Haematococcus astaxanthin: Is it time to rethink astaxanthin use in white shrimp feeds?

By Martin Guerin

The dietary inclusions of astaxanthin in this shrimp have been constrained by low pigmentation efficiency of synthetic sources, despite known human health benefits of astaxanthin as well as anti-stress and disease resistance properties observed in shrimp. All these may change with higher efficacy and competitive prices of natural astaxanthin.

Astaxanthin (AX) is a natural carotenoid pigment found in many animals, for example in fish such as the salmon and red seabream, in crustaceans such as shrimp or krill, or in algae such as *Haematococcus pluvialis*. Astaxanthin is nature's most powerful antioxidant. Indeed, astaxanthin has demonstrated largely superior singlet oxygen and free radical scavenging properties versus other carotenoids or vitamin E (Miki, 1991). In recent years, its potential applications for human health have attracted a growing interest leading to numerous astaxanthin nutritional supplements with applications in eye and skin health, fights against inflammation and cancer, or even sports health (Guerin et al. 2003; Nakao et al. 2010). In these human health applications, natural astaxanthin extracted from *Haematococcus* algae (HAX) has become the standard, especially since the form of astaxanthin it supplies reflects the form in human diet throughout the ages. *Haematococcus* astaxanthin supplements have reached the GRAS (Generally Recognised As Safe) status in the USA since 2010.

Production of natural astaxanthin Haematococcus pluvialis in raceways in Atacama Desert, Chile. Photo by Atacama BioNatural Products SA.

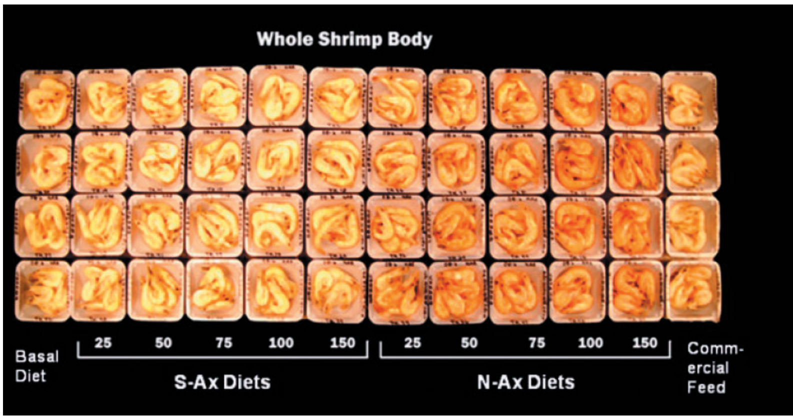
The increased interest in the health benefits of algal astaxanthin could also mean its increased value for shrimp. Today, salmon is a major source of astaxanthin in the human diet and shrimp can be one too as the growing production capacities of *Haematococcus* algae are allowing the supply of larger quantities of natural astaxanthin at more attractive prices.

In nature, astaxanthin comes in several forms. This can be confusing even to the initiated. Astaxanthin has several geometrical (cis and trans) and optical isomers (3S,3'S, 3R,3'R, 3R,3'S), and can come as free astaxanthin or esterified astaxanthin. To further complicate matters, these forms have varying degrees of utilisation and effectiveness among species.

Synthetic astaxanthin (SAX) comes as free astaxanthin in a combination of three optical isomers (50% of 3R,3'S, 25% of 3R,3'R, and 25% of 3S,3'S forms). It has become the norm for supplementing salmon feeds as to date it is the lower priced product and is very effective in the pigmentation of the salmon. This preference for SAX in salmon diets continues despite the fact that the astaxanthin profile of farmed salmon meat reflects the one of SAX and does not resemble that of wild salmon, where the 3S,3'S form largely dominates (Megdal et al, 2009). This same 3S,3'S form is also the main isomer found in shrimp (Moretti et al. 2006).

Natural pigmentation is better

Haematococcus pluvialis algae contain almost exclusively the desirable 3S,3'S isomer (Moretti et al. 2006), with AX representing about 90% of their total carotenoids content. These algae can accumulate higher than 3% astaxanthin in the dried biomass during their vegetative growth stage. As they also have an excellent image for human health, they offer a very attractive natural alternative to the synthetic form for use in aquafeeds, not only in organic or functional feeds but also regular feeds.

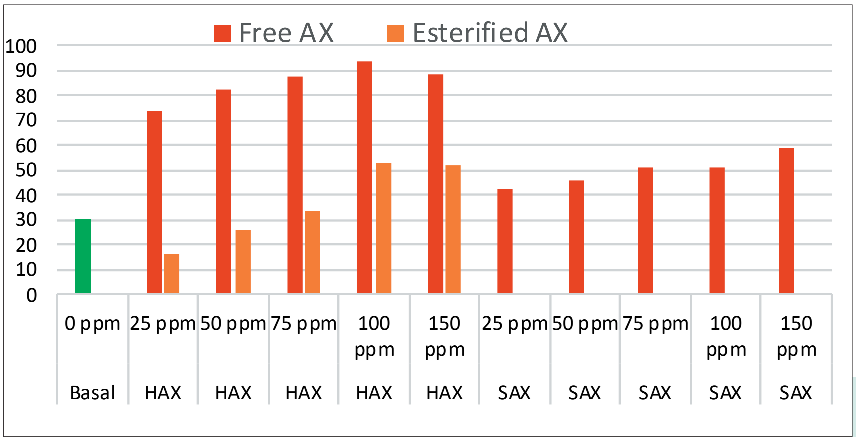


Colouration of cooked vannamei shrimp fed graded levels of synthetic astaxanthin (SAX) or Haematococcus astaxanthin (HAX) (Source: Ju et al. 2011).

In 2013, a study (Capelli et al. 2013) confirmed the superiority of HAX in the invitro capture of superoxide and peroxide free radicals over vitamin C, E, beta-carotene or pyctogenol but also over synthetic astaxanthin. One additional benefit of HAX is that it comes in an esterified form, which is naturally much more stable than free astaxanthin, and is found abundantly in the epidermis of shrimp or seabream. Finally, in both shrimp and red seabream and related species, algal astaxanthin has demonstrated superior biological and pigmentation properties versus the synthetic pigment.

In red seabream (*Pagrus major*), feeding 20ppm HAX for 45 days achieved superior red colour and astaxanthin deposition levels in the skin vs 40ppm SAX (Guerin & Hosokawa. 2001). Similarly, in red porgy (*Pagrus pagrus*), HAX gave better pigmentation results than the synthetic form (Kalinowsky et al, 2005).

Figure 1. Free and esterified astaxanthin (AX) deposition in the *vannamei* shrimp fed *Haematococcus* astaxanthin (HAX) over shrimp fed synthetic astaxanthin (SAX) (adapted from Ju et al. 2011).



(b) Deposition in the head and shell

Pigmentation of cooked shrimp

This is complex and is influenced by a number of factors:

- ***Genetics***
  In shrimp exoskeleton, astaxanthin binds to a protein: crustacyanin. Intense red colouration of cooked *Penaeus monodon* shrimp seems related to the abundance of this protein which is directly coded by specific genes. Pigmentation may vary with the degree of expression of these genes. Several of these genes have been found within and between crustacean species, which will lead to different pigmentation results (Wade, 2010).
- ***Environment***
  Penaeus vannamei reared with external light favours the deposition of astaxanthin against culture without light (You et al. 2006). On the other hand, shrimp (*P. monodon* or *P. vannamei*) reared in a black tank tend to have a stronger red colour when cooked (Pan et al. 2001, Parisenti et al. 2011). In the *monodon* shrimp, this appears to be due to higher expansion of the chromatophores and higher percentage of free astaxanthin deposition, leading to a stronger red pigmentation when cooked, compared to this shrimp reared in a light-colour tank, where astaxanthin is mainly deposited as esters, in shrunken chromatophores (Wade et al. 2017).
- ***Diet***
  The type and level of carotenoids ingested play a critical role. Shrimp can convert beta-carotene into astaxanthin but the process is highly inefficient, slower and variable as it requires several enzymatic reactions (Wade et al. 2017). Higher levels of astaxanthin fed will lead to higher astaxanthin deposition, higher pigmentation and stronger red colour when cooked.
- ***Species***
  Monodon shrimp respond well to SAX, although not as well as HAX. However, the *vannamei* shrimp respond poorly to SAX but well to HAX, as the algal source of astaxanthin helps achieve satisfactory pigmentation with much lower levels of supplementation than SAX (Ju et al. 2011).

Recently, researchers in Australia feeding the *monodon* shrimp with graded levels of SAX or HAX found that the minimum level and duration of feeding SAX to reach highest pigmentation levels were up to 20% higher and up to 14% longer than with HAX, depending on the season (Angell et al.2018). It also seems that in the monodon shrimp astaxanthin deposits to higher levels in the shell than in the flesh where astaxanthin levels seem to plateau after only 2 weeks of feeding 50ppm of SAX (Menasveta et al. 1993).

In the *vannamei* shrimp, Ju et al. (2011) found that feeding up to 150 ppm of SAX for 8 weeks failed to improve significantly colouration of cooked shrimp, even though they observed an increase in free astaxanthin deposition. On the contrary, *vannamei* shrimp fed levels as low as 25ppm HAX improved significantly the pigmentation which was superior to shrimp fed 150ppm suggesting that HAX was at least 6-folds more efficient.

They also observed that the lack of pigmentation efficacy of the synthetic pigment in the *vannamei* shrimp was accompanied with minimal deposition of esterified astaxanthin, unlike the shrimp fed algal astaxanthin which saw an increase in esterified astaxanthin deposition. Although feeding SAX helped raise the free astaxanthin deposition, it was also less efficient than HAX which led to approximately 20% higher levels of free astaxanthin. This lack of pigmentation efficacy of synthetic astaxanthin and the

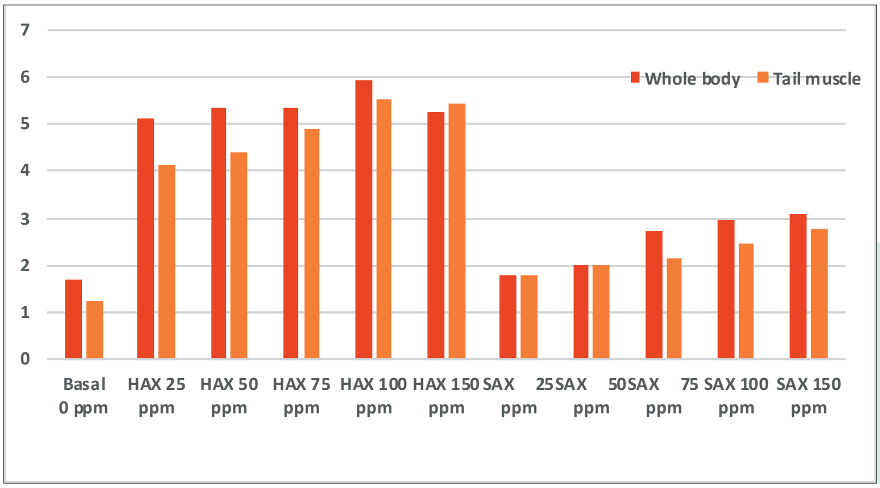


Figure 2. Redness A value (measured by colorimeter) of tails and whole body muscle in cooked *vannamei* shrimp, after feeding graded levels of *Haematococcus* astaxanthin (HAX) and synthetic astaxanthin (SAX) (adapted from Ju et al. 2011). HAX was significantly superior to SAX ($P<0.05$).

lack of supply of algal astaxanthin for commercial shrimp feeds may explain why to date astaxanthin is hardly used in grower feeds for *vannamei* shrimp, although it is used in specialty feeds where other functions of astaxanthin are needed, such as in larval feeds or maturation diets.

Superior anti-stress and disease resistance properties of algal astaxanthin

Interestingly, the superior properties of HAX in shrimp over SAX for pigmentation extend to the antioxidant properties and related anti-stress benefits of astaxanthin.

In Thailand, researchers (Darachai et al. 1998) reported that HAX led to higher survival in zoea, mysis or post larvae (PL15) of the *monodon* shrimp versus larvae and post larvae fed the same amount of SAX. In addition, feeding HAX also resulted in better resistance to the low-salinity stress challenge. Earlier this year, researchers (Xiaohui et al. 2018) reported that the supplementation of 50ppm HAX to *vannamei* post larvae (PL15) for 35 days, led to better growth and astaxanthin deposition than 70ppm SAX supplementation, while supplementation of 90ppm HAX led to higher activity levels of the key antioxidant enzymes, superoxide dismutase and glutathione peroxidase versus the supplementation of 140ppm or 70 ppm SAX.

The 90ppm HAX level gave also the best survival in a *Vibrio parahaemolyticus* disease challenge superior to other levels of supplemented SAX or HAX. If *V. parahaemolyticus* strains were the leading bacterial pathogen in shrimp farming in recent years, the leading viral disease remains to date white spot syndrome virus (WSSV). In WSSV challenge studies, recent research demonstrated that astaxanthin can also improve resistance and survival against WSSV infection (Wang et al. 2015).

Conclusion

Vannamei shrimp largely dominate the world shrimp farming production, but the industry has so far been using astaxanthin sparingly due to the reported poor efficiency of the synthetic version in pigmenting this species. However, the increased supply of *H. pluvialis* astaxanthin at more competitive prices as compared to previous years, could see this situation change drastically.

Indeed, use of the HAX could help processors seek better shrimp prices with better pigmented shrimp. The superior biological efficacy, shrimp health benefits, as well as improved marketability of HAX-fed shrimp- thanks to HAX beneficial human health attributes, and the better consumer appeal through better colouration of cooked shrimp, are reasons for shrimp farmers and feed companies to take a new look at this source of natural astaxanthin.

References are available on request.

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