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A Review of Carotenoid Utilisation and Function in Crustacean Aquaculture

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Abstract

Studies over a number of years have consistently shown that dietary carotenoid supplementation is beneficial for crustacean aquaculture across a range of commercially relevant parameters. Most obvious is the effect on pigmentation, where carotenoid inclusion levels in feeds and duration of feeding diets with carotenoids have been extensively optimised across many species to improve product colour, and subsequently quality and price. However, beneficial effects of carotenoid inclusion have increasingly been demonstrated on other parameters including survival, growth, reproductive capacity, disease resistance and stress resistance. A number of natural and synthetic carotenoid sources have been utilised in crustacean aquaculture. This review focuses on the type, metabolic conversion and function of carotenoids used in crustacean nutrition, and explores the physiological benefits this class of molecules brings to these animals.

Keywords:
Shrimp, color, astaxanthin

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1 Introduction

Carotenoids form the basis of the pigmentation of a wide variety of aquatic organisms (Matsuno, 2001, Britton and Goodwin, 1982, Maoka, 2011), and marine animals extensively utilise a variety of properties that carotenoids possess. Due to their diversity and broad distribution, carotenoid types, structure, metabolism and function have been extensively studied across a wide range of organisms (Britton et al., 2008). Among those organisms studied, crustaceans utilise a range of different carotenoids that vary across species, within individual crustacean tissues or are dependent on various physiological, geographic or ecological parameters (Castillo et al., 1982).

Very little attention has been paid to the specific effects of carotenoid supplementation in crustacean aquaculture, aside from the affect on pigmentation (Bjerkeng, 2008). Up until recently, the physiological effects beyond pigmentation have been inferred from other studies, mostly from fish. The present review summarises the recent progress in the use of carotenoids as a dietary nutrient in crustacean aquaculture, and outlines the effects of this dietary carotenoid supplementation on various aspects specific to crustacean physiology.

2 Carotenoids in Crustaceans

2.1 Tissue Distribution and Carotenoid Types

The majority of crustaceans and crustacean tissues attribute their colouration to the presence of various carotenoids. This topic has largely been covered extensively in the past (Castillo et al., 1982, Lenel et al., 1978) and is not the focus of this review. All wild and cultured crustacean species report the presence of free and esterified forms of various carotenoids, predominantly astaxanthin (Axn) (Castillo et al., 1982, Lenel et al., 1978, Tanaka et al., 1976a). The distribution of these forms of carotenoids also varies with species, life history stages, developmental stage, moult stage and the organ or tissue of the animals (Ribeiro et al., 2001, Lenel et al., 1978, Sachindra et al., 2005, Okada et al., 1994, Pan and Chien, 2000, Dall, 1995, Petit et al., 1998, Pan et al., 1999, Valin et al., 1987, Katayama et al., 1971, Petit et al., 1997). The esterification of Axn with specific fatty acids and the presence of carotenoid isomers can significantly
increase the complexity of the interaction between the carotenoid and other biological molecules or membranes (Britton, 1995, Goodwin, 1986, Liaaen-Jensen, 1997). The accumulation of certain carotenoids in the tissues of different crustaceans not only indicates that these animals are able to interconvert one carotenoid to another, but also implies that there is a specific function for particular carotenoid in certain tissues.

2.2 Carotenoid Interconversion and Metabolism

Like most animals, crustaceans cannot synthesise carotenoids and must obtain them from their diets (Goodwin, 1952). However, for some time there has been strong evidence that various Decapod crustaceans can convert different dietary carotenoids (including canthaxanthin, lutein or zeaxanthin) into the predominant carotenoid Axn (Castillo and Lenel, 1978, Castillo et al., 1980, Chien and Jeng, 1992, Kour and Subramoniam, 1992, Petit et al., 1991, Yamada et al., 1990, Castillo and Negre-Sadargues, 1995, Negre-Sadargues et al., 1993, Mantiri et al., 1995, Vernon-Carter et al., 1996, D'Abraham et al., 1983, Tanaka et al., 1976b, Tanaka et al., 1976a). Many years ago, the carotenoid metabolic transformative capacity of crustaceans was summarised (Negre-Sadargues, 1978, Schiedt et al., 1993, Castillo et al., 1982). The major pathway by which β-carotene is converted to Axn is summarised in Figure 1, although it would appear that crustaceans are able to perform a variety of other carotenoid transformations (Castillo et al., 1982). Crustaceans fall into two broad classes based on their metabolic conversion capacity: those that can convert β-carotene to Axn in their internal organs, such as Penaeid shrimp; or those that can convert β-carotene to Axn in their internal organs but also convert metabolic intermediates in other tissues of their body, such as lobsters and crabs (Katayama et al., 1973). Dietary paprika has been used as a source of carotenoids in P. monodon broodstock diets, suggesting that the carotenoids α-carotene, α-cryptoxanthin and capxanthin present in paprika were converted into Axn (Wyban et al., 1997). Similarly, M. japonicus has been shown to produce Axn from α-carotene, canthaxanthin, echinenone or zeaxanthin (Chien and Jeng, 1992, Tanaka et al., 1976b, Yamada et al., 1990). Carotenoid metabolic capacity is active throughout crustacean early larval and post-larval development (Mantiri
et al., 1995, Mantiri et al., 1996, Petit et al., 1991, Berticat et al., 2000), where the carotenoids may be metabolised as a source of retinoids (Dall, 1995). Yet despite the increase in genomic knowledge of crustaceans, including the sequencing of the complete genome of Daphnia, there has been very little progress in defining the biochemical pathways responsible carotenoid metabolism in this Class of animals. The variation in different carotenoid types across different developmental, physiological and ecological parameters strongly suggests that crustaceans utilise specific carotenoids for different functions during developmental processes or in response to environmental circumstances.

2.3 Carotenoid Sources in Crustacean Aquaculture

Sources of carotenoids that have been used in crustacean diets include synthetic carotenoids (Castillo and Negre-Sadargues, 1995, Chien and Jeng, 1992, Negre-Sadargues et al., 1993), Antarctic krill (Maoka et al., 1985), brine shrimp (Pan and Chien, 2003), shrimp by-products (Mandeville et al., 1991, Chakrabarti, 2002, Meyers and Bligh, 1981), microalgae (Sommer et al., 1991, Supamattaya et al., 2005, Armenta-Lopez et al., 2002, Chien and Jeng, 1992), blue green algae (Liao et al., 1993, Okada et al., 1991), and plant extracts (Vernon-Carter et al., 1996, D’Abrahmo et al., 1983, Arredondo-Figueroa et al., 2003). More recently, other potential sources of carotenoids for crustacean aquaculture have been investigated, including genetic engineering of higher plants to accumulate high levels of ketocarotenoids such as Axn (Han et al., 2013). Studies assessing the effect of different sources of carotenoids on pigmentation in crustaceans are summarised in Table 1.

3 Carotenoid Function in Crustaceans

Carotenoids are known to be involved in a large number of physiological functions in plants and animals, and these functions are largely based on the structure of the carotenoid (Britton, 2008, Goodwin, 1986). As the major carotenoid in crustacean tissues, Axn provides functions that include pigmentation, photoprotection, antioxidant and a source of provitamin A (Britton, 2008). Benefits to the animal include the enhancement of growth, higher survival, increased stress resistance and improved reproductive potential.
(Kumar et al., 2009, Supamattaya et al., 2005, Niu et al., 2014, Paibulkichakul et al., 2008, Linan-Cabello et al., 2002a). An example of these benefits was observed in crayfish exposed to pollution, which had lower levels of vitamins and carotenoids in the hepatopancreas, suggesting these may play a role in tolerating polluted environments (Barim and Karatepe, 2010). The conversion of carotenoids into other biologically active molecules, such as Provitamin A and retinoids has also been implicated (Linan-Cabello et al., 2002a). Since the initial proposals of carotenoid function in crustaceans, there has been substantial progress in gathering scientific evidence to support the range of proposed functions of Axn and its effects on crustacean physiology, which will be discussed in further detail in the following sections.

3.1 Carotenoids and Crustacean Colouration

The best-established function of carotenoids in crustaceans is pigmentation. Colour plays a major role in consumer acceptability, perceived quality and price paid for commercial crustacean species (Parisenti et al., 2011b, Shahidi et al., 1998, Chien and Jeng, 1992, Erickson et al., 2007). Many species of crustacean lose or do not develop pigmentation if not supplied a diet with sufficient carotenoids. Among these included hermit crabs (Castillo and Negre-Sadargues, 1995), red king crabs (Daly et al., 2013), crayfish (Sommer et al., 1991), clawed lobsters (Tlusty and Hyland, 2005), spiny lobsters (D’Abrahmo et al., 1983, Barclay et al., 2006), and shrimp (Dall, 1995). In shrimp, poor pigmentation was initially described as a disease status (Howell and Matthews, 1991), although this was subsequently shown to be ameliorated by dietary carotenoid supplementation (Menasveta et al., 1993). Recently, pigmentation in banana shrimp has been shown to be heritable (Nguyen et al., 2014), potentially through improvements in pigment retention. Crustacean colour variations have also been observed that are unrelated to dietary carotenoids. Indeed, much of the colour variation between species is thought to be attributable to differences in the sequence and expression pattern of pigment gene crustacyanin (Wade et al., 2009), which will be discussed in more detail in later sections. Rare genetic colour mutations have been observed in clawed lobsters, predominantly Homarid species (Haggin, 2012), but also in prawns and crabs. The spiny lobster
Panulirus cygnus undergoes a colour change from deep red to pale pink during a migratory period (Phillips, 1983). This colour change has been attributed to a developmental ontogenic change that provides protective camouflage during migration, as it was not prevented by dietary carotenoid supplementation or triggered by background substrate colour (Wade et al., 2008). In another example of colour variation, seasonal appearance of pink crab disease was shown to be caused by a parasitic infection (Stentiford et al., 2002). Similarly, colour transitions have been observed between juvenile and adult stages of crabs (Krause-Nehring et al., 2010).

3.1.1 Carotenoid Type, Inclusion Levels and Feed Duration
The majority of the focus of dietary carotenoid inclusion has been on the effects on crustacean pigmentation, having been studied over many years across a range of crustacean species. These studies have been summarised in Table 1. In general, pigment development is largely dependent on the amount of carotenoid in the feed and the duration for which it is fed. Dietary Axn concentrations between 50-100 mg/kg fed for one month were sufficient to produce optimal pigmentation in a range of shrimp species (Niu et al., 2012, Niu et al., 2014, Yamada et al., 1990, Petit et al., 1997). However, 80-100 mg/kg dietary Axn supplementation produced a darker external colour more rapidly, although similar pigmentation levels were achieved over a longer duration of feeding at 50 mg/kg (Chien and Jeng, 1992, Tlusty and Hyland, 2005, Barclay et al., 2006). Pigmentation of red king crabs was also significantly improved over a 56 day period when diets were supplemented with 380 mg/kg Axn (Daly et al., 2013), but no lower inclusion levels or shorter feeding periods were tested. There is clear evidence that as dietary carotenoid levels increase, so does the Axn content of the animal, particularly the Axn esters (Yamada et al., 1990, Supamattaya et al., 2005, Boonyaratpalin et al., 2001, Barclay et al., 2006, Kumar et al., 2009, Wade et al., 2008, Wade et al., 2015b). In order to maintain initial carotenoid levels, spiny lobsters required 90 or 120 mg/kg dietary Axn (Barclay et al., 2006). In some cases, the body concentration of carotenoids (mg/kg dry weight) decreased as shrimp grew (Pan et al., 2001, Pan et al., 1999), while in others the carotenoid concentration was maintained as the animals grew (Yamada et al.,
1990, Wade et al., 2015b). Accordingly, some studies report that the whole body
tissue Axn concentration is an appropriate indicator of body color of shrimp
(Menasveta et al., 1993, Negre-Sadargues et al., 2000), while others suggest Axn
collection isn’t necessarily reflective of body colour (Tume et al., 2009).
Clearly, further work is required to provide some clarity to the objectivity of this
method of assessment.
The type of dietary carotenoid also affects the rate at which pigmentation is
developed. Shrimp (P. monodon) fed dietary Axn at 100 mg/kg showed the
highest levels of tissue Axn (16.5 mg/kg body weight) which was 23% and 43%
higher than animals fed 100 mg/kg canthaxanthin or β-carotene, respectively
(Yamada et al., 1990). Pigmentation of juvenile Kuruma shrimp, Marsupenaeus
japonicus, was better when animals were fed 100 mg/kg Axn for one month,
compared with animals fed 50 mg/kg Axn or 20 – 200 mg/kg β-carotene (Chien
and Jeng, 1992). A similar improved carotenoid tissue deposition was also
observed in shrimp fed 100 mg/kg Axn, compared with either canthaxanthin or
an Axn-canthaxanthin mixture (Negre-Sadargues et al., 1993). For P. monodon to
achieve a similar colour to that achieved using 50 mg/kg dietary Axn over 4
weeks, β-carotene was required at 125 mg/kg over 7-8 weeks, which was
reduced to 5-6 weeks by using 175 mg/kg (Boonyaratpalin et al., 2001). Shrimp
fed a diet supplemented with Artemia nauplii (which were enriched with 80%
canthaxanthin) for 4 weeks had improved deposition of free and esterified Axn
compared with those fed a diet supplemented with mauxia shrimp (55% β-
carotene) (Pan and Chien, 2003). Dietary supplementation of 200-300 mg/kg of
the β-carotene enriched microalgal pigment from Dunaliella was required for
optimal pigmentation in Penaeus monodon (Supamattaya et al., 2005). These
observations support that the efficiency with which carotenoid intermediates are
converted to Axn depends on their position in the relevant metabolic conversion
pathways. Dietary Axn levels greater than 200 mg/kg did not lead to
improvements in pigmentation or tissue carotenoid accumulation (Yamada et al.,
1990, Merchie et al., 1998), but other potential benefits of these high dietary
carotenoid levels were not examined in these studies. Later sections of this
review will explore further research in this area.
3.1.2 Chromatophores and Pigmentory Effectors

The colour of crustaceans is present in either the exoskeleton, or in pigment structures within the underlying hypodermal layer known as chromatophores (Rao, 1985). These structures are able to expand and contract, which strongly contributes to the degree of individual colouration, particularly for species with thin opaque shells like shrimp (Fingerman, 1965, Fingerman, 1966). Such physiological colour changes can be rapid, are reversible and often rhythmic in some species of crustaceans. This expansion and contraction is controlled by hormones secreted from glands in the eyestalks of crustaceans: pigment dispersing hormone (PDH) and red pigment concentrating hormone (RPCH), as a response to various physiological cues (Bagnara and Hadley, 1973, Rao, 2001). These cues can span aspects such as background colour, light source and photoperiod (Latscha, 1990, Rao, 1985).

Short-term exposure to black substrates has been shown to improve prawn pigmentation through expansion of hypodermal chromatophores (Parisenti et al., 2011a, Tume et al., 2009, Wade et al., 2015a). An example of the effect that background exposure has on the chromatophores in shrimp epithelial tissue is shown in Figure 2. In addition to expanding and contracting, the chromatophores completely change their pigment content in response to different substrates. In response to dark backgrounds, animals with expanded chromatophores contained high levels of free Axn, while white adapted animals with contracted chromatophores contained high levels of Axn mono-esters (Tume et al., 2009, Wade et al., 2015b). This expansion was also shown to be linked with the accumulation of the colour protein crustacyanin in the hypodermal tissues (Wade et al., 2012), presumably bound to free Axn to create the darker colouration. Tank colour was also shown to affect larval colour, survival and development in crabs (Rabbani and Zeng, 2005). When exposed to constant light, the body color of shrimp (P. aztecs) faded and chromatophores lost their diurnal rhythm (Lakshmi et al., 1976). Similarly, the body color of P. monodon also became faint when cultured indoors under low light intensity less than 1000 lx (Tseng et al., 1998). However, shrimp (P. monodon) subjected to constant light maintained higher carotenoid levels as they grew (Pan et al., 2001). Without addition of Axn in diet, metal halide illumination at 2500 lux resulted in the
significant accumulation of Axn in whole body of *L. vannamei* to over 4 mg/kg, compared with animals held in complete darkness at just over 2 mg/kg (You et al., 2006).

Lastly, the colour of *P. monodon* has been observed to become redder when subjected to thermal and hypoxic stress, but this pigment effect was reversible when the stress was removed and hypoosmotic stress had no effect on colour (de la Vega et al., 2007). Hypoxia was shown to increase the levels of CRCN-C1 abundance in the hepatopancreas of *Litopenaeus vannamei* (Jiang et al., 2009), although why this may be occurring is not understood. Other reports of the effect of stress on pigmentation are largely anecdotal, and there is presently very little understanding of why this might be occurring.

### 3.1.3 Carotenoproteins and Crustacyanin

Carotenoids and associated carotenoprotein complexes have been found in many invertebrate species with tissue distribution ranging from the skin and gonads to the blood, eggs and shell (Zagalsky, 1985, Lakshman and Okoh, 1993, Cheesman et al., 1967, Bhosale and Bernstein, 2007). Carotenoprotein complexes can be divided into two types: lipovitellins and true carotenoproteins. Lipovitellins possess a less stable and non-specific association of the carotenoid with the lipid portion of a lipoprotein and are responsible colouration of such tissues as the blood, epithelium, eggs and ovaries (Zagalsky, 1985, Cheesman et al., 1967). True carotenoproteins display a highly specific and stoichiometric relationship between the carotenoid and a carotenoid binding protein (CBP), and appear to be particularly widespread among the animals in class *Crustacea* as the mechanism of shell colour production (Zagalsky, 1985, Lakshman and Okoh, 1993, Cheesman et al., 1967).

Pigmentation in crustaceans is produced by a combination of the abundance and degree of expansion of different coloured chromatophores, yellow, blue and red (Rao, 1985), although visibility of chromatophores can be influenced by the thickness of the exoskeleton in some species. As noted earlier, dietary Axn supplementation increases the abundance of epithelial Axn, particularly Axn esters (Yamada et al., 1990, Supamattaya et al., 2005, Boonyaratpalin et al., 2001, Barclay et al., 2006, Kumar et al., 2009, Wade et al., 2015b). Similarly, background colour modifies pigment proportions in epithelial tissues, with
contracted chromatophores containing high levels of carotenoid esters, and expanded chromatophores containing high levels of free Axn (Tume et al., 2009, Wade et al., 2015b, Wade et al., 2015a) Within the exoskeleton and hypodermal tissue of crustaceans, free Axn is often bound within a multimeric protein complex called crustacyanin (CRCN) (Wald et al., 1948). CRCN is a member of the lipocalin protein family, a functionally diverse group of proteins that bind small hydrophobic molecules such as steroid hormones, carotenoids, odourants and pheromones (Flower, 1996, Flower et al., 2000). The interaction of CRCN and Axn modifies the naturally red carotenoid to blue or any other colour in the visible spectrum, producing the diverse array of colours seen in the exoskeleton of crustaceans (Cianci et al., 2002). During cooking, this interaction is disrupted, releasing the distinct red colouration of cooked seafood. The dimeric β-crustacyanin (β-CRCN) is formed by two types of CRCN subunits (A and C, also called H₁ and H₂) in association with two Axn molecules (Cianci et al., 2002). Eight of these dimers form a larger molecular weight complex known as α-crustacyanin (α-CRCN), which has been extensively studied using crystallographic techniques (reviewed in, (Chayen et al., 2003, Zagalsky, 2003)). At present, two genes that encode CRCN-A and CRCN-C have been identified across a range of crustaceans (Wade et al., 2009, Ertl et al., 2013, Wang et al., 2007). Their expression is restricted to the outer layer of the hypodermis (Wade et al., 2009, Wang et al., 2007), and the spatial regulation of the CRCN genes is thought to define the species-specific shell colors and patterns that different crustaceans display (Wade et al., 2009). In further support of this theory, reconstitution of recombinant CRCN monomers (either A or C) formed complexes with distinct absorption spectra, and the presence of CRCN in various species correlated with the ability to produce certain shell colours (Ferrari et al., 2012).

The development of colour over time in pigment deficient clawed lobsters (H. americanus) was dependent on dietary carotenoid concentration, and progressed over three months through either a predominantly red or a predominantly blue phase before achieving a colour considered equivalent to those from the wild (Tlusty and Hyland, 2005). In freshwater shrimp (M. rosenbergii), external colour was removed by specific knockdown of a CRCN
homolog using RNAi (Yang et al., 2011). In this study, the blue pigment attributed
to the Axn-CRCN interaction was removed by decreasing CRCN gene expression,
and hence protein abundance, which modified the shrimp colour to red.
Although not directly measured, the red colour that remained was likely the
underlying red chromatophores containing predominantly Axn esters. This
suggests that colour could be preferentially deposited in different
chromatophores, although how this might be regulated is not understood.
Exposure to white substrates significantly decreased the amount of CRCN
protein in shrimp hypodermal tissue, along with decreased free Axn levels and
increased Axn ester levels (Wade et al., 2012). Exposure to black substrates
significantly increased the abundance of epithelial CRCN protein (Wade et al.,
2012), indicating the presence of this protein was critical to redistributing
hypodermal pigments and achieving optimal cooked colour (Wade et al., 2012).
However, CRCN gene expression did not vary across the moult cycle or in
response to substrate colour (Wade et al., 2012). Albino colour morphs of shrimp
(F. merguiensis) displayed significantly reduced expression of the CRCN-A and C
genes compared with other shrimp, as well as a range of other genes potentially
involved in the regulation of crustacean colour (Ertl et al., 2013). However,
expression levels of CRCN were not significantly different between light and dark
coloured shrimp, and there was no correlation between levels of CRCN gene
expression and Axn content (Ertl et al., 2013). Despite extensive knowledge of
the mechanism by which CRCN binds Axn to produce crustacean colour, there is
very little known about how CRCN gene expression is regulated or how the CRCN
protein complexes form or are modified in the crustacean exoskeleton.

3.2 Carotenoids and Growth and Survival
Reports of the effect of dietary carotenoid supplementation on growth and
survival in crustaceans have been mixed, with virtually all research having been
conducted on shrimp. Some studies reported no significant difference in growth
in shrimp that had received dietary carotenoid supplementation (Pan et al.,
2001, Negre-Sadargues et al., 1993, Boonyaratpalin et al., 2001). However, an
increasing number of studies have shown that either growth or survival, or both,
are significantly improved when shrimp are fed a diet that contains carotenoids

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Early reports describing the beneficial effects of Axn on shrimp growth were assessed on postlarvae (Darachai et al., 1998, Chien, 1996) with evidence that Axn supplementation shortened the moult frequency (Petit et al., 1997). Larval stages and postlarvae of P. monodon showed greater survival and were longer when fed algal Axn (Haematococcus pluvialis) supplemented diets (Darachai et al., 1998). Studies on M. japonicus juveniles demonstrated that growth performance was similar in shrimp over 8-weeks whether or not 100 mg/kg carotenoid was included (Yamada et al., 1990). However, by the end of 8 weeks animals without dietary carotenoid contained significantly less total carotenoid than those fed 100 mg/kg, and their survival had dropped from 91.3% to 57.1% (Yamada et al., 1990). In a separate experiment by the same authors but using smaller animals, animals that had received 100 mg/kg Axn for 8 weeks had grown significantly better than those that had not been fed Axn, while survival was unaffected (Yamada et al., 1990). Between these two experiments, there was a marked difference in the total carotenoid content prawns at the beginning of the experiment, with poor survival over 8 weeks recorded when initial carotenoid content was low (15.6±0.8 mg/kg). Significant correlations have been observed between tissue carotenoid concentration and survival (Chien and Jeng, 1992) or specific growth rate (You et al., 2006).

Since this initial work, the vast majority of studies have focused on the giant tiger shrimp, Penaeus monodon. Shrimp fed 125-300 mg/kg of algal extract for 8-weeks showed higher weight gain and survival compared with controls (Supamattaya et al., 2005). When fed with 100mg/kg Axn combined with 1% cholesterol for 74 days, shrimp showed higher weight gain and survival compared with those fed diets without carotenoids (Niu et al., 2012), with apparent Axn digestibility of approximately 98%. In a similar study, shrimp fed 100 mg/kg Axn combined with 1% cholesterol also showed significantly higher weight gain and survival (Niu et al., 2014), and showed similarly high (>90%) Axn digestibility. Although less studied, other species have shown a similar
response. Post-larval shrimp (*L. vannamei*) fed 80 mg/kg Axn for 6 weeks showed an increased daily growth coefficient and a reduced moult frequency compared with those animals that had not been fed dietary Axn, but survival was unaffected (Flores *et al.*, 2007). Shrimp (*L. vannamei*) fed either 100, 200 or 400 mg/kg Axn for 30 days showed improved weight gain and survival compared with those without dietary carotenoids (Niu *et al.*, 2009). After 56 days, shrimp (*L. vannamei*) fed 125 or 150 mg/kg Axn had higher weight gain than those fed 25, 50, 75 or 100 mg/kg Axn (Zhang *et al.*, 2013), but survival was unaffected. In freshwater *Macrobrachium*, inclusion of 50, 100 or 200 mg/kg Axn improved growth over the reference (Kumar *et al.*, 2009). Shrimp (*M. japonicus*) had improved survival from 37% to over 50% when fed diets containing carotenoids over 9 weeks, (Chien and Shiau, 2005), with a complementary increase in body Axn levels, but no effect on growth. Improved survival, but not growth, was also recorded in red king crab juveniles fed 380 mg/kg Axn for 56 days (Daly *et al.*, 2013).

Combined, these data suggest that survival is not affected when carotenoids are maintained at a certain level, perhaps between 10-15 mg/kg body weight for *P. monodon*, but survival is compromised below that level without carotenoid supplementation. Where tissue carotenoid levels are initially high, perhaps above 20 mg/kg, further carotenoid supplementation allows improved growth. Variability in animal performance in growth trials may be explained by a range of factors, including animal health, quality of feed ingredients, system design and animal husbandry. Detection of growth differences in shrimp fed dietary carotenoids in more recent studies may reflect improvements in trial maintenance and animal husbandry. The study by (Pan *et al.*, 2001) had shown there was no significant increase in survival in animals fed carotenoids compared with those that were not, although overall survival was less than <30% across the experiment, and this low level of survival casts aspersions on the validity of this work. Despite this, it was demonstrated that higher tissue carotenoid levels were correlated with higher survival (Pan *et al.*, 2001). Carotenoid levels in shrimp at the beginning of the study will also be critical, as carotenoid stores in animal tissues may compensate for the lack of dietary carotenoids at least through the initial stages of an experimental growth trial.
3.3 Carotenoids and Tolerance to Disease and Stress

This section will focus on studies where dietary carotenoids have been supplied, then the capacity to tolerate an induced stress has been directly tested under controlled conditions, and the effects on survival or other biochemical parameters assessed. The improved survival described in the previous section was reported after a period of 8-9 weeks of a growth feeding trial in experimental systems using different carotenoids (Axn, β-carotene or canthaxanthin). However, more recent studies have been designed to specifically assess whether responses to acute and chronic stresses, such as hypoxia, salinity or viral infection, are improved after long periods of dietary carotenoid supplementation. Analysis on shrimp (F. chinensis) showed that hypoxia alone triggered significant up-regulation of proteins involved in immunity (chymotrypsin and carboxypeptidase), and down regulation of proteins involved in energy production (citrate synthase, ATP synthase), metabolism (transketolase and esterases) and antioxidant capacity (glutathione peroxidase and cMnSOD) (Jiang et al., 2009). Dietary levels of 125 and 150 mg/kg Axn fed to shrimp (L. vannamei) for 56 days lowered total antioxidant status, superoxide dismutase (SOD), and catalase activities than those animals fed 25, 50, 65 or 100 mg/kg (Zhang et al., 2013). Carotenoids were found to be less abundant in the digestive gland and ovary of farmed L. vannamei compared with wild animals, and levels were concluded to be insufficient to neutralise oxidative stress during ovarian development (Linan-Cabello et al., 2003). Crayfish exposed to pollution had lower levels of vitamins and carotenoids in the hepatopancreas, suggesting these may play a role in tolerating polluted environments (Barim and Karatepe, 2010).

Similar to growth and survival, the majority of work on tolerance to stress has been performed on shrimp. Early studies showed that larval stages of P. monodon supplemented with algal carotenoids were more resistant to low salinity stress than those with synthetic Axn or controls (Darachai et al., 1998). Similarly, survival of P. monodon postlarvae during a low salinity stress test exposure to 4 hours of low dissolved oxygen (< 1.0 mg/L) was improved in
shrimp (*P. monodon*) fed 360 mg/kg Axn for one week (Chien et al., 1999). In a separate test, these shrimp were also shown to be more tolerant of lower oxygen levels in a lethal oxygen test (Chien et al., 1999). Dietary Axn supplementation at 80 mg/kg enhanced antioxidant capacity in tiger shrimp (*P. monodon*) postlarvae, which resulted in a significant improvement in recovery to both thermal and osmotic stress (Chien et al., 2003). In this study, higher body Axn levels were recorded, total antioxidant status (TAS) was reduced and superoxide dismutase (SOD) levels were reduced. The authors also speculated that hepatopancreas function was improved due to lower levels of aspartate aminotransferase (AST), a blood marker of liver integrity in mammalian systems, being identified in the circulating hemolymph. However, both AST and alanine aminotransferase (ALT) levels were reduced by thermal and osmotic stress, which was opposite to the expected effect of stress. The inclusion of 80 mg/kg in diets for 8-weeks improved shrimp (*P. monodon*) resistance to ammonia stress, and animals showed higher total antioxidant status and lower SOD levels (Pan et al., 2003). AST and ALT levels were lowered by Axn supplementation, and were negatively correlated with TAS. However, aminotransferase levels were not correlated with survival, and may indicate that shrimp mortality was unrelated to hepatopancreas damage. When fed 300 mg/kg of algal carotenoids for 8 weeks, *P. monodon* showed improved tolerance to a nine day period of daily hypoxic stress (<1.0 mg/L) and also higher resistance to WSSV infection (Supamattaya et al., 2005).

Studies in other shrimp also showed similar effects. In *M. japonicus*, inclusion of at least 50 mg/kg dietary Axn, from either synthetic or algal sources, resulted in improved survival to low oxygen stress (Chien and Shiau, 2005). Significantly greater levels of Axn had accumulated during the 9-week feeding trial, along with a reduced oxygen consumption rate, suggesting that Axn may be acting as an intracellular oxygen reserve or as a potent cellular antioxidant. Total carotenoid levels were highest in animals that showed the highest survival, yet total hemocyte count was lower and hemolymph phenoloxidase activity was unchanged. Post-larval shrimp (*L. vannamei*) fed 80 mg/kg Axn for 6-weeks showed significantly higher osmoregulatory capacity than those without dietary Axn after salinity was reduced from 35 to 3 gL⁻¹ (Flores et al., 2007). This was
coupled with significantly increased levels of hemocytes, hemocyanin and glucose in the hemolymph, and reduced levels of hemolymph lactate (Flores et al., 2007). In a hypoxia stress test, postlarval shrimp (L. vannamei) fed either 200 or 400 mg/kg Axn recorded significantly higher survival, but no other physiological parameters were measured (Niu et al., 2009). More recently, freshwater prawns showed a significant increase in phenoloxidase activity and total hemocyte count after 28 days of consuming carotenoid fortified diets (Kumar et al., 2009), although no direct stress test was performed on the animals in this study. Systemic injection of Axn into the same species caused an increase in the total hemocyte count and an increased resistance to bacterial infection, although there was no complementary increase in antioxidant indicators (Angeles et al., 2009). After low dissolved oxygen challenge, shrimp (L. vannamei) fed 75-150 mg/kg Axn for 56 days had higher survival than those animals fed 25 or 50 mg/kg Axn, and this was potentially linked with higher expression of hypoxia inducible factor 1 alpha (HIF-1α), cytosolic manganese superoxide dismutase (cMnSOD) and catalase in Axn fed animals (Zhang et al., 2013). After 74 days feeding 100 mg/kg Axn or 250 mg/kg β-carotene, improved growth performance and survival in juvenile P. monodon was coupled with lower malondialdehyde levels (an indicator of lipid peroxidation) after a simulated live transport test (Niu et al., 2014). In addition, expression levels of heat shock protein 70 (Hsp-70) were significantly elevated under hypoxia compared with normoxia, and further up-regulated under hypoxic conditions without dietary carotenoids (Niu et al., 2014). Although counter-intuitive, the expression of hypoxia inducible factor 1 alpha (HIF-1α) was decreased under hypoxic condition, but were higher in animals fed β-carotene suggesting that the response to hypoxia had been alleviated (Zhang et al., 2013, Niu et al., 2014).

In summary, data consistently demonstrate that dietary carotenoids increase the total antioxidant capacity in the haemolymph of crustaceans, coupled with decreased activity of other antioxidant enzymes. This may occur through increased Axn levels in the haemolymph and tissues, improved oxygen carrying capacity, decreased oxidation of polyunsaturated fatty acids or cellular proteins or decreased activation of stress response systems. Combined, these data suggest that the stress response is reduced in animals receiving dietary carotenoids.
which improves survival to that stress, and that Axn is performing a broad
protective role against the detrimental effects of oxidative damage in tissues.

Similar to growth, many factors can affect survival in experimental systems,
which is especially problematic when survival is a key measure of performance
against stress. However, clear experimental evidence now exists to show that
carotenoid supplementation improves a range of factors to enable crustaceans to
tolerate stresses such as disease, hypoxia, temperature and salinity. These effects
appear to link the proposed antioxidant function of carotenoids themselves, with
physiological improvements in antioxidant capacity in the animals, and
improved performance under various stressful conditions. Some inconsistency
exists in the physiological responses of animals to dietary carotenoids, which
may highlight differences in the way different crustaceans deal with a variety of
stressors.

3.4 Carotenoids and Reproductive Performance

Nutrition plays a critical role in the reproductive success of crustaceans, and the
accumulation of nutrients in the developing ovaries, particularly lipids and
carotenoids, has a direct effect on reproductive measures such as egg number,
hatching rate and total nauplii produced (Wouters et al., 2001). Very little progress has been made in understanding the basis by which dietary carotenoids
improve crustacean reproduction since it was summarised more than ten years
ago (Linan-Cabello et al., 2002a). During early maturation, carotenoids
accumulate in the hepatopancreas in both free and esterified form, after which
they are transported via the haemolymph to the ovaries during secondary
vitellogenesis (Harrison, 1990, Vincent et al., 1988). Carotenoid content and type
varies greatly during ovarian development (Dall et al., 1995, Linan-Cabello et al.,
2002b, Linan-Cabello et al., 2003, Vincent et al., 1988, Vincent et al., 1989). The
darkening that occurs with this accumulation forms the basis of “staging” female
ovaries during ovarian maturation (Wouters et al., 2001). Free and esterified Axn
is known to accumulate in the hepatopancreas during ovarian maturation, while
levels in the integument remain relatively constant (Dall et al., 1995). Captive
shrimp contained less carotenoids, particularly in stage IV ovaries, than their
wild caught counterparts (Linan-Cabello et al., 2003), strongly suggesting that
broodstock nutrition was deficient. Paprika as a source of dietary carotenoids (α-carotene, α-cryptoxanthin and capxanthin) was shown to improve nauplii quality in P. monodon broodstock (Wyban et al., 1997), with the assumption that these carotenoids were able to be converted into Axn. Axn supplemented in broodstock diets for Penaeus monodon showed improved spawning and fecundity (Pangantihon-Kuhlmann et al., 1998). In the only recent study, high levels of dietary fish oil and Axn have been linked to improved reproductive performance, as measured by egg and spermatozoa number, in P. monodon broodstock (Paibulkichakul et al., 2008). As might be expected, increased dietary fish oil led to accumulation of polyunsaturated fatty acids (PUFAs) in hepatopancreas and ovary tissues, particularly 22:6n-3. However, extremely high levels of dietary Axn (300 mg/kg) also led to an accumulation of Axn along with these long chain PUFAs in ovary tissue (Paibulkichakul et al., 2008). Increased focus may be required on the use of carotenoids in conjunction with other nutrients of reproductive significance, such as long chain PUFAs.

The positive effects of Axn can potentially be attributed its extremely high capacity to scavenge oxygen free radicals, and the prevention of peroxidation of PUFAs in tissues and diets (Britton, 2008, Miki, 1991). In various fish species, the accumulation of carotenoids in reproductive tissues through dietary carotenoid supplementation has been shown to improve a number of performance characteristics, such as egg number, egg quality and number of larvae (Bjerkeng, 2008). Oxygen free radicals have been shown to attack biomembrane lipids and proteins, leading to deterioration in egg quality (Bromage and Roberts, 1995). In crustaceans, in conjunction with a depletion of carotenoids in the hepatopancreas and ovary, an elevation of superoxide dismutase (SOD) activity was observed in the haemolymph of captive shrimp compared with wild shrimp (Linan-Cabello et al., 2003). This was suggested to reflect the insufficient scavenger activity to neutralize oxidative stress processes during spawning. Normal developmental and physiological processes, such as ovarian development and reproduction, are also potential sources of oxygen free radicals.

Although not initially identified as necessary for embryonic development, carotenoids are lost from fish and crustacean embryos prior to the first feeding
stages (Bjerkeng, 2008, Dall et al., 1995). This implies the carotenoids present in eggs and pre-feeding embryos are metabolised into other colourless molecules, that in turn potentially perform biological functions. Axn has been proposed to be an important source of Provitamin A and retinoids in eggs and early embryos (Dall et al., 1995, Linan-Cabello et al., 2002a, Miki, 1991). Evidence from a number of different crustaceans suggests that the retinols and other retinoid derivatives play a critical role in developmental processes of crustaceans, including ovarian and larval development (Linan-Cabello et al., 2002a). Crustaceans possess a number of retinoids and retinoic acid receptors in crustaceans and the enhancement of the ovarian development in shrimp suggests an important role of these metabolites in shrimp physiology for their successful aquaculture. Carotenoids are the sole source of retinoids in crustaceans, and their role as bioactive molecules may have been largely overlooked (Linan-Cabello et al., 2002a).
4 Conclusion

Carotenoids are considered a semi-essential nutrient that promotes optimal survival and growth at low dietary inclusion levels, approximately 25 mg/kg dietary Axn. Studies demonstrate that some form of dietary carotenoid intake is required in order to maintain carotenoid levels over time as animals grow, whether that intake is from natural pond biota or formulated into feeds. This amount is estimated at 50 mg/kg dietary Axn to maintain between 20-25 mg/kg body weight Axn for juvenile *P. monodon*. Increasingly, evidence suggests that specific carotenoids accumulate in different crustacean tissues over various life history stages. At present this minimum body Axn level is poorly defined, but whole body Axn levels may improve survival and growth across various stages of commercial production.

Optimal shrimp pigmentation can be achieved within several weeks by including Axn in the diet at levels of between 50-100 mg/kg, which can be reduced by using higher dietary inclusion levels. At these and even higher inclusion levels, utilisation efficiency of dietary carotenoids is extremely high and often exceeds 90%. In Penaeid shrimp, the amount of carotenoid required to be deposited in the tissues to achieve optimal colour is around 30-50 mg/kg body weight. However, this amount does not result in the same overall colour of different species, i.e. *P. monodon* is darker than *L. vannamei* at the same body Axn level. In other crustaceans, this body Axn level may need to be significantly higher. Background colour and light intensity are highly effective at redistributing carotenoid pigments, both to make shrimp darker or lighter in colour. Optimal pigmentation can lead to substantially higher sale prices, but there can be a preference for either darker or lighter shrimp depending on the target market.

Although presently poorly defined, the carotenoid levels required to elicit the physiological improvements in disease resistance, hypoxia or reproductive performance may be considerably higher than those for pigmentation. These beneficial effects have been demonstrated on various physiological characteristics such as survival, growth and resistance to stress. However, unlike colour, accurate measurement of these effects is often difficult due to a range of external factors. Improvements in research methods and techniques have led to
a stronger understanding of the physiological mechanisms underlying carotenoid function in crustaceans. Very little is known about the genetic mechanisms that underlie the absorption, transport, tissue accumulation or metabolic transformations of carotenoids in any animal species. It is reasonable to assume that the accumulation of these carotenoids underpins the physiological changes that lead to improved performance of a variety of commercially relevant traits in aquaculture. More detailed studies are required to define the basis of the benefits of carotenoids in crustacean aquaculture. Although some functions of carotenoids may be preserved, we cannot continue to rely on research from vertebrate systems to draw conclusions on their effect in crustaceans.
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Figure 1. Schematic diagram of the major conversion pathway of \( \beta \)-carotene to astaxanthin in crustacean tissues.
Figure 2 The response of crustacean abdominal epithelial chromatophores when exposed to black (A and C) or white (B and D) coloured substrates.
Table 1. Summary of carotenoid research in crustacean diets that improves pigmentation.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Inclusion range</th>
<th>Carotenoid</th>
<th>Source</th>
<th>Optimal Pigmentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giant Tiger Prawn (<em>Penaeus monodon</em>)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Yamada et al., 1990)</td>
<td>0 – 400 mg/kg</td>
<td>Astaxanthin / β-carotene / Canthaxanthin</td>
<td>Synthetic</td>
<td>200 mg/kg Astaxanthin</td>
</tr>
<tr>
<td>(Liao et al., 1993)</td>
<td>3%</td>
<td>β-carotene / Zeaxanthin</td>
<td>Spirulina / Krill Oil</td>
<td>3% Spirulina</td>
</tr>
<tr>
<td>(Menasveta et al., 1993)</td>
<td>0 – 50 mg/kg</td>
<td>Astaxanthin</td>
<td>Synthetic</td>
<td>50 mg/kg</td>
</tr>
<tr>
<td>(Merchie et al., 1998)</td>
<td>230 – 810 mg/kg</td>
<td>Astaxanthin</td>
<td>Synthetic</td>
<td>inconclusive</td>
</tr>
<tr>
<td>(Boonyaratpalin et al., 2001)</td>
<td>125 – 175 mg/kg</td>
<td>β-carotene</td>
<td>Algal</td>
<td>125 mg/kg</td>
</tr>
<tr>
<td>(Supamattaya et al., 2005)</td>
<td>125 – 300 mg/kg</td>
<td>β-carotene</td>
<td>Algal</td>
<td>200 – 300 mg/kg</td>
</tr>
<tr>
<td>(Niu et al., 2012)</td>
<td>70 – 200 mg/kg</td>
<td>Astaxanthin / Canthaxanthin</td>
<td>Synthetic</td>
<td>100 mg/kg Astaxanthin + cholesterol</td>
</tr>
<tr>
<td>(Niu et al., 2014)</td>
<td>100 – 250 mg/kg</td>
<td>Astaxanthin / β-carotene</td>
<td>Synthetic</td>
<td>100 mg/kg Astaxanthin + cholesterol</td>
</tr>
<tr>
<td>Pacific White Shrimp (<em>Litopenaeus vannamei</em>)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Vernon-Carter et al., 1996)</td>
<td></td>
<td>Astaxanthin / Lutein</td>
<td>Synthetic / Marigold</td>
<td>Marigold</td>
</tr>
<tr>
<td>(Arredondo-Figueroa et al., 2003)</td>
<td>200-250 mg/kg</td>
<td>Capsanthin</td>
<td><em>Capsicum annuum</em></td>
<td></td>
</tr>
<tr>
<td>(Niu et al., 2009)</td>
<td>0 – 400 mg/kg</td>
<td>Astaxanthin</td>
<td>Synthetic</td>
<td>100 - 200 mg/kg</td>
</tr>
<tr>
<td>(Ju et al., 2011)</td>
<td>25 – 150 mg/kg</td>
<td>Astaxanthin</td>
<td>Algal and Synthetic</td>
<td>75 – 100 mg/kg</td>
</tr>
<tr>
<td>Species</td>
<td>Range of Astaxanthin Concentration</td>
<td>Source and Formulation</td>
<td>Source and Formulation Details</td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
<td>Kuruma Shrimp (<em>Marsupenaeus japonicus</em>)</td>
<td>50 – 200 mg/kg</td>
<td>Astaxanthin / β-carotene</td>
<td>Synthetic / algal 100 mg/kg Astaxanthin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 mg/kg</td>
<td>Astaxanthin / Canthaxanthin</td>
<td>Synthetic 50 mg AX + 50 mg CX</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 – 220 mg/kg</td>
<td>Astaxanthin / Canthaxanthin</td>
<td>Synthetic / Artemia 60 mg/kg Astaxanthin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 – 100 mg/kg</td>
<td>Astaxanthin</td>
<td>Synthetic / algal 100 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Giant Freshwater Prawn <em>Macrobrachium rosenbergii</em></td>
<td>0 – 200 mg/kg</td>
<td>Astaxanthin</td>
<td>Synthetic 200 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Hermit Crab (<em>Clibanarius erythropus</em>)</td>
<td>200 mg/kg</td>
<td>Astaxanthin / β-carotene / Canthaxanthin</td>
<td>Synthetic 200 mg/kg Astaxanthin</td>
<td></td>
</tr>
<tr>
<td>Red King Crab (<em>Paralithodes camtschaticus</em>)</td>
<td>0 – 380 mg/kg</td>
<td>Astaxanthin</td>
<td>Synthetic / algal 380 mg/kg</td>
<td></td>
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<tr>
<td>American Clawed Lobster (<em>Homarus americanus</em>)</td>
<td>0 – 220 mg/kg</td>
<td>Astaxanthin</td>
<td>Synthetic 220 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Tropical Spiny Crayfish (<em>Panulirus ornatus</em>)</td>
<td>30 – 120 mg/kg</td>
<td>Astaxanthin</td>
<td>Synthetic 120 mg/kg</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Summary of carotenoid research in crustacean diets that improves physiological performance.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Inclusion level</th>
<th>Species</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Growth and Survival</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Yamada <em>et al.</em>, 1990)</td>
<td>100 mg/kg Axn</td>
<td><em>M. japonicus</em></td>
<td>Improved survival or growth</td>
</tr>
<tr>
<td>(Darachai <em>et al.</em>, 1998)</td>
<td>various</td>
<td><em>P. monodon</em></td>
<td>Improved post-larval survival</td>
</tr>
<tr>
<td>(Chien and Shiau, 2005)</td>
<td>50-100 mg/kg</td>
<td><em>M. japonicus</em></td>
<td>Improved survival</td>
</tr>
<tr>
<td>(Supamattaya <em>et al.</em>, 2005)</td>
<td>300 mg/kg β-carotene</td>
<td><em>P. monodon</em></td>
<td>Greater weight gain and improved survival</td>
</tr>
<tr>
<td>(Flores <em>et al.</em>, 2007)</td>
<td>80 mg/kg Axn</td>
<td><em>L. vannamei</em></td>
<td>Improved growth and moult frequency</td>
</tr>
<tr>
<td>(Kumar <em>et al.</em>, 2009)</td>
<td>50-200 mg/kg Axn</td>
<td><em>M. rosenbergii</em></td>
<td>Greater weight gain and improved survival</td>
</tr>
<tr>
<td>(Niu <em>et al.</em>, 2009)</td>
<td>100-400 mg/kg Axn</td>
<td><em>L. vannamei</em></td>
<td>Greater weight gain and improved survival</td>
</tr>
<tr>
<td>(Niu <em>et al.</em>, 2012)</td>
<td>100 mg/kg Axn + cholesterol</td>
<td><em>P. monodon</em></td>
<td>Greater weight gain and improved survival</td>
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<tr>
<td>(Daly <em>et al.</em>, 2013)</td>
<td>380 mg/kg</td>
<td><em>Paralithodes camtschaticus</em></td>
<td>Improved survival</td>
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<tr>
<td>(Zhang <em>et al.</em>, 2013)</td>
<td>125-150 mg/kg Axn</td>
<td><em>L. vannamei</em></td>
<td>Improved growth</td>
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<tr>
<td>(Niu <em>et al.</em>, 2014)</td>
<td>100 mg/kg Axn + cholesterol</td>
<td><em>P. monodon</em></td>
<td>Greater weight gain and improved survival</td>
</tr>
</tbody>
</table>

*Tolerance to Disease and Stress*

<table>
<thead>
<tr>
<th>Reference</th>
<th>Inclusion level</th>
<th>Species</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Darachai <em>et al.</em>, 1998)</td>
<td>various</td>
<td><em>P. monodon</em></td>
<td>Improved tolerance to low salinity</td>
</tr>
<tr>
<td>(Chien <em>et al.</em>, 1999)</td>
<td>360 mg/kg Axn</td>
<td><em>P. monodon</em></td>
<td>Improved survival to low dissolved oxygen</td>
</tr>
<tr>
<td>(Chien <em>et al.</em>, 2003)</td>
<td>80 mg/kg Axn</td>
<td><em>P. monodon</em></td>
<td>Improved recovery from thermal and osmotic stress, enhanced anti-oxidant capacity.</td>
</tr>
<tr>
<td>Reference</td>
<td>Treatment Details</td>
<td>Species</td>
<td>Effects in Organisms</td>
</tr>
<tr>
<td>----------------------------</td>
<td>-------------------</td>
<td>------------------</td>
<td>-------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>(Pan et al., 2003)</td>
<td>80 mg/kg Axn</td>
<td><em>P. monodon</em></td>
<td>Improved resistance to ammonia stress, higher anti-oxidant status, lower SOD levels.</td>
</tr>
<tr>
<td>(Chien and Shiau, 2005)</td>
<td>50 mg/kg Axn</td>
<td><em>M. japonicus</em></td>
<td>Improved survival to low oxygen</td>
</tr>
<tr>
<td>(Supamattaya et al., 2005)</td>
<td>300 mg/kg Axn</td>
<td><em>P. monodon</em></td>
<td>Improved survival to daily hypoxia stress, increased resistance to WSSV infection</td>
</tr>
<tr>
<td>(Flores et al., 2007)</td>
<td>0-150 mg/kg Axn</td>
<td><em>L. vannamei</em></td>
<td>Improved survival to daily hypoxia stress</td>
</tr>
<tr>
<td>(Niu et al., 2009)</td>
<td>200-400 mg/kg Axn</td>
<td><em>L. vannamei</em></td>
<td>Improved survival to bacterial infection</td>
</tr>
<tr>
<td>(Angeles et al., 2009)</td>
<td>1.34 nmol g⁻¹ Axn injected</td>
<td><em>M. rosenbergii</em></td>
<td>Improved survival to hypoxia stress, increased HIF-1α, cMnSOD and catalase expression.</td>
</tr>
<tr>
<td>(Zhang et al., 2013)</td>
<td>75-150 mg/kg Axn</td>
<td><em>L. vannamei</em></td>
<td>Improved survival in live transport test, reduced malondialdehyde and HSP-70 levels</td>
</tr>
<tr>
<td>(Niu et al., 2014)</td>
<td>100 mg/kg Axn</td>
<td><em>L. vannamei</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>250 mg/kg β-carotene</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Reproductive Performance

<table>
<thead>
<tr>
<th>Reference</th>
<th>Treatment Details</th>
<th>Species</th>
<th>Effects in Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Wyban et al., 1997)</td>
<td>Various</td>
<td><em>L. vannamei</em></td>
<td>Improved nauplii quality</td>
</tr>
<tr>
<td>(Pangantihon-Kuhlmann et al., 1998)</td>
<td>100 mg/kg Axn</td>
<td><em>P. monodon</em></td>
<td>Improved spawning and fecundity</td>
</tr>
<tr>
<td>(Paibulkichakul et al., 2008)</td>
<td>50-300 mg/kg Axn</td>
<td><em>P. monodon</em></td>
<td>Increased number of eggs and spermatozoa, accumulation of Axn in ovary tissue</td>
</tr>
</tbody>
</table>