chirality. Astaxanthin has two asymmetric carbons in the benzenoid ring where the hydroxyl groups are located. This asymmetry gives three unequal spatial configurations to the astaxanthin molecule. One of these isomers, the so-called 3S,3'S configuration, can only be sourced from *H. pluvialis*.

Chiral isomers show different biological and chemical behaviours when reacting with other chiral molecules, just as a left-hand interacts differently with left and right-handed gloves. Animal molecules such as enzymes, proteins, DNA and nucleotides are also chiral and are affected during binding and interaction with astaxanthin.

Figure 2. Healthy female broodstock resulting in active mating, better egg quality and quantity, robust nauplii and zoea.

Due to the exposure to the intense sunlight of the Atacama Desert during its culture cycle, *H. pluvialis* reinforces its antioxidant power by up to 15% compared with other carotenoids. Cerezal-Mezquita et al. (2022) said that the main pigment is astaxanthin esters (55.13%), followed by lutein (5.67%), free astaxanthin (5.54%), β-carotene (2.76%) and canthaxanthin (2.65%). *H. pluvialis* astaxanthin is esterified with fatty acids, providing more stability and shelf life.

Experiences from industrial uses

In recent years, two major Asian companies have carried out tests at different levels using natural astaxanthin from *H. pluvialis*. Following the good results, they have scaled its use to an industrial level. These results presented in this article were obtained by using Red Meal powder 1.5% in the industrial-scale production of shrimp. Red Meal 1.5% is the brand name of a line of products developed specifically for the animal feed market, which uses the microencapsulated cracked biomass of *H. pluvialis* microalgae. The supplemented amount of Red Meal powder 1.5% in various feeds is given in (Table 1).

Diets	Broodstock	Larvae/post larvae	Nursery PL12-PL42	Grow-out
Supplementation of Red Meal powder 1.5%/kg of feed	20-40g	5g	3-5g	3-5g (14 weeks) 10-15g (5 days)

Table 1. Supplementation levels of Red Meal powder 1.5% in test diets for commercial trials.

Empirical results

In the hatchery, broodstock fed with the diets supplemented with the Red Meal powder showed the following

- An excellent absorption of astaxanthin with dark colouration, a glossy body and a hard, slippery shell. The absorption is explained by the high affinity between the 3S, 3'S natural astaxanthin stereoisomer from H. pluvialis and the tissue cells. The enantio-selectivity of chiral astaxanthin molecules allows good binding to chiral protein molecules.
- There were no wounds after clashing. The more rigid shells and the active immune system with robust, in-condition haemocytes can quickly activate prophenoloxidase (proPO) to produce melanin synthesis involved in wound healing and enzymes of the clotting system.
- Healthy broodstock results in active mating, better egg quality and quantity, robust nauplii and zoea. This difference is explained by the accumulation of astaxanthin which protects cell and mitochondria membranes from oxidation for all high-energy-consuming metabolisms (Figure 2).

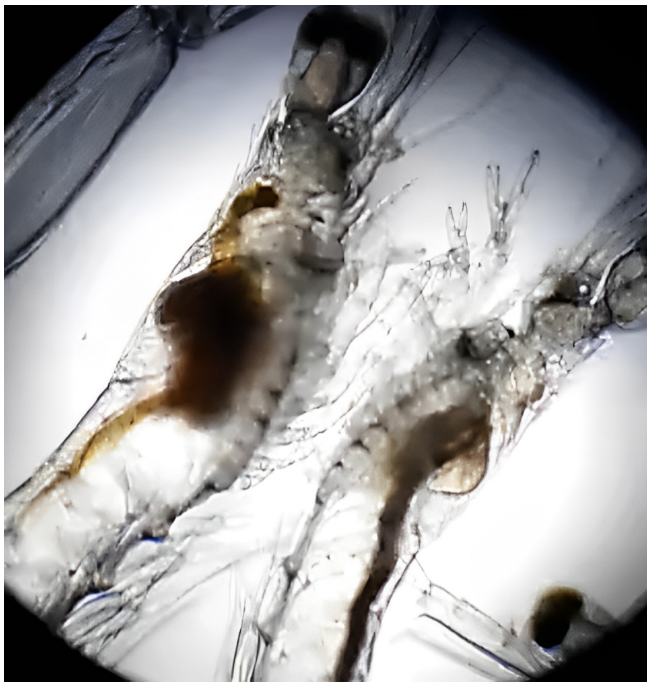

Figure 3. Larvae and post larvae with well-defined and coloured hepatopancreas produced by supplementation with Artemia enriched with astaxanthin.

Larvae and post larvae fed with diets supplemented with 5g Red Meal powder 1.5%/kg of feed using enriched rotifers or mixed over pellets showed dark colour heads, glossy bodies, strong and vigorous PL12, lipids in large dark hepatopancreas and excellent positive rheotaxis (Figure 3 and 4).

Due to astaxanthin's antioxidant action, reactive oxygen species (ROS) are quenched, thus protecting mitochondria and cell membranes from oxidative stress produced by high energy needs for rapid body differentiation and moulting during metamorphosis.

Figure 4. Vigorous larvae with dark-coloured heads and excellent results in a positive rheotaxis test.

Figure 5. Notable difference in colouration after cooking of the shrimp Penaeus vannamei; between natural colouration with astaxanthin (above), vs synthetic pigmentation (below).

In the nursery, PL12 to PL42 fed with 3-5g Red Meal powder 1.5% per kg feed showed noticeable stress resistance to low salinity, moulting and disease. Over 90% survival at PL42 was achieved. Post larvae also showed an active, ready to work immune system. Strong and healthy haemocytes are produced abundantly by well-guarded haematopoietic tissue cells. Astaxanthin is linked and coupled to phospholipids in cellular and mitochondria membranes quenching ROS/RNS (reactive nitrogen species) that produce oxidative stress and lipid peroxidation membrane destruction.

Shrimp in the grow-out pond were also fed diets supplemented with 3 to 5g of Red Meal powder at1.5%/kg of feed for 14 days before harvesting or with inclusions of 10 to 15g/kg of feed for 5 days. The main benefit observed was a 27+ colour on the salmon fan after cooking (Figure 5).

The high affinity between 3S, and 3'S natural astaxanthin stereoisomer and tissue proteins resulted in these positive results. Enantio selectivity of chiral astaxanthin molecules allows for good binding to chiral proteins in the crustacyanin complex.

Reference

Cerezal-Mezquita, P., Espinosa-Álvarez, C., Jáuregui-Tirado, M., Jaime-Matus, C., Palma-Ramírez, J., Ruiz-Domínguez, M.C. 2022. Physical-chemical characteristics of "Red Meal", a novel non-defatted additive in the fish feed from cracked biomass of Haematococcus pluvialis, Animal Feed Science and Technology, Volume 285, 115247.
https://doi.org/10.1016/j.anifeedsci.2022.115247

Patricio Hidalgo is Technical Sales Specialist for feed applications.
Email: phidalgo@atacamabionatural.com