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REVIEW

REVIEWS IN Aquaculture

Carotenoids modulate stress tolerance and immune responses in aquatic animals

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Abstract
Aquaculture continues to expand swiftly and remains the fastest-growing food industry worldwide amidst ever-present threats from chronic stressors and emerging diseases. Nutrition plays a pivotal role in the profitability and viability of the aquaculture industry that steered a paradigm shift to therapeutic nutrition. Carotenoids, also termed tetraterpenoids, have garnered considerable attention owing to their therapeutic attributes and immeasurable health benefits, which incited a surge in global demand. These biological pigments are recognized to promote immune systems and antioxidant defence mechanisms in both aquatic vertebrates and invertebrates. This review brings forth existing scientific evidence and underscores the notable roles of carotenoids as biologically active constituents with anti-stress and immunostimulatory potentials in farmed aquatic animals whilst explicating possible mechanisms of action. Empirical data unequivocally established the modulatory functions of carotenoids on endogenous antioxidant enzymes, innate and adaptive arms of the immune response, as well as the expression of multiple antioxidant and immune-related genes. The comprehensive information presented is beneficial to deepen our understanding of the utilization of carotenoids as potent stress alleviators and immunostimulants in cultured aquatic animals, which is translated into improved health. Advancements in aquatic animal health and welfare could principally contribute to reconstructing a more sustainable aquaculture industry. This article may be useful for subsequent investigations towards further advances in research and innovation to a greener blue revolution in solving the challenge of global food security.

KEYWORDS
antioxidant defence, immunity, metabolism, oxidative stress, pigment, reactive oxygen species

1 | INTRODUCTION

Aquaculture made its mark as the world's most dynamic and fastest-growing livestock sector with a multibillion-dollar profit margin, broadly fuelled by the ever-growing human population and demand for animal protein. Today, aquaculture is rapidly expanding with a compound annual growth rate (CAGR) of 5.3% yearly (2001–2018).[1] Global aquaculture production attained another record high of 114.5 million tonnes in 2018 (valued at USD 263.6 billion), supplying more than 60% of the total harvest weight of aquatic animals for human consumption.[1] Despite phenomenal growth, the industry has been enduring recurring boom-and-bust cycles linked to devastating disease outbreaks with eventual production collapse. The outbreak of threatening diseases is a primary constraint to the production of many aquaculture species. Indeed, disease was listed ahead of all other causes of production losses in the questionnaire for the Census of

Aquaculture 2018.[1] The World Bank estimates that aquaculture diseases entail economic losses of more than USD 6 billion annually.[2] Routinely, prophylactic treatments (e.g. antimicrobial therapeutic agents or drugs) are adopted as a primary option in treating infectious diseases.[3,4] Unregulated and imprudent use of antimicrobials accelerates the emergence of drug-resistant pathogens while inducing host immunosuppression due to potential oxidative stress that arises following treatments.[5–7] Moreover, intensive aquaculture has been linked to numerous environmental and husbandry-related stressors, posing challenges to the sector. Good nutrition, successful health management and eco-friendly farming practices serve as the strongest pillars for sustainable aquaculture.

Carotenoids or tetraterpenoids represent a diverse group of organic pigments that are synthesized exclusively by plants, phytoplankton, photosynthetic bacteria and some species of archaea and fungi. These pigments provide a broad variety of conspicuous hues (e.g. orange, red and yellow) to various animals, fruits and leaves of plants. Carotenoid pigments (e.g. astaxanthin, β-carotene, canthaxanthin, fucoxanthin and lutein) are renowned for commercial applications in food, cosmetic, nutraceutical, pharmaceutical and animal feed industries; owing to their anti-oxidative and therapeutic properties.[8–10] High-performance compounded aquaculture feeds are frequently integrated with carotenoids from natural and synthetic origins. Animals lack the capacity to biosynthesize carotenoids de novo and thus rely upon dietary sources for these compounds (food-borne carotenoids) or partly modified via metabolic reactions.[11,12] The fundamental roles of carotenoids in all photosynthetic organisms include light absorption and photoprotection against photooxidative stress while acting as precursors of plant hormones.[13–15] In animals, carotenoids serve critical functions as essential precursors to vitamin A (retinol), antioxidants, photo-protectors, immunostimulants, anti-inflammatories and growth promoters.[16–18] The antioxidant potency of carotenoids is of particular significance to aquatic animal health, which augments the efficiency of immune responses and elicits innate immune activation.[19–22] Furthermore, as potent singlet oxygen quenchers, carotenoids can potentially halt the toxic effects of reactive oxygen species (ROS) generated during mounting host defence activities without raising the associated costs of immunity.[23,24] The overproduction of ROS could conceivably entail host immunosuppression and severe oxidative damages since the immune response is a costly physiological activity when initiated against foreign microorganisms (non-self entities) or pathogenic processes.[25,26] From this perspective, supplementation of carotenoids for a well-integrated antioxidant system is imperative for enhanced stress tolerance and immunity in cultured animals.

Recent progress infused direct insights into the biological roles of carotenoids in signal transduction and expression of multiple antioxidant and immune-related genes, particularly in aquatic vertebrates and invertebrates.[27–32] The aforementioned has prompted new enthusiasm and investigation on the intricate interplay between carotenoids, antioxidant defence system and immunity; and how this may be associated with disease aetiology and prevention in commercial aquatic species. As a consequence, great advances in immunology and animal health are anticipated with the rapid development and application of such technologies in aquatic organisms. Research investigating the multi-faceted roles of carotenoids in the stress tolerance and immunoenhancement of aquatic organisms had thrived over the years through well-controlled feeding trials and time-series studies; nonetheless, the information remained poorly assembled as a whole.[16,24] It is necessary, however, to incorporate these findings so that the knowledge gained and implications of the outcomes are focalized. This review seeks to explore and bring forth the available scientific evidence on the beneficial application of carotenoids as stress alleviators and immunostimulants in aquatic animals while elucidating possible mechanisms of action. More generally, the information expands our knowledge of the anti-stress and immunomodulatory functions of carotenoids. Opportunities and challenges are also addressed, along with implications for future research.

2 | STRUCTURE OF CAROTENOIDS

The properties and natural functions of a carotenoid molecule are fundamentally dependent on its molecular or chemical structure. A systematic scheme has been exercised to define and delineate the chemical structure of various carotenoids. Several hundred carotenoid structures have been classified from a wide assortment of biological systems. Most carotenoids typically contain a 40-carbon skeleton built from eight isoprene units that are covalently linked together, forming multiple conjugated double bonds (usually termed the 'polyene chain') (Figure 1). This general structure (polyene chain) may be cyclized at one or both ends of the molecule or possess various oxygen-containing functional groups or hydrogenation levels, resulting in structural diversity.[33,34] Structurally, carotenoids have different terminal groups at both ends of the principal polyene chain (Figure 2). The long central chain of conjugated double bonds is primarily responsible for the shape, coloration, chemical reactivity, spectral properties and proper functioning of carotenoids.[35–37] There exist two main groups of carotenoids: carotenes and xanthophylls (Figure 3a,b). Carotenes (e.g. α-carotene, β-carotene, γ-carotene, lycopene, neurosporene and phytoene) are polyunsaturated hydrocarbons composed exclusively of carbon and hydrogen. Contrarily, xanthophylls (e.g. astaxanthin, canthaxanthin, fucoxanthin, lutein, neoxanthin, violaxanthin and zeaxanthin) carry oxygen atoms in their structures (oxygenated carotenes). Xanthophylls are amongst the essential components in the photosynthetic tissues of plants. In nature, some xanthophylls are present as glycosides, fatty acid esters, protein complexes and sulfates.[13,38] Presently, approximately 50 types of carotenes and almost 800 types of xanthophylls have been described.[17,39]

Additionally, apocarotenoids are a subclass of carotenoids, or carotenoid derivatives, composing less than 40 carbon atoms derived from the oxidative cleavage of C40 carotenoids (i.e. carotenes and xanthophylls). Some carotenoids with 45 or 50 carbon atoms are collectively known as higher carotenoids. Nearly 120 naturally occurring apocarotenoids have been identified in plants and animals, while about 40 higher carotenoids are present in archaea.[17,39] Furthermore,

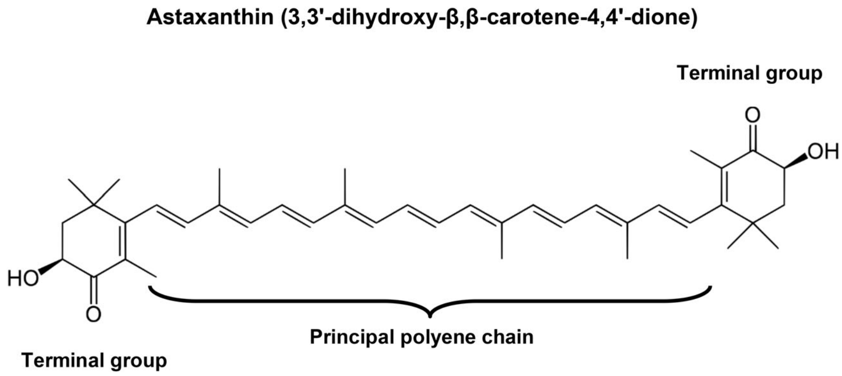

FIGURE 1 General structure of a selected carotenoid and its terminal groups.

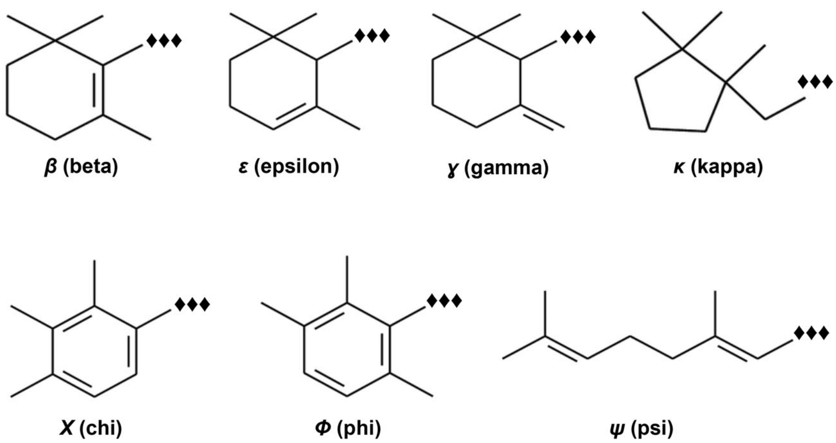

FIGURE 2 Various terminal groups present in carotenoids.

carotenoids can exist as cis (Z) or all-trans (all-E) isomers depending on the number of conjugated double bonds in their molecules, which differ markedly in shape and size.[40–42] In most cases, the all-trans configuration is more prevalent in natural sources due to its relatively higher stability, as the cis isomers of carotenoids are particularly vulnerable to oxidation. Carotenoids are lipophilic isoprenoid compounds (hydrophobic molecules) present typically in cell membranes and lipoproteins. The lipophilicity of carotenoids directly impacts their absorption, distribution and metabolism in living organisms.[13,36,43] Research on the structure and properties of carotenoids has been ongoing for many decades, and progress reflects advances in all areas of chemistry and biochemistry. The structural characteristics of carotenoids are the ultimate key to understanding their diverse functional roles and applications.

3 | SOURCES, BIOAVAILABILITY AND METABOLISM OF CAROTENOIDS

3.1 | Natural and synthetic sources

Experts have been actively investigating new biological sources of carotenoids due to the exceptional qualities of these fascinating molecules. Carotenoids are richly hued pigments that are ubiquitous and, in general, wholly accountable for the striking colours of many plants (including leaves, flowers, fruits and stems) and animals. More than 1000 structurally different carotenoids of natural origins have been isolated and characterized until recently.[17,44] Prominent sources of unique carotenoids are primarily represented by terrestrial and marine plants but also by numerous marine organisms and simple microorganisms (photosynthetic and non-photosynthetic), specifically archaea, bacteria, fungi and yeast, with complex carotenoid metabolism (Table 1).[45–47] The deposition of carotenoids in animal tissues truly reflects their dietary sources along the food chain, as they cannot biochemically synthesize these compounds de novo.[16,54] However, the contribution of animal-derived carotenoids (e.g. meat and dairy products, seafood and crustacean by-products) must not be disregarded, as these sources may provide a substantial quantity of important carotenoids (e.g. astaxanthin, canthaxanthin, zeaxanthin and lutein).[55,56] Naturally sourced carotenoids are gaining traction worldwide, directly driven by surging consumer demand and preference for natural products, along with end-use applications, particularly in the North American and European regions.[57,58] Consequently, global manufacturers have been actively pursuing the production and commercialization of natural carotenoids at a lucrative pace with new-age technologies.

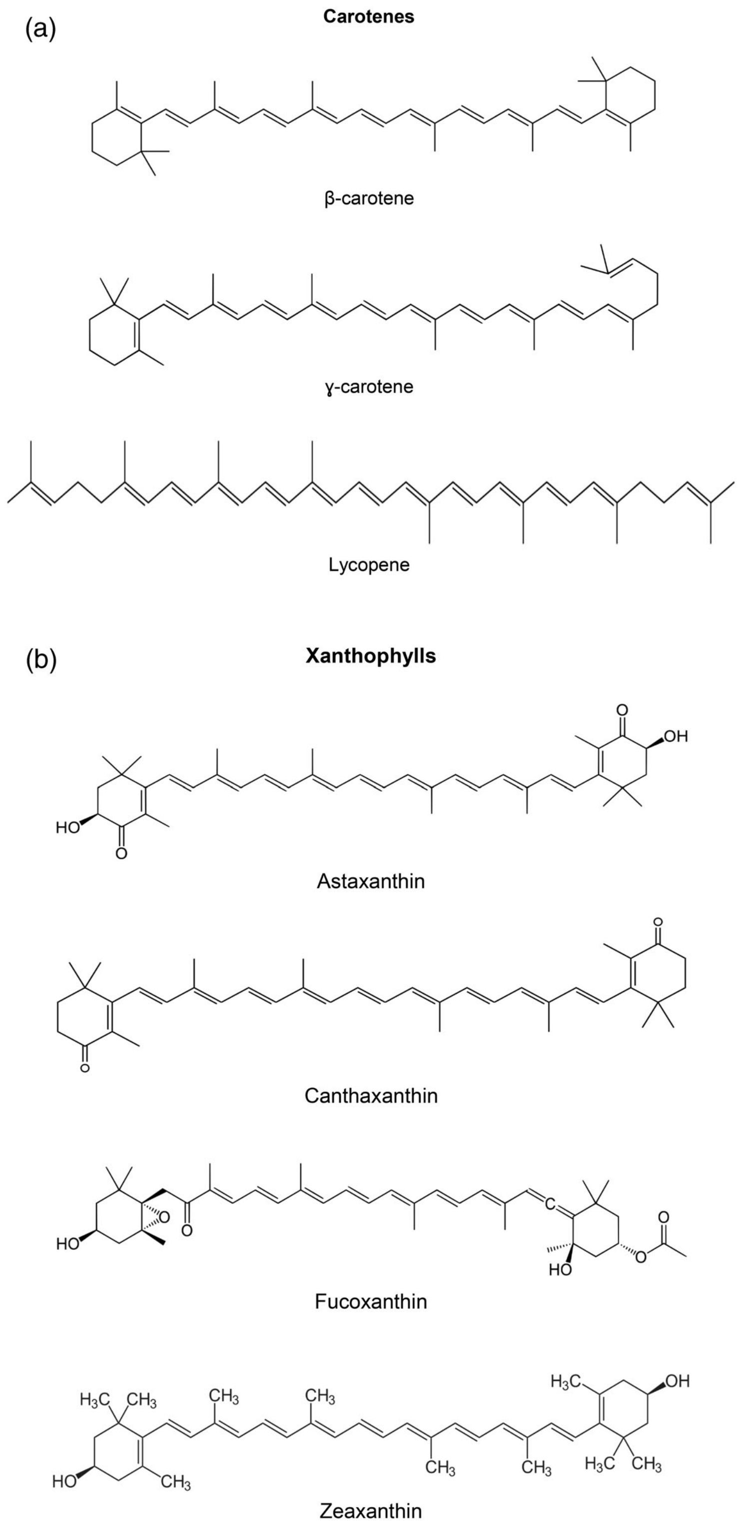

FIGURE 3 Chemical structures of (a) typical carotenes and (b) typical xanthophylls

Most commercialized carotenoids are synthetically derived from chemical sources (e.g. inorganic chemicals, organic acids and petrochemical-derived precursors) with inexpensive labour, negating the requirement of biological organisms and subsequent costs of harvesting and extraction.[16,59,60] Chemically synthesized carotenoids constitute 90% of the current global production, whereas natural counterparts account for the remaining 10%. Both the Dutch State Mines (DSM) and the Baden Aniline and Soda Factory (BASF) are the leading global manufacturers in the synthetic market, with more than 60% production. Synthetic carotenoids, nonetheless, exhibit less

TABLE 1 Common types and prominent sources of carotenoids

Pigment	Colour	Source	References
Astaxanthin	Pink-red	Crustacean, microalgae and yeast	16
α-carotene	Orange	Terrestrial plants	48
β-carotene	Yellow-orange	Cyanobacteria, microalgae and terrestrial plants	49
Canthaxanthin	Orange-red	Crustacean, microalgae, archaea, bacteria and fungi	8
Fucoxanthin	Brown	Diatoms, brown macro- and microalgae	50
Lutein	Yellow-orange	Terrestrial plants	51
Lycopene	Red	Terrestrial plants	52
Zeaxanthin	Yellow	Terrestrial plants	53

efficacy in terms of their biological functions and therapeutic properties, despite a speedier production at considerably lower costs.[57,61–63] The market price of microalgal-derived pigments, for instance, could fetch up to USD 7500 kg^{-1}, while synthetic equivalents may be proportionately cheaper at half the price (approximately USD 250–3000 kg^{-1}).[64,65] Secondary carotenoids, such as canthaxanthin and astaxanthin, are amongst the priciest pigments on the market. Chemical synthesis for the production of synthetic carotenoids on an industrial scale involves a variety of generally complex reaction processes (i.e. dehydration and elimination, carbonyl condensation, Wittig reaction and the selective cross-coupling reaction) that often induce the formation of hazardous wastes.[57,66,67] Such wastes could potentially pose a severe threat to human and environmental health upon indiscriminate disposal. The synthetic forms of carotenoids, with the potential carryover of synthesis intermediates or impurities, are only permitted for commercial applications in the animal feed industry (aquatic animals and poultry) and have not been approved exclusively for direct human consumption thus far.[65,68,69] These undesired adverse effects have reverberated in many discussions, which raised public concerns associated with environmental pollution, food safety (inherent toxicity) and sustainability, even though chemical synthesis can supply a steady source of carotenoids.[57,64,70] Hence, natural carotenoids represent a much favoured and premium option for animal nutrition or direct human consumption.

The global market has witnessed remarkable growth in the production of carotenoids to cater to the extraordinarily high demands from multiple industries comprising food, cosmetics, aquaculture, nutraceutical and pharmaceutical, over the last decade. Carotenoids are of great dietary importance and perform a versatile role in contributing to therapeutic effects and health benefits with myriad applications. These include anti-inflammatory, immune system boosting, anti-cancer, anti-ageing, sun proofing, anti-diabetic activities and amongst others, owing to their impressive antioxidant capacity.[16,71,72] Moreover, scientific efforts devoted through the years persistently substantiated the instrumental role of carotenoids in promoting animal health (e.g. various improvements in stress tolerance, innate immunity, immune-related gene expression and disease resistance).[19,22,28,73,74] The future of the global carotenoid market appears promising, which is likely to expand steadily from USD 1.5 billion in 2020 to USD 2 billion by the end of 2026, registering a compound annual growth rate (CAGR) of 4.2% during the forecast period.[75] Amongst the available carotenoids, the market is dominated by astaxanthin, shared fairly by β-carotene, lutein, lycopene and zeaxanthin, in light of their established functions. Some carotenoids, including canthaxanthin and fucoxanthin, are beginning to penetrate the market with the potential scope for economic importance due to recent scientific discoveries on their pharmacological effects and health benefits.[63,75] Most carotenoid producers are currently emphasizing sustained growth over the long-term future, as the impacts of the unprecedented COVID-19 pandemic crisis are being felt globally across operations in multiple dimensions. Despite that, the carotenoid industry can play a significant role as the driving force of economic recovery and is most anticipated to witness potential opportunities over the short-term period. The market continues to build momentum with biotechnological advancements and the ever-expanding demand for carotenoids in the healthcare industry and animal feed production.

3.2 | Bioavailability and metabolic aspects

Humans and animals are incapable of biosynthesizing carotenoids, and, hence, these pigments must be acquired exclusively from dietary sources. The bioavailability of carotenoids merely refers to the proportion of consumed carotenoids that enters the systemic circulation in their active forms to be readily absorbed and utilized by the host body. Bioavailability is an intricate process involving separate phases, including digestion (liberation), gastrointestinal absorption, transport, tissue distribution, metabolism and bioactivity.[76,77] Conversely, the bioaccessibility of carotenoids has been described as the fraction or portion of the ingested carotenoids that are released from the food matrix and thus become potentially available for gastrointestinal absorption.[77,78] Bioaccessibility is part and parcel of bioavailability, and both increase simultaneously. The latter, in particular, serves as a key concept for assessing the bioactivities and health benefits of carotenoids. The bioavailability of carotenoids is complicated by multiple factors that influence their digestion, absorption, transport and storage in aquatic animals. Several factors include but are not limited to the following: type of carotenoid and their origin (synthetic or natural), the interaction between carotenoids, dietary source (natural or formulated diet), as well as the dose and route of administration.[79,80]

Specific efforts have been made over the years within these scenarios in aquatic species to mimic identical strategies established to explore carotenoid bioavailability in mammals.[81–84] Nevertheless, data are not adequately comparable to enable a comprehensive systematic comparison of outcomes. Beyond simple consideration, exogenous components linked to the food matrix and structure and how the food is processed are influential in dictating the bioavailability of carotenoids. Carotenoids ought to be liberated from the food matrix in absorbable forms and transferred into the systemic circulation to exert their biological activities after ingestion. Delivery strategies, namely nanoemulsion and nanoencapsulation systems, offer great avenues to bypass the limiting factors on the bioavailability of carotenoids, usually achieving a great deal of improvement in absorption.[77,85–87] The expeditious development of sophisticated techniques, such as the stable isotope dilution method, permits an accurate yet precise assessment of carotenoid activities.[88,89] Comprehending the bioavailability of carotenoids delivers a solid foundation for an effective design strategy and critical interpretation of dietary interventions in aquatic animals. In defining future approaches, it is imperative to grasp the underlying mechanisms by which the bioavailability of carotenoids can be altered and leveraged for enhanced health benefits.

Carotenoids are substantially hydrophobic and lipid-soluble metabolites, bound solely to circulating plasma lipoproteins, alongside animal fats and cholesterol, determined in large part by their structure and physicochemical properties.[36,43] Hence, as with their absorption, carotenoids pursue an identical absorptive pathway to other lipid components. Poor solubility in the aqueous milieu of the gastrointestinal tract impedes their absorption nonetheless.[77,90] The association with dietary lipids (e.g. cholesterol and fatty acids) has been documented to promote digestion, intestinal absorption and metabolism of carotenoids in animals,[16,91,92] particularly so in humans.[76,77] Although mammals and other non-mammalian vertebrates typically absorb and deposit carotenoids in their body tissues, many crucial aspects of metabolism remain unresolved. Various research unravelled wide variations in the absorption and metabolism of carotenoids from one aquatic species to another, while some are not absorbed in any way. Such disparities can be explained by the properties of the carotenoid in question (e.g. polarity, hydrophobicity and isomerism) and also host factors (e.g. species, growth stage, diet and genetics).[16,83,93–95] The differential distribution in terms of specific tissue uptake of carotenoids from the plasma and retention, along with the governing selective mechanisms, has not been clarified until the present. New investigations evidenced the intricacy of endogenous bioconversion and deposition of carotenoids in aquatic animals, which for the most part, was related to structural isomerism.[95,96] It was not until recently that researchers began to characterize and elucidate the diverse range of genes and pathways involved in the metabolism and deposition of exogenously derived carotenoids.[90,97–99] Exciting varieties of enzymes and transporter molecules linked to carotenoid-based integumentary colouration in fish were suggested to be genetically controlled, analogous to findings in mammals and avians.[94,100–102] Accordingly, the main regulators in the molecular mechanisms of carotenoid utilization and storage amongst living animals can be potentially enhanced through genetic improvement.[103–105] Several articles thus far defined the fundamental mechanisms of absorption, transport, tissue uptake and metabolism of this broad family of molecules in some aquatic species and humans.[16,43,79,106,107] The colourful world of carotenoids inspired classic research on their metabolic aspects in aquaculture species. Surprisingly, experimental studies are somewhat scarce, as a whole, with only a handful on salmonids[16,82,92,108] and some crustaceans.[95,96,109] The physiological relevance of such studies still needs to be entirely established for a wide variety of domesticated species. Knowledge gaps, however, still exist concerning the metabolic fate of carotenoids and their potential to influence biological processes while serving as modulators of disease in aquatic animals. The continuous development of assessment techniques (e.g. molecular and structural biology), robust analytical approaches for identification and quantification (e.g. protein, gene and metabolite levels), as well as the adoption of ideal animal models for thorough mechanistic investigations, are required to extend our profound understanding of the subject.

4 | SAFETY ASPECTS OF CAROTENOIDS

Aquaculture stands as one of the most environmentally sustainable alternatives for food security and human nutrition. Good nutrition is the foundation of aquaculture production systems and is imperative to the economical production of healthy, top-quality aquatic products. Supporting evidence has emerged concerning the biological functions of carotenoids in aquatic animals, which are necessary to ensure optimal development, reproductive success and excellent health.[18,24,110] Most importantly, the desired pigmentation rendered by carotenoids as feed additives contributes to characteristic quality criteria for marketing aquaculture products and satisfying consumer demand and acceptability. Safety and regulatory requirements of carotenoids vary in different countries and regions, being governed by a region-specific authority. The safety of carotenoids in animal nutrition, along the feed and food chain, is systematically evaluated by regulatory agencies, including the Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA), ensuring they are innocuous before being permitted for use.[56,63] The use of such additives is held under constant observation and is subject to reassessment by the agencies should the need arises.[111,112] These safety evaluations lead to the publication of scientific opinions (publicly available at https://www.efsa.europa.eu and https://www.fda.gov) defining the safety aspects of the pigments (i.e. risks to target species, consumers and the environment) under their proposed conditions of use, including under certain circumstances the acceptable intake.

A series of scientific opinions dealing with the safety and efficacy of several carotenoids (i.e. astaxanthin, β-carotene, canthaxanthin and adonirubin) have been previously delivered by the EFSA's Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) for crustaceans and salmonids, as well as other fishes.[111,113–117] Based on the former assessments, the FEEDAP Panel declared that the evaluated carotenoids are safe and efficacious as pigmenting agents in aquatic species (up to 100 mg kg^{-1} diet). The panel further testified that the proposed use of the pigments is of no particular concern for the health and safety of consumers nor constitutes any additional unintended or harmful ecological risks. Notwithstanding, the bulk of experimental data from various feeding trials through recent years afforded solid evidence that dietary carotenoids are generally innoxious and well-tolerated by many aquatic species, even at larger doses as purified supplements. Historically, carotenoids have been incorporated into animal nutrition for decades without any reports of adverse effects on animal performance and health. Contrariwise, the lack of adequate pigments may negatively influence their general performance. These were incisively demonstrated in numerous commercially important species such as the kuruma shrimp, *Marsupenaeus japonicus*,[118,119] oriental river prawn, *Macrobrachium nipponense*,[120] ridgetail white prawn, *Exopalaemon carinicauda*,[121] Asian seabass, *Lates calcarifer*,[11,19,22] Atlantic cod, *Gadus morhua*,[122] blood parrot cichlid, *Cichlasoma citrinellum* x *Cichlasoma synspilum*,[123] rainbow trout, *Oncorhynchus mykiss*,[73,124] red porgy, *Pagrus pagrus*,[125,126] and yellow catfish, *Pelteobagrus fulvidraco*,[20] supplemented with individual or a blend of carotenoids in vivo (up to 400 and 600 mg kg^{-1} diet in fish and crustaceans, respectively). Some investigators, nonetheless, emphasized that carotenoid residues in animal flesh could represent a prominent source of carotenoids for promoting human health; as an ideal alternative to dietary supplements.[127–129] Acute and sub-chronic toxicity studies of some carotenoids (i.e. astaxanthin, β-carotene, fucoxanthin and lutein) performed in rodents had consistently reported the absence of relevant behavioural alterations, systemic toxicity and mortality through detailed clinical observations (administered gavagely up to a daily dose of 2000 mg kg^{-1} body weight).[130–135] Several investigations also raised no specific concerns for teratogenic and genotoxic potentials of carotenoids (i.e. astaxanthin and zeaxanthin) that could lead to congenital abnormalities in rodents (administered orally up to a daily dose of 750 mg kg^{-1} body weight).[133,134,136] Similarly, no deleterious side effects of carotenoids have been identified thus far in healthy human subjects when provided sufficiently, which strongly supports the safety profile of the pigments for epidemiological studies and clinical trials.[72,137–139] For instance, a review on the safe dosing of astaxanthin has been conducted earlier from 87 human intervention studies (with oral doses ranging from 12 to 45 mg day^{-1}), and none of which found safety concerns.[140] Feng et al.[141] through meta-analysis, suggested that lutein supplementation is safe in humans (10–20 mg day^{-1}) and may be beneficial to reduce the risks of age-related macular degeneration (AMD). Moreover, another study indicated no apparent detrimental health effects in men upon oral administration of 25 mg lycopene day^{-1} for 12 weeks through a randomized, double-blind, placebo-controlled trial.[142] Much information manifested that dietary carotenoids exert protective actions against atherosclerotic cardiovascular diseases (CVDs), cancer, chronic degenerative diseases, cognitive impairments, rheumatoid arthritis, ultraviolet B-induced erythema and depressive disorders in humans via their potential as a therapeutic agent to inhibit oxidative stress.[138,143,144] Pharmacological and mechanistic data had most recently confirmed the bright future of carotenoids (i.e. astaxanthin and fucoxanthin) in alleviating the complications associated with COVID-19.[145,146] Given the above, it is indisputable that an adequate intake of carotenoids has been found safe in animals and human subjects without any identifiable negative consequences while offering multiple health benefits.

5 | BIOACTIVE CAROTENOIDS AS ANTIOXIDANTS

Carotenoids are amongst nature's most potent biological antioxidants, assuming a central role in neutralizing the harmful effects of ROS, thus prohibiting oxidative damage related to all living organisms. These bioactive molecules may act directly from scavenging free radicals (e.g. $OH^{\bullet}$, $O_2^{\bullet-}$, $HO_2^{\bullet}$, $HOO^{\bullet}$ and $ROO^{\bullet}$) to promoting oxidative stress defences or resistance in the host at all levels of complexity.[45,147–149] Antioxidative attributes of carotenoids are principally governed by the extensive system of conjugated double (C=C) bonds in their polyene chains.[150–152] The structural feature of conjugated double bonds leads to a high reducing potential and antioxidant activity of these tetraterpene compounds.[153,154] Any structural modifications, such as the number of conjugated double bonds alongside the addition of oxygen-containing functional groups, could altogether alter the chemical reactivity (i.e. quenching capability and antioxidant capacity) of a carotenoid molecule.[15,155] The antioxidative function of carotenoids is also dependent on their immediate environment and the nature of the oxidizing free radicals.[35,156] There are several mechanisms of carotenoid action as an antioxidant, including: (i) serving as efficient physical quenchers of excited singlet molecular oxygen ($^1O_2$) (Equations 1 and 2)[157–160]; (ii) reacting rapidly with free radicals of different origins and convert them into more stable compounds or non-radical products (Equations 3–6)[161–164]; (iii) preventing the formation of free radicals through the interruption of free radical-induced chain reactions and terminating free radical oxidations[165–167]; and (iv) acting as metal chelators by facilitating the conversion of iron and copper derivatives into stable chelate complexes (harmless molecules).[168–170] Mechanisms of carotenoid action as physical quenchers of singlet molecular oxygen and free radical scavengers are represented by the followings:

- Physical quenching of $^1O_2$ by a carotenoid (Crt) molecule that involves the removal of excitation energy ensued by thermal deactivation

$$\mathrm{Crt} + {}^1O_2 \rightarrow {}^3\mathrm{Crt}^* + {}^3O_2 \quad (1)$$

$$ {}^3\mathrm{Crt}^* \rightarrow \mathrm{Crt} + \mathrm{heat} \quad (2)$$

- Electron transfer reaction between free radicals ($R^{\bullet}$) and Crt leading to the formation of a carotenoid radical anion ($\mathrm{Crt}^{\bullet-}$) or cation ($\mathrm{Crt}^{\bullet+}$)

$$\mathrm{Crt} + \mathrm{R}^{\bullet} \rightarrow \mathrm{Crt}^{\bullet -} + \mathrm{R}^{+} \quad (3)$$

$$\mathrm{Crt} + \mathrm{R}^{\bullet} \rightarrow \mathrm{Crt}^{\bullet +} + \mathrm{R}^{-} \quad (4)$$

- The mechanism of radical adduct formation (RCrt$^{\bullet}$)

$$\mathrm{Crt} + \mathrm{R}^{\bullet} \rightarrow \mathrm{RCrt}^{\bullet} \quad (5)$$

- Hydrogen atom transfer or abstraction resulting in the formation of a neutral Crt radical (Crt$^{\bullet}$)

$$\mathrm{Crt} + \mathrm{R}^{\bullet} \rightarrow \mathrm{RH} + \mathrm{Crt}^{\bullet} \quad (6)$$

Numerous investigations through the years have focused on the antioxidant properties of carotenoids as potent $^1O_2$ quenchers and free radical scavengers with reference to their molecular structures.[37,171] The availability of novel carotenoids isolated from various plants and microorganisms facilitated the continuous development of carotenoid research to define their functions, both in vivo and in vitro. Much progress has been made in the genetic manipulation of carotenoid biosynthesis and composition within organisms, which offers excellent opportunities for studying the antioxidant roles of carotenoids and their actions directly in living cells.[172–176] Notwithstanding, there are still many other biochemical and physiological functions to be linked to carotenoids owing to their structural diversity.

6 | IMMUNITY IN FISH AND AQUATIC INVERTEBRATES

The immune system is an interconnected network of broadly distributed biological processes of splendid complexity that safeguard an organism against infections.[177,178] On the whole, there is a heightened interest in comprehending the immune functions of aquatic animals to develop and protect both wild and farmed populations sustainably. A deeper understanding of these immune processes is paramount for the betterment of aquaculture practices and the prevention of aquatic diseases in commercial species. Profound insights into the immune responses of aquatic animals accelerated the novel discovery and development of various prophylactic and therapeutic solutions, leading the way to robust immunization and immunomodulation strategies.[179–181] Moreover, marked advances in genetic and genomic sciences have fostered new progressions within veterinary immunology.[182–184]

Conceptually, all aquatic animals are endowed with a primitive system of defence, which is further classified into innate (non-specific) and adaptive (specific or acquired) immunity beyond structural and chemical barriers to pathogens. Constitutive mechanisms of innate immunity provide the front line of host defence that inhibits the entry or annihilates infiltrating pathogens almost instantly in a non-specific manner. Innate immunity is well elucidated as a primitive form of host defence against pathogenic invasion and functions as a fundamental defence mechanism.[185,186] Unequivocally, the innate immune system plays an instructive role in activating and determining the nature of its adaptive counterparts.[187,188] The adaptive immune responses, in contrast, are tailored to repel specific or unique non-self pathogens (antigen-specific recognition) while acting as the second line of host defence.[189,190] Both overlapping immunological mechanisms occur via the collective interaction of the cellular and humoral components. The adaptive immune system establishes immunological memory after a previous initial exposure or response to a specific pathogen, thus delivering enhanced responsiveness and long-term protection against recurrent infections. Nonetheless, the capacity for immunological memory is classically believed to be less complex in the innate defence mechanism, which has been much debated in recent years.[191–193] Together, the innate and adaptive immune systems orchestrate an extremely close communication to improve antimicrobial host defence, despite distinct differences in their mechanisms and properties. Much of the previous work has focused on the developmental aspects of the immune systems in fish and aquatic invertebrates.[194–196] Noteworthy progress has been achieved in the characterization of diverse immune mechanisms and pathways.[195,197–199] However, knowledge gaps in numerous immune mechanisms, including cellular and molecular basis, of aquatic animals persist, and the available information differs according to species. The complete development of immunology as a scientific discipline remains equitably incorporated in both fundamental and clinical research.

Teleosts are by far the most speciose (boasting almost 30,000 species) of any living vertebrates today, which have conceivably undergone rapid adaptive radiations in the evolutionary emergence of vertebrates, primarily driven by ecological and morphological disparity.[200,201] Enhanced knowledge of fish immune systems heralded comparative outgroups for understanding the immune system of higher vertebrates over evolutionary time.[202,203] In general terms, the structural and functional organization of the piscine immune system is physiologically analogous to those in other vertebrates, albeit less evolved and with some discrepancies (mainly due to body compartments, cellular organization and poikilothermic nature).[202,204] Teleosts have all the vital organs dedicated to immune defence, while regarded as the earliest class of vertebrates with all known essential elements of innate and adaptive immunity, though with some specializations and unique attributes.[180,205] Fish are capable of deploying effective host defence mechanisms through early embryonic development. The innate immune system of teleosts is broadly separated into three compartments composing the mucosa-associated lymphoid tissues (gills, skin and gut) (local barriers), as well as cellular and humoral repertoires (systemic responses).[185,186] Cellular components of the innate immune system of teleosts consist of assorted cell types, including granulocytes, monocytes, natural killer cells and dendritic cells, engineered to facilitate cell-mediated immunity. Moreover, humoral factors comprise mainly natural antibodies, antimicrobial peptides

(AMPs), chemokines, cytokines, lytic enzymes and various components of the complement pathways, amongst others.[186,206] The innate immunity in teleosts is served by a restricted number of germ-line encoded pattern recognition receptors (PRRs) that identify pathogen-associated molecular patterns (PAMPs) (i.e. prevalent biomolecules of pathogens, such as lipopolysaccharides, glycoproteins, peptidoglycans and single- or double-stranded RNA) while detecting endogenous danger signals from malignant tissues or apoptotic cells.[207,208] Adaptive immune responses of teleosts, on the other hand, are firmly regulated by the crosstalk mechanisms between cellular and humoral responses that involve an intricate network of highly specialized systemic cells, biochemical messages, proteins and genes. The adaptive immune system mounts a full-fledged response through the generation of T-cell receptors (TCRs), major histocompatibility complex (MHC), immunoglobulins (Igs), as well as B and T lymphocytes.[185,189] These components are highly specific to the antigens of foreign pathogens. The proliferation and activation of B and T lymphocytes are crucial factors that trigger the initiation of the adaptive immune system. Nevertheless, the initiation of adaptive immunity is commonly delayed due to requirements for cellular proliferation, protein synthesis and specific receptor selection but is assuredly effective for long-lasting immunity. Comprehensive descriptions of teleost immunity are available in past literature devoted completely to surveying all facets of fish immunology.[186,203,209,210] There is an expeditious expansion of studies employing fish models in comparative immunology to improve the understanding of fish immunity and the evolution of immune systems. Significant contributions from molecular genomic research and integrations of multi-omics strategies to identify previously unrecognized functions and unresolved paradigms for piscine immune reactions can be anticipated henceforth.

Contrariwise, invertebrates respond, whether local or systemic, through a non-adaptive innate immunity. Invertebrates possess inarguably less developed adaptive immune responses as they depend more readily on their potent and rather complex innate immune system to contain invading pathogens.[24,211] Generally, a multitude of cellular and humoral responses partake in defending these organisms from pathogenic agents that successfully compromised their exoskeletons, gastrointestinal tracts and internal tissues.[212] A plethora of information is available on the extent of the involvement of distinct cell types in invertebrate defence reactions.[196,211] Typically, the cellular components of the invertebrate immune system are represented by an assembly of freely circulating and sessile blood cells (coelomocytes and hemocytes) that are solely responsible for cell-mediated immunity (i.e. phagocytosis, melanization or encapsulation, generation of reactive oxygen species, as well as the production of antimicrobial peptides and hydrolytic enzymes).[213,214] The sessile phagocytic cells may be scattered throughout body tissues or localized in haematopoietic organs. In humoral responses, invertebrates utilize an assortment of soluble factors or effector molecules secreted by the cellular components, such as AMPs, agglutinins, lectins, complement factors and some hydrolytic enzymes.[215,216] These factors act in synergy with phagocytes to exterminate noxious microorganisms and other foreign agents that have intruded into the host. Recent molecular and genomic studies uncovered astonishingly diversified immune molecules and PRRs.[198,207,217,218] Most aquatic invertebrates have well-developed alternative approaches to initiate complex immune processes, although lacking a great deal of the prominent features of vertebrate immunity.[219,220] Consequently, invertebrates can adapt and thrive in a wide variety of ecosystems and, in some instances, under extreme conditions. Apprehending the broad armamentarium of invertebrate body defences is vitally important as these animals continue to confront all sorts of challenges to self-integrity from their pathogen-laden habitats. Research interests in the immune system of invertebrates have been somewhat astounding over the past decade, spurred entirely by their economic significance in global aquaculture and phylogenetic position, as well as evolutionary history.

7 | BIOACTIVITY AND RELATED MECHANISMS OF CAROTENOIDS ON THE STRESS TOLERANCE AND IMMUNITY OF AQUATIC ANIMALS

7.1 | Robust modulation of the antioxidant system

The antioxidant defence or antioxidative system is a multilevel and complex network that counteracts and regulates endogenous cytotoxic ROS, which is deemed paramount in the life activities of aquatic animals. All animals subsisted in ecologically diverse environments, confronted with multiple stressors, by altering their physiological attributes, specifically antioxidant systems.[221,222] The primary enzymatic components (antioxidant enzymes) of the antioxidant defence grid comprise catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD).[223,224] These molecules collectively work against superoxide radicals: reduced into hydrogen peroxide by SOD and further converted into water and molecular oxygen by CAT and GPx.[24,225] Structural and functional features of antioxidant enzymes appear exceptionally well-conserved across aquatic vertebrates and invertebrates.[24] Reactive oxygen species are physiologically relevant molecules perpetually generated by living cells as normal cellular metabolic byproducts of oxidative metabolism. Strictly speaking, ROS perform essential roles in host immune responses, gene transcription and signal transduction.[28,226] The fine-tune of ROS homeostasis is ensured by the enzymatic mechanism of the antioxidant system during physiological aberrations. Nevertheless, the excessive proliferation of ROS induced by oxidative stress can cause pathological damage to tissues and organs while irreversibly impairing DNA, proteins and lipids, with adverse consequences on host cellular functions if homeostasis is not restored.[221,227] Chronic oxidative stress frequently leads to an elevated risk of negative health outcomes and the prevalence of animal diseases. In-depth studies on antioxidant activity ushered in new lines of research in aquatic physiology, forging novel scientific knowledge that benefits numerous aspects of animal husbandry and artificial propagation.

TABLE 2 Effects of carotenoids on antioxidant defence systems in various aquatic species

Carotenoid	Source and inclusion level	Species	Response	References
Astaxanthin	Synthetic; 50–100 mg kg^{-1} diet	Rainbow trout (*Oncorhynchus mykiss*)	↑ total antioxidant capacity; ↓ activities of CAT and SOD	228
	Natural; 400 mg kg^{-1} diet	Blood parrot cichlid (*Cichlasoma citrinellum* × *Cichlasoma synspilum*)	↑ total antioxidant capacity; ↓ activities of CAT and SOD	123
	Synthetic; 25–50 mg kg^{-1} diet	Large yellow croaker (*Larimichthys crocea*)	↑ total antioxidant capacity; ↓ activity of SOD	229
	Synthetic; 50–200 mg kg^{-1} diet	Northern snakehead (*Channa argus*)	↑ activities of CAT, GPx and SOD	230
	Natural; 91 mg kg^{-1} diet	Leopard coral trout (*Plectropomus leopardus*)	↑ total antioxidant capacity; ↑ activities of CAT, GPx and SOD	231
	Synthetic; 30–60 mg kg^{-1} diet	Swimming crab (*Portunus trituberculatus*)	↓ activities of CAT and SOD	232
	Natural; 68 mg kg^{-1} diet	Chinese mitten crab (*Eriocheir sinensis*)	↓ total antioxidant capacity; ↓ activity of SOD	233
	Synthetic; 160 mg kg^{-1} diet	Pacific white shrimp (*Litopenaeus vannamei*)	↑ total antioxidant capacity; ↑ activity of SOD	234
	Synthetic; 40–160 mg kg^{-1} diet	Pacific white shrimp (*Litopenaeus vannamei*)	↑ total antioxidant capacity; ↓ activities of CAT and SOD	32
β-carotene	Natural; 105 mg kg^{-1} diet	Pacu (*Piaractus mesopotamicus*)	↓ activities of CAT and SOD	235
	Natural; 150–200 mg kg^{-1} diet	Yellow catfish (*Pelteobagrus fulvidraco*)	↑ total antioxidant capacity; ↑ activities of CAT and SOD	20
	Natural; 50 mg kg^{-1} diet	Nile tilapia (*Oreochromis niloticus*)	↑ total antioxidant capacity; ↑ activities of CAT, GPx and SOD	30
	Synthetic; 100–500 mg kg^{-1} diet	Pacific white shrimp (*Litopenaeus vannamei*)	↑ total antioxidant capacity; ↑ activity of SOD	236
Canthaxanthin	Synthetic; 25 mg kg^{-1} diet	Rainbow trout (*Oncorhynchus mykiss*)	↑ activities of GPx and SOD	73
	Synthetic; 100–120 mg kg^{-1} diet	Chinese soft-shelled turtle (*Pelodiscus sinensis*)	↑ total antioxidant capacity; ↑ RNA expression levels of antioxidant genes (CAT and SOD2)	237
	Synthetic; 50–400 mg kg^{-1} diet	Pacific white shrimp (*Litopenaeus vannamei*)	↑ total antioxidant capacity; ↓ activities of CAT and GPx	238
Lutein	Natural; 100 mg kg^{-1} diet	Marbled spinefoot rabbitfish (*Siganus rivulatus*)	↑ total antioxidant capacity; ↑ activities of CAT and GPx	239
	Natural; 50–200 mg kg^{-1} diet	Oriental river prawn (*Macrobrachium nipponense*)	↑ total antioxidant capacity; ↓ activities of CAT and SOD	120
	Natural; 62.5–75 mg kg^{-1} diet	Pacific white shrimp (*Litopenaeus vannamei*)	↓ total antioxidant capacity; ↓ RNA expression levels of antioxidant genes (CAT and GPx)	29

Abbreviations: CAT, catalase; GPx, glutathione peroxidase; RNA, ribonucleic acid; SOD, superoxide dismutase; SOD2, superoxide dismutase 2.

Comparative investigations established the dual role of carotenoids in antioxidant defence systems of various aquatic species, i.e. as potent ROS scavengers while also exerting specific stimulatory effects on the activities of enzymatic antioxidants (Table 2). One primary role is explicitly associated with their antioxidant capacities to directly scavenge ROS, thus averting a dynamic imbalance between pro- and anti-inflammatory status.[18] This balance is crucial for regulating oxidative stress, predominantly linked to inflammatory responses. Accordingly, carotenoids could effectively assume the function of antioxidant enzymes (as a compensatory response to ROS) whilst reducing their relative activities and physiological costs of production. The synthesis of antioxidant enzymes, including each enzyme-catalyse event, typically requires investments from anabolic and catabolic processes within hosts (e.g. escalated amino acid assimilation and metabolic rate).[240,241] Such additional resources in this regard are allocable to other competing physiological processes (primarily immune activation). Compelling evidence revealed a reduced need for endogenous antioxidant enzymes in different fishes when supplemented with dietary carotenoids, but at the same time, enhancing their overall antioxidant status.[123,228,229,235] These authors proposed that carotenoids are comparatively more potent free radical quenchers than antioxidant enzymes and, as such, superseded their functional importance. The results were consistent with recent discoveries in several crustaceans, in which dietary carotenoids improved their total antioxidant status while lowering the activities of CAT, GPx and SOD.[32,120,232,238] Correspondingly, the RNA expression levels of antioxidant genes (CAT, GPx and SOD) were observed to be considerably lower in the hepatopancreas of the Pacific white shrimp (*Litopenaeus vannamei*) when fed lutein-supplemented diets (62.5–75 mg kg^{-1}) up to 56 days.[29] Moreover, in some instances, carotenoids can potentially diminish oxidative stress by swiftly boosting the levels and actions of endogenous antioxidant enzymes. Several available reports contradictorily documented the prominent role of carotenoids in rapidly encouraging the activities of endogenous antioxidant enzymes of some fishes and crustaceans, which resulted in much enhanced antioxidant capacity.[20,30,73,230,231,234,236,239] Wang et al.[237] likewise reported an induced antioxidant capacity and upregulated expression of antioxidant genes (CAT and SOD2) in the canthaxanthin-fed soft-shelled turtle (*Pelodiscus sinensis*). In this context, it should be reasonably fair to infer that the interactions between carotenoids and antioxidant enzymes might be, to a large extent, influenced by environmental or pathological conditions, together with other physiological aspects, but this warrants clarification. Babin et al.[23] had earlier described the interaction of supplemental carotenoids with antioxidant enzymes in an amphipod (*Gammarus pulex*) while emphasizing the potential significance of carotenoids in the evolution of antioxidant mechanisms of the crustacean. Therefore, in both ways, supplemented carotenoids grant immeasurable benefits to the antioxidant defence systems of aquatic animals. This is particularly pertinent for animals reserving immense quantities of carotenoids in their body tissues. A robust antioxidant status generally translates into a stronger host immunity, as both are closely interrelated. Given the manifestation of a specific interplay between carotenoids and endogenous antioxidant enzymes, it would be insightful to delve further into the synergistic influences of multiple carotenoids and the fundamental molecular mechanisms accountable for the interactions, including explicit functional collaboration with immunity.

7.2 | Induction of stress tolerance

Stress responses compose coordinated suites of biological and physiological processes to any perceived threats that disrupt homeostasis. These responses are generally adaptive, but prolonged exposure to stress (chronic) impairs the health and welfare of animals, predisposing them to severe consequences, such as poor growth performance, weakened immune system, susceptibility to infectious diseases and eventual mortality.[242,243] Intensive aquaculture practices under artificial settings or conditions have witnessed an insurgence of multiple environmental and husbandry stressors (e.g. biological, physical, chemical and procedural). Thus, it appears necessary to recognize what induces stress in aquatic animals, particularly concerning physiological mechanisms and responses (molecular and cellular), which steers to the transformation in behavioural characteristics, metabolism and immune functions. Whilst stress factors are not entirely avoidable, optimizing the stress tolerance of cultured species (through dietary interventions) is of foremost importance to ensure sustainable production.

Reactive oxygen species are constantly generated and annihilated in all animals during normal physiological processes, usually in a steady-state (dynamic equilibrium). Stressful conditions lead to the excessive production of ROS (oxidative stress), causing progressive oxidative damage, lipid peroxidation and subsequent cell death.[221,227] Much empirical research demonstrated the critical function of carotenoids in augmenting the stress tolerance of aquatic animals (Table 3). Existing data indicated enhanced resistance to oxygen-depleted conditions,[32,238] osmotic shock,[118,245,246] acute crowding,[244] exposure to toxic ammonia,[21,32] lipopolysaccharide-induced oxidative stress,[230] as well as temperature and pH fluctuations[20,233] amongst aquatic vertebrates and invertebrates associated with the supplementation of carotenoids. Nevertheless, more attention was devoted to astaxanthin due to its popularity amongst researchers and producers alike as one of nature's most potent and sought-after antioxidants. As elaborated earlier, the distinctive role of carotenoids in exterminating harmful ROS improves the overall function of the integrated antioxidant system. Notably, carotenoids were also discovered to directly modulate the mRNA expression levels of various other essential mitochondrial-localized stress and antioxidative-related proteins (e.g. glutamate dehydrogenase [GDH], glutamine synthetase [GS], glutathione-S-transferase [GST], heat shock protein 70 [HSP70], hypoxia-inducible factor-1α [HIF-1α] and manganese superoxide dismutase [MnSOD]), apart from the primary endogenous enzymatic antioxidants (i.e. CAT, GPx and SOD). These biological molecules are vital in defying oxidative stress and sustaining the reducing environment of the cell.[20,32] Intracellular signal transduction pathways are often influenced by carotenoids in response to circulating ROS and the degree of oxidative stress, rendering modulations in the expression of antioxidant genes.[18,247] A recent investigation showed that *L. vannamei* supplemented with astaxanthin (40–160 mg kg^{-1} diet) for 56 days exhibited remarkably upregulated mRNA expression levels of several stress and antioxidant-related genes (i.e. GDH-β, GS, GST, HIF-1α, HSP70 and MnSOD).[32] This apparently boosted the tolerance of the shrimp to hypoxia and ammonia stress. In separate studies, Liu et al.[20,244] similarly observed that the HSP70 gene was considerably expressed in the yellow catfish (*Pelteobagrus fulvidraco*) that received dietary astaxanthin (80 mg kg^{-1} diet) and β-carotene (150–200 mg kg^{-1} diet) over 60 days, respectively. Both studies emphasized the functional significance of dietary carotenoids in stimulating the antioxidative capacity and stress tolerance of fish against acute

TABLE 3 Effects of carotenoids on the stress tolerance of several aquatic species

Carotenoid	Source and inclusion level	Species	Response	References
Astaxanthin	Natural; 80 mg kg^{-1} diet	Yellow catfish (*Pelteobagrus fulvidraco*)	↓ ALT, AST, cortisol, glucose and MDA levels; ↑ mRNA expression levels of HSP70; ↑ tolerance to acute crowding stress	244
	Synthetic; 50–200 mg kg^{-1} diet	Northern snakehead (*Channa argus*)	↓ MDA level; ↑ tolerance to lipopolysaccharide-induced oxidative stress	230
	Natural; 68 mg kg^{-1} diet	Chinese mitten crab (*Eriocheir sinensis*)	↓ ALT and AST levels; ↑ tolerance to high pH stress	233
	Natural; 30–120 mg kg^{-1} diet	Chinese mitten crab (*Eriocheir sinensis*)	↓ MDA level; ↑ tolerance to ammonia-N stress	21
	Synthetic; 50–1600 mg kg^{-1} diet	Kuruma shrimp (*Marsupenaeus japonicus*)	↑ tolerance to osmotic stress	118,245,246
	Synthetic; 40–160 mg kg^{-1} diet	Pacific white shrimp (*Litopenaeus vannamei*)	↓ MDA level; ↑ mRNA expression levels of antioxidant-related genes (GDH-β, GS, GST, HIF-1α, HSP70 and MnSOD) ↑ tolerance to hypoxia and ammonia stress	32
β-carotene	Natural; 150–200 mg kg^{-1} diet	Yellow catfish (*Pelteobagrus fulvidraco*)	↓ ALT, cortisol and MDA levels; ↑ mRNA expression level of HSP70; ↑ tolerance to high-temperature stress	20
Canthaxanthin	Synthetic; 50–400 mg kg^{-1} diet	Pacific white shrimp (*Litopenaeus vannamei*)	↓ ALT, AST and MDA levels; ↑ tolerance to hypoxia	238

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GDH-β, glutamate dehydrogenase; GR, glutathione reductase; GS, glutamine synthetase; GST, glutathione-S-transferase; HIF-1α, hypoxia-inducible factor-1α; HSP70, heat shock protein 70; MDA, malondialdehyde; MnSOD, manganese superoxide dismutase; mRNA, messenger ribonucleic acid.

crowding and high-temperature conditions. Most available reports also presented evidence on the suppression of the plasmatic levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), cortisol and malondialdehyde (MDA) in experimented animals with regard to the intake of carotenoids.[20,21,32,230,233,238,244] Alterations in the activities of ALT and AST have been broadly used as relevant biomarkers of stress response and health status in animal research. The reduced activities of ALT and AST are indicative of stress alleviation and enhanced liver function resulting from the supplementation of carotenoids in aquatic animals.[19,22,238] Some authors postulate that dietary carotenoids could diminish cortisol secretion by inter-renal cells of the head kidney via the inhibition of adrenocorticotropic hormone (ACTH) release.[19,22,248] Cortisol is well-described to suppress many elements of the antioxidant system and immunity in aquatic animals that further exacerbate the deleterious effects of stressors in synergism.[242,249,250] Furthermore, MDA is recognized as a convenient biomarker of lipid degradation in cells, denoting the extent of oxidative stress and antioxidant status within hosts.[230,238] An upsurge in ROS generation in response to stressful circumstances brings about the overproduction of MDA. Dietary carotenoids have been implicated in chain termination against lipid peroxidation, safeguarding cells from the destructive action of ROS while maintaining membrane dynamics and cellular functions.[18,230,232,251] In this regard, the repression of MDA could serve as a potential intervention strategy for curtailing oxidative stress. Correspondingly, inadequate ingestion of carotenoids might increase the vulnerability of an organism to oxidative stress and related diseases. These collective interactions and mechanisms, as a whole, bolster stress tolerance in aquatic species, with reference to the alleviation of oxidative stress.

7.3 | Augmentation of host defence mechanisms and immune gene expression

Modern aquaculture pivots around the rapid intensification, diversification and commercialization of aquatic products. Domesticated animals, under intensive rearing, are usually subjected to stressful situations that restrict the efficacy of their immune system and disease resistance against opportunistic pathogens (e.g. fungi, parasites, bacteria and viruses).[16,252] The industry has seen a noteworthy shift in terms of nutrition over the last decade. Nutritional status is seemingly a central aspect that influences the immune capacity of animals to stave off diseases. Nutritional intervention inevitably keeps the immune system on track to withstand and prevent infections.[19,22] This could benefit animals through health optimization and enhanced protection during periods of heightened vulnerability.

Bendich and Shapiro[253] were the first to document the specific roles of carotenoids on the immune responses in animals. Thereafter, substantial research efforts have focused predominantly on boosting the immunity of aquatic animals by incorporating dietary carotenoids. Recent findings unambiguously established the augmentation effects of supplemented carotenoids (i.e. astaxanthin, β-carotene,

TABLE 4 Effects of carotenoids on the immune defences of various aquatic species

Carotenoid	Source and inclusion level	Species	Response	References
Astaxanthin	Natural; 50–150 mg kg^{-1} diet	Asian seabass (*Lates calcarifer*)	↑ leucocyte count; ↑ lysozyme activity, phagocytic activity, respiratory burst activity and serum total Ig	19
	Synthetic; 50–200 mg kg^{-1} diet	Northern snakehead (*Channa argus*)	↑ lysozyme activity; ↑ levels of C3, C4, IL-1β and TNF-α	230
	Natural; 50–150 mg kg^{-1} diet	Asian seabass (*Lates calcarifer*)	↑ leucocyte count; ↑ lysozyme activity, phagocytic activity, respiratory burst activity and serum total Ig; ↑ disease resistance to *Vibrio alginolyticus* infection	22
	Natural; 91 mg kg^{-1} diet	Leopard coral trout (*Plectropomus leopardus*)	↑ lysozyme activity and serum total IgM; ↑ levels of C3 and C4; ↑ mRNA expression levels of immune-related genes (*c3*, *c4-b*, *igm* and *lz-c*) ↑ disease resistance to *Vibrio harveyi* infection	231
	Synthetic; 200–800 mg kg^{-1} diet	Red swamp crayfish (*Procambarus clarkii*)	↑ lysozyme activity and serum total protein	254
	Source not specified; 25–200 mg kg^{-1} diet	Pacific white shrimp (*Litopenaeus vannamei*)	↑ activities of lysozyme and phenoloxidase; ↑ total hemocyte count and phagocytic activity; ↑ disease resistance to *Vibrio harveyi* infection	255
	Synthetic; 160 mg kg^{-1} diet	Pacific white shrimp (*Litopenaeus vannamei*)	↑ lysozyme activity; ↑ mRNA expression level of lysozyme gene; ↑ disease resistance to *Vibrio alginolyticus* infection	234
β-carotene	Natural; 150–200 mg kg^{-1} diet	Yellow catfish (*Pelteobagrus fulvidraco*)	↑ lysozyme activity; ↑ levels of C3, C4 and Ig; ↑ mRNA expression level of *igm* and *lz-g* genes; ↑ disease resistance to *Proteus mirabilis* infection	20
	Natural; 50 mg kg^{-1} diet	Nile tilapia (*Oreochromis niloticus*)	↑ lysozyme and phagocytic activities; ↑ serum total IgM; ↑ mRNA expression levels of immune-related genes (IFN-γ and IL-1β)	30
	Synthetic; 100–500 mg kg^{-1} diet	Pacific white shrimp (*Litopenaeus vannamei*)	↑ lysozyme activity	236
Canthaxanthin	Synthetic; 50–400 mg kg^{-1} diet	Pacific white shrimp (*Litopenaeus vannamei*)	↑ lysozyme activity	238
Lutein	Natural; 50–200 mg kg^{-1} diet	Oriental river prawn (*Macrobrachium nipponense*)	↑ lysozyme activity and total hemocyte count	120

Abbreviations: *c3*, complement component 3 gene; C3, complement component 3; C4, complement component 4; *c4-b*, complement component 4 gene; IFN-γ, interferon-gamma; Ig, immunoglobulin; *igm*, immunoglobulin M gene; IgM, immunoglobulin M; IL-1β, interleukin-1β; *lz-c*, lysozyme C gene; *lz-g*, lysozyme G gene; mRNA, messenger ribonucleic acid; TNF-α, tumour necrosis factor-alpha.

canthaxanthin and lutein) on several dominant facets of innate and adaptive immunity that ultimately strengthen disease resistance, primarily in fish and crustaceans (Table 4). Most investigations denoted marked stimulatory actions of carotenoids on the enzymatic activity of lysozyme, leucocyte proliferation and phagocytosis, and respiratory burst.[19,20,22,30,120,230,231,234,236,238,254,255] Additionally, increments in humoral factors, including immunoglobulins and pro-inflammatory molecules (e.g. complement component 3 [C3], complement component 4 [C4] and tumour necrosis factor-alpha [TNF-α]), were observed in several fish species supplemented with carotenoids.[19,20,22,30] Natural antioxidants, including carotenoids, have been described to prevent peroxidation damage in various immune cells and tissues while preserving structural integrity.[16,19,22] This may well imply the superior impacts of carotenoids on immune system activation and functions of haematopoietic organs (e.g. head kidney, spleen and thymus). Hence, against this background, carotenoids could be intimately linked to the modulation of haematopoiesis and immune cell differentiation. An upsurge in lysozyme and phagocytic activities could, therefore, be ascribed to an increased leucocyte count in view of the improved haematopoiesis. Phagocytes eliminate pathogens principally via the generation of ROS, lysozyme-catalysed hydrolysis and phagocytosis.[256,257] Moreover, dietary carotenoids have been demonstrated to exert distinguished dual antioxidant and prooxidant activities under particular biochemical and physiological conditions. Both in vitro and in vivo evidence elucidate that carotenoids exhibit a tendency to scavenge or induce more ROS in biological systems, which may be dose-dependent or cell-type specific.[258–263] Some studies suggest the prooxidative role of astaxanthin in stimulating the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-generated ROS for a robust respiratory burst activity in fish.[19,22] There was also mounting evidence that dietary carotenoids can improve in vitro and in vivo immunoglobulin production in humans[264–266] and some terrestrial animals,[267–270] substantiating the findings in aquatic species. Notwithstanding the foregoing, the precise underlying mechanisms are somewhat ambiguous and have not been completely explicated. However, it has been proposed that, with dietary carotenoids, T lymphocytes could be better activated to incite the proliferation of B lymphocytes that mediate immunoglobulin synthesis.[19,22,269] Most importantly, immunostimulants, particularly carotenoids, possess the capability to directly trigger the defence pathway against diseases via their action on PRRs as signalling molecules. The receptors on the target immune cells indiscriminately identify diversified PAMPs located on carotenoids as high-risk or foreign molecules, leading to the activation of innate and adaptive defence mechanisms by a cascade of cellular signals, which is crucial in developing a hostile environment for pathogens.[271–274] This initiated an overall defence response, consequently expediting the detection and clearance of a broad spectrum of infectious agents. In fact, the defence system remains reinforced even after returning to the pre-stimulation level, as noticed in shrimps.[273,275] The administration of carotenoids is widely accepted with rising awareness for eco-friendly aquaculture. Thus, dietary manipulation with carotenoids holds enough potential to improve health status and confer stronger disease resistance to aquatic animals, which is amongst the best alternatives to preventive healthcare. More intensive research is required to further comprehend their roles in disease prevention, specifically in the context of newly emerging diseases, taking into account all aspects of pharmacology, including optimal doses, toxicity and possible side effects.

Genetic regulation of innate and adaptive immune systems is paramount to ensure appropriate elicitation of immune responses for a rapid and vigorous defence against challenges when necessary.[276,277] The function of immune cells is finely regulated through the expression of diverse immune-relevant genes. Pro-inflammatory cytokines (e.g. interferon-gamma [IFN-γ], interleukin-1β [IL-1β], interleukin-6 [IL-6] and tumour necrosis factor-alpha [TNF-α]), a broad group of signalling proteins, are most frequently assessed in the study of immune gene expression.[272] These immunoregulatory molecules are mainly secreted by activated macrophages and lymphocytes. The primary functions of cytokines comprise the mediation and regulation of immunity (both innate and adaptive), immune-cell communication, haematopoiesis and inflammation.[278,279] A complex signalling cascade involving the nuclear factor-kappa B (NF-κB) protein transcription factor regulates the gene transcription of pro-inflammatory cytokines in immune cells.[272,280] In a recent study, Hassaan et al.[30] revealed prominent upregulations in the mRNA expression levels of IFN-γ and IL-1β in Nile tilapia (*Oreochromis niloticus*) supplemented with β-carotene (50 mg kg^{-1} diet) for 70 days. Besides, Li et al.[230] noted a pronouncedly higher level of TNF-α in the northern snakehead (*Channa argus*) fed with dietary astaxanthin (50–200 mg kg^{-1} diet) over 56 days. These findings could indicate the potential mechanism of carotenoids in modulating the NF-κB signalling pathway, as postulated by some authors.[272,280–282] Furthermore, some recent research on carotenoid-supplemented fish similarly discovered improvements in mRNA expression levels of several immune genes related to the complement system.[20,231] Nonetheless, our understanding of the mechanisms in the modulation of immune-related gene expression by carotenoids is incomplete and fragmented. Oftentimes, when stimulation is identified, there is little consensus on the mechanism by which the pigment affects the molecular process generating variation in immune gene expression. Perhaps, experimentations are better off advocating whole-genome and transcriptome approaches[18,283,284] to explore the complexity and broadly address the research hypothesis. The progress in genotyping technologies (high-throughput) and availability of genome resources allowed genome-wide association studies of immune response and disease resistance in aquatic animals.

8 | CHALLENGES IN THE DIETARY INTERVENTION WITH CAROTENOIDS

Carotenoids have been the most explored from multifarious aspects (e.g. chemical structure, physicochemical properties, stability, biosynthesis and metabolism) amongst all phytochemicals.[17,37] Dietary intervention with carotenoids will continue to be intensively investigated in the future as a sustainable strategy for developing stress tolerance, immunocompetence and disease resistance in numerous animals. Notwithstanding, some key challenges and concerns are worth contemplating but are not limited to the following: (i) vulnerability of carotenoids to oxidation and isomerization during feed processing and storage, (ii) variations in the nature of carotenoids and feed matrices, (iii) uneven distribution of carotenoids within the processed feed, (iv) erroneous quantification of carotenoid content, (v) leaching of carotenoids from the pelleted ration and (vi) effective and optimal administrative dosages as influenced by environmental rearing conditions of target animals and species-specificity (including distinct stages of life cycle). These factors cause, at least in part, discrepancies between results in studies that associate carotenoid intake with the physiological responses of animals. Perhaps the most challenging issue is the loss or alteration of pigments during the processing and storage of feed. Unesterified carotenoids are inclined to enzymatic or non-enzymatic oxidative degradation and geometrical isomerization.[16,285] It is particularly important, however, that carotenoids must be stable and unaltered upon incorporation into feed formulations for maximum effectiveness. Precautionary measures to steer clear of quantitative losses of carotenoids should be standard practice. The prudent solutions remain to be the refinement of feed processing methods, identification of origins of errors and measures to circumvent them, and the implementation of comprehensive feed quality assurance. Nevertheless, carotenoids stand their ground as bioactive molecules with anti-stress and immunostimulatory potentials for aquatic animals, regardless of seeming obstacles and challenges.

9 | CONCLUSION

Amid the aquaculture boom, disease and environmental problems continue to threaten the sustainability of the lucrative sector. Good nutrition is crucial to grant beneficial and lasting effects on animal health and immunity. Carotenoids are essential bioactive molecules primarily known for their outstanding antioxidant capacities to disarm free radicals and mitigate oxidative stress. Scientific discoveries over the years have proven the numerous physiological benefits (e.g. robust antioxidant system and improved stress tolerance and survival) associated with dietary carotenoids. While conferring multiple benefits, carotenoids play an even more prominent role in supporting the immune systems of aquatic species. These biological pigments are generally well-acknowledged to promote host defence systems and disease tolerance in an array of aquatic vertebrates and invertebrates. The aquaculture industry may exploit the findings from dietary intervention studies to develop stress-alleviating and immune-potentiating carotenoid-fortified feeds customized per the requirements of different stages of the production cycle. Current advances in immunogenomics and genomic technologies spurred substantial progress toward our knowledge of the immunomodulatory action of carotenoids in aquatic animals. More comprehensive and rigorous research is still necessary to establish the explicit functional association between antioxidant defence and immune mechanisms modulated by carotenoids and their positive contribution to aquatic animal health.

AUTHOR CONTRIBUTIONS

Keng Chin Lim: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; validation; visualization; writing – original draft; writing – review and editing. **Fatimah Md. Yusoff:** Conceptualization; funding acquisition; resources; supervision; validation; visualization; writing – review and editing. **Murni Karim:** Resources; supervision; validation; visualization. **Fatin M. I. Natrah:** Resources; validation; visualization.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

1. FAO. *The state of world fisheries and aquaculture: sustainability in action*. FAO; 2020. doi:10.4060/ca9229en
2. Saura M, Carabano MJ, Fernandez A, et al. Disentangling genetic variation for resistance and endurance to scuticociliatosis in turbot using pedigree and genomic information. *Front Genet*. 2019;10:539. doi:10.3389/fgene.2019.00539
3. Culot A, Grosset N, Gautier M. Overcoming challenges of phage therapy for industrial aquaculture: a review. *Aquaculture*. 2019;513:734423. doi:10.1016/j.aquaculture.2019.734423
4. Chen J, Sun R, Pan C, Sun Y, Mai B, Li QX. Antibiotics and food safety in aquaculture. *J Agric Food Chem*. 2020;68(43):11908-11919. doi:10.1021/acs.jafc.0c03996
5. Ibrahim M, Ahmad F, Yaqub B, et al. Current trends of antimicrobials used in food animals and aquaculture. In: Hashmi MA, ed. *Antibiotics and Antimicrobials Resistance Genes in the Environment*. Elsevier; 2020:39-69. doi:10.1016/B978-0-12-818882-8.00004-8
6. Lulijwa R, Rupia EJ, Alfaro AC. Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers. *Rev Aquac*. 2020;12(2):640-663. doi:10.1111/raq.12344
7. Limbu SM, Chen L, Zhang M, Du Z. A global analysis on the systemic effects of antibiotics in cultured fish and their potential human health risk: a review. *Rev Aquac*. 2021;13(2):1015-1059. doi:10.1111/raq.12511
8. Rebelo BA, Farrona S, Ventura MR, Abranches R. Canthaxanthin, a red-hot carotenoid: applications, synthesis, and biosynthetic evolution. *Plants (Basel)*. 2020;9(8):1039. doi:10.3390/plants9081039
9. Yusoff FM, Banerjee S, Nagao N, Imaizumi Y, Shariff M, Toda T. Use of microalgae pigments in aquaculture. In: Jacob-Lopes B, Queiroz M, Zepka L, eds. *Pigments from Microalgae Handbook*. Springer Nature; 2020:471-513.
10. Foo SC, Khoo KS, Ooi CW, Show PL, Khong NMH, Yusoff FM. Meeting sustainable development goals: alternative extraction processes for fucoxanthin in algae. *Front Bioeng Biotechnol*. 2021;8:546067. doi:10.3389/fbioe.2020.5460067
11. Lim KC, Yusoff FM, Shariff M, Kamarudin MS. Dietary administration of astaxanthin improves feed utilization, growth performance and survival of Asian seabass, *Lates calcarifer* (Bloch, 1790). *Aquacult Nutr*. 2019;25(6):1410-1421. doi:10.1111/anu.12961
12. Jin Y, Yu Y, Zhang C, Li S, Zhang X, Li F. Characterization and function analysis of the beta-carotene oxygenase-like genes in carotenoids metabolism of the ridgetail white prawn *Exopalaemon carinicauda*. *Front Physiol*. 2020;11:745. doi:10.3389/fphys.2020.00745
13. Rodriguez-Concepcion M, Avalos J, Bonet ML. A global perspective on carotenoids: metabolism, biotechnology, and benefits for nutrition and health. *Prog Lipid Res*. 2018;70:62-93. doi:10.1016/j.plipres.2018.04.004
14. Muzzopappa F, Kirilovsky D. Changing color for photoprotection: the orange carotenoid protein. *Trends Plant Sci*. 2020;25(1):92-104. doi:10.1016/j.tplants.2019.09.013
15. Swapnil P, Meena M, Singh SK, Dhuldhaj UP, Harish MA. Vital roles of carotenoids in plants and humans to deteriorate stress with its structure, biosynthesis, metabolic engineering and functional aspects. *Curr Plant Biol*. 2021;261(1):100203. doi:10.1016/j.cpb.2021.10023
16. Lim KC, Yusoff FM, Shariff M, Kamarudin MS. Astaxanthin as feed supplement in aquatic animals. *Rev Aquac*. 2018;10(3):738-773. doi:10.1111/raq.12200
17. Maoka T. Carotenoids as natural functional pigments. *J Nat Med*. 2020;74(1):1-16. doi:10.1007/s11418-019-01364-x
18. Nakano T, Wiegertjes G. Properties of carotenoids in fish fitness: a review. *Mar Drugs*. 2020;18(11):568. doi:10.3390/md18110568

19. Lim KC, Yusoff FM, Shariff M, Kamarudin MS, Nagao N. Dietary supplementation of astaxanthin enhances hemato-biochemistry and innate immunity of Asian seabass, *Lates calcarifer* (Bloch, 1790). *Aquaculture*. 2019;512:734339. doi:10.1016/j.aquaculture.2019.734339
20. Liu F, Qu Y, Wang A, et al. Effects of carotenoids on the growth performance, biochemical parameters, immune responses and disease resistance of yellow catfish (*Pelteobagrus fulvidraco*) under high-temperature stress. *Aquaculture*. 2019;503:293-303. doi:10.1016/j.aquaculture.2019.01.008
21. Jiang X, Zu L, Wang Z, Cheng Y, Yang Y, Wu X. Micro-algal astaxanthin could improve the antioxidant capability, immunity and ammonia resistance of juvenile Chinese mitten crab, *Eriocheir sinensis*. *Fish Shellfish Immunol*. 2020;102(8):499-510. doi:10.1016/j.fsi.2020.05.021
22. Lim KC, Yusoff FM, Shariff M, Kamarudin MS. Dietary astaxanthin augments disease resistance of Asian seabass, *Lates calcarifer* (Bloch, 1790), against *Vibrio alginolyticus* infection. *Fish Shellfish Immunol*. 2021;114:90-101. doi:10.1016/j.fsi.2021.03.025
23. Babin A, Saciat C, Teixeira M, et al. Limiting immunopathology: interaction between carotenoids and enzymatic antioxidant defences. *Dev Comp Immunol*. 2015;49(2):278-281. doi:10.1016/j.dci.2014.12.007
24. Tan K, Zhang H, Lim L, Ma H, Li S, Zheng H. Roles of carotenoids in invertebrate immunology. *Front Immunol*. 2020;10:3041. doi:10.3389/fimmu.2019.03041
25. Eikenaar C, Hegemann A, Packmor F, Kleudgen I, Isaksson C. Not just fuel: energy stores are correlated with immune function and oxidative damage in a long-distance migrant. *Curr Zool*. 2020;66(1):21-28. doi:10.1093/cz/zoz009
26. Petitjean Q, Jacquin L, LeHenaff M, et al. Dose- and time-dependent effects of an immune challenge on fish across biological levels. *J Exp Zool A: Ecol Integr Physiol*. 2021;335(2):250-264. doi:10.1002/jez.2430
27. Zhang H, Cheng D, Tan K, et al. Identification of two ferritin genes and their expression profiles in response to bacterial challenge in noble scallop *Chlamys nobilis* with different carotenoids content. *Fish Shellfish Immunol*. 2019;88:9-16. doi:10.1016/j.fsi.2019.02.051
28. Liu D, Lazado CC, Pedersen L, Straus DL, Meinelt T. Antioxidative, histological and immunological responses of rainbow trout after periodic and continuous exposures to a peracetic acid-based disinfectant. *Aquaculture*. 2020;520(2):734956. doi:10.1016/j.aquaculture.2020a.734956
29. Fang H, He X, Zeng H, Liu Y, Tian L, Niu J. Replacement of astaxanthin with lutein in diets of juvenile *Litopenaeus vannamei*: effects on growth performance, antioxidant capacity, and immune response. *Front Mar Sci*. 2021;8:803748. doi:10.3389/fmars.2021.803748
30. Hassaan MS, Mohammady EY, Soaudy MR, Sabae SA, Mahmoud AMA, El-Haroun ER. Comparative study on the effect of dietary β-carotene and phycocyanin extracted from *Spirulina platensis* on immune-oxidative stress biomarkers, genes expression and intestinal enzymes, serum biochemical in Nile tilapia, *Oreochromis niloticus*. *Fish Shellfish Immunol*. 2021;108:63-72. doi:10.1016/j.fsi.2020.11.012
31. Zhang H, Tan K, Li S, Ma H, Zheng H. Genome-wide analysis of TRAF gene family and its response to bacterial infection in noble scallop *Chlamys nobilis* with different carotenoids contents. *Aquaculture*. 2021;535(5625):736309. doi:10.1016/j.aquaculture.2020.736309
32. Zhao X, Wang G, Liu X, et al. Dietary supplementation of astaxanthin increased growth, colouration, the capacity of hypoxia and ammonia tolerance of Pacific white shrimp (*Litopenaeus vannamei*). *Aquac Rep*. 2022;23:101093. doi:10.1016/j.aqrep.2022.101093
33. Lakey-Beitia J, Kumar J, Hegde ML, Rao KS. Carotenoids as novel therapeutic molecules against neurodegenerative disorders: chemistry and molecular docking analysis. *Int J Mol Sci*. 2019;20(22):5553. doi:10.3390/ijms20225553
34. Perez-Galvez A, Viera I, Roca M. Carotenoids and chlorophylls as antioxidants. *Antioxidants*. 2020;9(6):505. doi:10.3390/antiox9060505
35. Young AJ, Lowe GL. Carotenoids: antioxidant properties. *Antioxidants*. 2018;7(6):345-351. doi:10.3390/antiox7020028
36. Melendez-Martinez AJ, Mapelli-Brahm P, Hornero-Mendez D, Vicario IM. Structures, nomenclature and general chemistry of carotenoids and their esters. In: Mercadante AZ, ed. *Carotenoid Esters in Foods: Physical, Chemical and Biological Properties*. Royal Society of Chemistry; 2019:1-50. doi:10.1039/9781788015851-00001
37. Wurtzel ET. Changing form and function through carotenoids and synthetic biology. *Plant Physiol*. 2019;179(3):830-843. doi:10.1104/pp.18.01122
38. Ambati RR, Gogisetty D, Aswathanarayana RG, et al. Industrial potential of carotenoid pigments from microalgae: current trends and future prospects. *Crit Rev Food Sci Nutr*. 2019;59(12):1880-1902. doi:10.1080/10408398.2018.1432561
39. Gupta AK, Seth K, Maheshwari K, et al. Biosynthesis and extraction of high-value carotenoids from algae. *Front Biosci (Landmark Ed)*. 2021;26(6):171-190. doi:10.52586/4932
40. Zhang Z, Xiao Y, Li D, Liu C. Identification and quantification of all-trans-zeaxanthin and its cis-isomers during illumination in a model system. *Int J Food Prop*. 2016;19(6):1282-1291. doi:10.1080/10942912.2015.1072209
41. Honda M, Kageyama H, Hibino T, et al. Evaluation and improvement of storage stability of astaxanthin isomers in oils and fats. *Food Chem*. 2021;352:12371. doi:10.1016/j.foodchem.2021.129371
42. Viazau YV, Goncharik RG, Kulikova IS, Kulikov EA, Vasilov RG, Selishcheva AA. E/Z isomerization of astaxanthin and its monoesters in vitro under the exposure to light or heat and in overilluminated *Haematococcus pluvialis* cells. *Bioresour Bioprocess*. 2021;8:55. doi:10.1186/s40643-021-00410-5
43. Reboul E. Mechanisms of carotenoid intestinal absorption: where do we stand? *Nutrients*. 2019;11(4):838. doi:10.3390/nu11040838
44. Liu C, Hu B, Cheng Y, Guo Y, Yao W, Qian H. Carotenoids from fungi and microalgae. *Bioresour Technol*. 2021;337:125398. doi:10.1016/j.biortech.2021.125398
45. Genc Y, Bardakci H, Yucel C, et al. Oxidative stress and marine carotenoids: application by using nanoformulations. *Mar Drugs*. 2020;18(8):423. doi:10.3390/md18080423
46. Ram S, Mitra M, Shah F, Tirkey SR, Mishra S. Bacteria as an alternate biofactory for carotenoid production: a review of its applications, opportunities and challenges. *J Funct Foods*. 2020;67:103867. doi:10.1016/j.jff.2020.103867
47. Sandmann G. Diversity and evolution of carotenoid biosynthesis from prokaryotes to plants. *Adv Exp Med Biol*. 2021;1261:79-94. doi:10.1007/978-981-15-7360-6_7
48. Zhao Z, Chen J, Ci F, Pang H, Cheng N, Xing A. α-carotene: a valuable carotenoid in biological and medical research. *J Sci Food Agric*. 2022;102(13):5606-5617. doi:10.1002/jsfa.11966
49. Singh RV, Sambyal K. An overview of β-carotene production: current status and future prospects. *Food Biosci*. 2022;47:101717. doi:10.1016/j.fbio.2022.101717
50. Leong YK, Chen CY, Varjani S, Chang JS. Producing fucoxanthin from algae-recent advances in cultivation strategies and downstream processing. *Bioresour Technol*. 2022;344:126170. doi:10.1016/j.biortech.2021.126170
51. Becerra MO, Contreras LM, Lo MH, Diaz JM, Herrera GC. Lutein as a functional food ingredient: stability and bioavailability. *J Funct Foods*. 2020;66:103771. doi:10.1016/j.jff.2019.103771
52. Przybylska S, Tokarczyk G. Lycopene in the prevention of cardiovascular diseases. *Int J Mol Sci*. 2022;23(4):1957. doi:10.3390/ijms23041957

53. Bouyahya A, El Omari N, Hakkur M, et al. Sources, health benefits, and biological properties of zeaxanthin. *Trends Food Sci Technol*. 2021;118:519-538. doi:10.1016/j.tifs.2021.10.017
54. Sathyaruban S, Uluwaduge DI, Yohi S, Kuganathan S. Potential natural carotenoid sources for the colouration of ornamental fish: a review. *Aquac Int*. 2021;29(4):1507-1528. doi:10.1007/s10499-021-00689-3
55. de Carvalho CCCR, Caramujo MJ. Carotenoids in aquatic ecosystems and aquaculture: a colorful business with implications for human health. *Front Mar Sci*. 2017;4:93. doi:10.3389/fmars.2017.00093
56. Melendez-Martinez AJ, Mandic AI, Bantis F, et al. A comprehensive review on carotenoids in foods and feeds: *status quo*, applications, patents, and research needs. *Crit Rev Food Sci Nutr*. 2022;62(8):1999-2049. doi:10.1810/10408398.2020.1867959
57. Yaqoob S, Riaz M, Shabbir A, Zia-Ul-Haq M, Alwakeel SS, Bin-Jumah M. Commercialization and marketing potential of carotenoids. In: Zia-Ul-Haq M, Dewanjee S, Riaz M, eds. *Carotenoids: Structure and Function in the Human Body*. Springer; 2021:799-826. doi:10.1007/978-3-030-46459-2_27
58. Mussagy CU, Khan S, Kot AM. Current developments on the application of microbial carotenoids as an alternative to synthetic pigments. *Crit Rev Food Sci Nutr*. 2022;62(25):6932-6946. doi:10.1080/10408398.2021.1908222
59. Saini RK, Keum Y. Carotenoid extraction methods: a review of recent developments. *Food Chem*. 2018;240:90-103. doi:10.1016/j.foodchem.2017.07.099
60. Koyande AK, Chew KW, Rambabu K, Tao Y, Chu D, Show P. Microalgae: a potential alternative to health supplementation for humans. *Food Sci Human Wellness*. 2019;8(1):16-24. doi:10.1016/j.fshw.2019.03.001
61. Shah MMR, Liang Y, Cheng JJ, Daroch M. Astaxanthin-producing green microalga *Haematococcus pluvialis*: from single cell to high value commercial products. *Front Plant Sci*. 2016;7:531. doi:10.3389/fpls.2016.00531
62. Young AJ, Pritchard J, White DA, Davies S. Processing of astaxanthin-rich *Haematococcus* cells for dietary inclusion and optimal pigmentation in rainbow trout, *Oncorhynchus mykiss* L. *Aquac Nutr*. 2017;23(6):1304-1311. doi:10.1111/anu.12505
63. Novoveska L, Ross ME, Stanley MS, Pradelles R, Wasiolek V, Sassi J. Microalgal carotenoids: a review of production, current markets, regulations, and future directions. *Mar Drugs*. 2019;17(11):640. doi:10.3390/md17110640
64. Venil CK, Dufosse L, Renuka DP. Bacterial pigments: sustainable compounds with market potential for pharma and food industry. *Front Sustain Food Syst*. 2020;4:100. doi:10.3389/fsufs.2020.00100
65. Rodriguez-Sifuentes L, Marszalek JE, Hernandez-Carbajal G, Chuck-Hernandez C. Importance of downstream processing of natural astaxanthin for pharmaceutical application. *Front Chem Eng*. 2021;2:601483. doi:10.3389/fceng.2020.601483
66. Alvarez R, Vaz B, Gronemeyer H, de Lera AR. Functions, therapeutic applications, and synthesis of retinoids and carotenoids. *Chem Rev*. 2014;114(1):1-25. doi:10.1021/cr400126u
67. Bogacz-Radomska L, Harasym J. β-Carotene—properties and production methods. *FQS*. 2018;2(2):69-74. doi:10.1093/fqsafe/fyy004
68. Capelli B, Bagchi D, Cysewski GR. Synthetic astaxanthin is significantly inferior to algal-based astaxanthin as an antioxidant and may not be suitable as a human nutraceutical supplement. *Forum Nutr*. 2013;12(4):145-152. doi:10.1007/s13749-013-0051-5
69. Diao J, Song X, Zhang L, Cui J, Chen L, Zhang W. Tailoring cyanobacteria as a new platform for highly efficient synthesis of astaxanthin. *Metab Eng*. 2020;61:275-287. doi:10.1016/j.ymben.2020.07.003
70. Oplatowska-Stachowiak M, Elliott CT. Food colors: existing and emerging food safety concerns. *Crit Rev Food Sci Nutr*. 2017;57(3):524-548. doi:10.1080/10408398.2014.889652
71. Bhatt T, Patel K. Carotenoids: potent to prevent diseases review. *Nat Prod Bioprospect*. 2020;10(3):109-117. doi:10.1007/s13659-020-00244-2
72. Maghsoudi S, Shahraki BT, Rabiee N, et al. The colorful world of carotenoids: a profound insight on therapeutics and recent trends in nano delivery systems. *Crit Rev Food Sci Nutr*. 2022;62(13):3658-3697. doi:10.1080/10408398.2020.1867958
73. Elia AC, Prearo M, Dorr AJM, et al. Effects of astaxanthin and canthaxanthin on oxidative stress biomarkers in rainbow trout. *J Toxicol Eviron Health Part A*. 2019;82(13):760-768. doi:10.1080/15287394.2019.1648346
74. Zhu Y, Yin L, Ge J, et al. Astaxanthin supplementation enriches productive performance, physiological and immunological responses in laying hens. *Anim Biosci*. 2021;34(3):443-448. doi:10.5713/ab.20.0550
75. Carotenoids Market Report. Carotenoids market by type (astaxanthin, β-carotene, lutein, lycopene, canthaxanthin, and zeaxanthin), application (feed, food and beverages, dietary supplements, cosmetics, and pharmaceuticals), source, formulation, and region—global forecast to 2026; 2020: 187 p.
76. Kopec RE, Failla ML. Recent advances in the bioaccessibility and bioavailability of carotenoids and effects of other dietary lipophiles. *J Food Compos Anal*. 2018;68:16-30. doi:10.1016/j.jfca.2017.06.008
77. Boonlao N, Ruktanonchai UR, Anal AK. Enhancing bioaccessibility and bioavailability of carotenoids using emulsion-based delivery systems. *Colloids Surf B Biointerfaces*. 2022;209(2):112211. doi:10.1016/j.colsurfb.2021.112211
78. Chacon-Ordonez T, Carle R, Schweiggert R. Bioaccessibility of carotenoids from plant and animal food. *J Sci Food Agric*. 2019;99(7):3220-3239. doi:10.1002/jsfa.9525
79. Desmarchelier C, Borel P. Overview of carotenoid bioavailability determinants: from dietary factors to host genetic variations. *Trends Food Sci Technol*. 2017;69:270-280. doi:10.1016/j.tifs.2017.03.002
80. Ferruzzi MG, Kruger J, Mohamedshah Z, Debelo H, Taylor JRN. Insights from in vitro exploration of factors influencing iron, zinc and provitamin a carotenoid bioaccessibility and intestinal absorption from cereals. *J Cereal Sci*. 2020;96(2):103126. doi:10.1016/j.jcs.2020.103126
81. Maltby JB, Albright LJ, Kennedy CJ, Higgs DA. Effect of route of administration and carrier on bioavailability and kinetics of astaxanthin in Atlantic salmon *Salmo salar* L. *Aquacult Res*. 2003;34(10):829-838. doi:10.1046/j.1365-2109.2003.00888.x
82. Choubert G, Cravedi J, Laurentie M. Pharmacokinetics and availabilities of ^{14}C-keto-carotenoids, astaxanthin and canthaxanthin, in rainbow trout, *Oncorhynchus mykiss*. *Aquac Res*. 2005;36(15):1526-1534. doi:10.1111/j.1365-2109.2005.01375.x
83. Choubert G. Response of rainbow trout (*Oncorhynchus mykiss*) to varying dietary astaxanthin/canthaxanthin ratio: colour and carotenoid retention of the muscle. *Aquacult Nutr*. 2010;16(5):528-535. doi:10.1111/j.1365-2095.2009.00692.x
84. Yuangsoi B, Jintasataporn O, Tabthipwon P, Kamel C. Utilization of carotenoids in fancy carp (*Cyprinus carpio*): astaxanthin, lutein and β-carotene. *World Appl Sci J*. 2010;11(5):590-598. http://www.idosi.org/wasj/wasj11(5)/14.pdf
85. Esfanjani AF, Assadpour E, Jafari SM. Improving the bioavailability of phenolic compounds by loading them within lipid-based nanocarriers. *Trends Food Sci Technol*. 2018;76:56-66. doi:10.1016/j.tifs.2018.04.002
86. Rehman A, Tong Q, Jafari SM, et al. Carotenoid-loaded nanocarriers: a comprehensive review. *Adv Colloid Interface Sci*. 2020;275:102048. doi:10.1016/j.cis.2019.102048
87. Sridhar K, Inbaraj BS, Chen BH. Recent advances on nanoparticle based strategies for improving carotenoid stability and biological activity. *Antioxidants*. 2021;10(5):713. doi:10.3390/antiox10050713

88. Sheftel J, Loechl C, Mokhtar N, Tanumihardjo SA. Use of stable isotopes to evaluate bioefficacy of provitamin a carotenoids, vitamin a status, and bioavailability of iron and zinc. *Adv Nutr*. 2018;9(5):625-636. doi:10.1093/advances/nmy036
89. Oxley A, Lietz G. Use of stable isotopes to study bioconversion and bioefficacy of provitamin a carotenoids. *Methods Enzymol*. 2022; 670:399-422. doi:10.1016/bs.mie.2022.01.018
90. Widjaja-Adhi MAK, Golczak M. The molecular aspects of absorption and metabolism of carotenoids and retinoids in vertebrates. *Biochim Biophys Acta - Mol Cell Biol Lipids*. 2020;1865(11):158571. doi:10.1016/j.bbalip.2019.158571
91. Chen L, Yokoyama W, Alves P, Tan Y, Pan J, Zhong F. Effect of encapsulation on β-carotene absorption and metabolism in mice. *Food Hydrocoll*. 2021;121:107009. doi:10.1016/j.foodhyd.2021.107009
92. Courtot E, Musson D, Stratford C, et al. Dietary fatty acid composition affects the apparent digestibility of algal carotenoids in diets for Atlantic salmon, *Salmo salar*. *Aquac Res*. 2022;53(6):2343-2353. doi:10.1111/are.15753
93. Chimsung N, Tantikitti C, Milley JE, Verlhac-Trichet V, Lall SP. Effects of various dietary factors on astaxanthin absorption in Atlantic salmon (*Salmo salar*). *Aquacult Res*. 2014;45(10):1611-1620. doi:10.1111/are.12108
94. Sefc KM, Brown AC, Clotfelter ED. Carotenoid-based coloration in cichlid fishes. *Comp Biochem Physiol B, Mol Integr Physiol*. 2014; 173(100):42-51. doi:10.1016/j.cbpa.2014.03.006
95. Yu W, Liu J. Astaxanthin isomers: selective distribution and isomerization in aquatic animals. *Aquaculture*. 2020;520(8):734915. doi:10.1016/j.aquaculture.2019.734915
96. Su F, Yu W, Liu J. Comparison of effect of dietary supplementation with *Haematococcus pluvialis* powder and synthetic astaxanthin on carotenoid composition, concentration, esterification degree and astaxanthin isomers in ovaries, hepatopancreas, carapace, epithelium of adult female Chinese mitten crab (*Eriocheir sinensis*). *Aquaculture*. 2020;523:735146. doi:10.1016/j.aquaculture.2020.735146
97. Tripathy PS, Devi NC, Parhi J, et al. Molecular mechanisms of natural carotenoid-based pigmentation of queen loach, *Botia dario* (Hamilton, 1822) under captive condition. *Sci Rep*. 2019;9(1):12585. doi:10.1038/s41598-019-48982-9
98. Judan Cruz KG, Landingin EP, Gajeton MB, Fernando SID, Watanabe K. Carotenoid coloration and coloration-linked gene expression in red tilapia (*Oreochromis* sp.) tissues. *BMC Vet Res*. 2021;17(1):314. doi:10.1186/s12917-021-03006-5
99. Schmeisser J, Verlhac-Trichet V, Madaro A, Lall SP, Torrissen O, Olsen RE. Molecular mechanism involved in carotenoid metabolism in post-smolt Atlantic salmon: astaxanthin metabolism during flesh pigmentation and its antioxidant properties. *Marine Biotechnol*. 2021;23(4):653-670. doi:10.1007/s10126-021-10055-2
100. Toews D, Hofmeister NR, Taylor SA. The evolutions and genetics of carotenoid processing in animals. *Trends Genet*. 2017;33(3):171-182. doi:10.1016/j.tig.2017.01.002
101. Ahi EP, Lecaudey LA, Ziegelbecker A, et al. Comparative transcriptomics reveals candidate carotenoid color genes in an east African cichlid fish. *BMC Genomics*. 2020;21:54. doi:10.1186/s12864-020-6473-8
102. Gazda MA, Araujo PM, Lopes RJ, et al. A genetic mechanism for sexual dichromatism in birds. *Science*. 2020;368(6496):1270-1274. doi:10.1126/science.aba0803
103. Madaro A, Torrissen O, Whatmore P, et al. Red and white Chinook salmon (*Oncorhynchus tshawytscha*): differences in the transcriptome profile of muscle, liver, and pylorus. *Marine Biotechnol*. 2020;22(4):581-593. doi:10.1007/s10126-020-09980-5
104. Du J, Chen H, Mandal BK, et al. HDL receptor/scavenger receptor B1-*Scarb1* and *Scarb1-like* mediate the carotenoid-based red coloration in fish. *Aquaculture*. 2021;545(2):737208. doi:10.1016/j.aquaculture.2021.737208
105. Mohammed Y, Ye D, He M, et al. Induction of biosynthesis of keto-carotenoid from β-carotene in fish embryos. *Aquaculture*. 2021;542:736863. doi:10.1016/j.aquaculture.2021.736863
106. Rajasingh H, Oyehaug L, Vage D, Omholt S. Carotenoids dynamics in Atlantic salmon. *BMC Biol*. 2006;4:10. doi:10.1186/1741-7007-4-10
107. Kotake-Nara E, Nagao A. Absorption and metabolism of xanthophylls. *Mar Drugs*. 2011;9(6):1024-1037. doi:10.3390/md9061024
108. Albrektsen S, Ostbye TK, Pedersen M, Ytteborg E, Ruyter B, Ytrestoyl T. Dietary impacts of sulphuric acid extracted fish bone compounds on astaxanthin utilization and muscle quality in Atlantic salmon (*Salmo salar*). *Aquaculture*. 2018;495:255-266. doi:10.1016/j.aquaculture.2018.05.047
109. Su F, Huang B, Liu JG. The carotenoids of shrimps (Decapoda: Caridia and Dendrobranchiata) cultured in China. *J Crustac Biol*. 2018; 38:523-530. doi:10.1093/jcbiol/ruy049
110. da Costa DP, Miranda-Filho KC. The use of carotenoid pigments as food additives for aquatic organisms and their functional roles. *Rev Aquac*. 2019;12(3):1567-1578. doi:10.1111/raq.12398
111. EFSA. Scientific opinion on the safety and efficacy of astaxanthin (CAROPHYLL® pink 10% CWS) for salmonids, crustaceans and other fish. *EFSA J*. 2019;17(12):e05920. doi:10.2903/j.efsa.2019.5920
112. EFSA. Safety of astaxanthin for its use as a novel food in food supplements. *EFSA J*. 2020;18(2):e05993. doi:10.2903/j.efsa.2020.5993
113. EFSA. Scientific opinion on modifications of the terms of authorisation of a red carotenoid-rich bacterium *Paracoccus carotinifaciens* (Panaferd-AX) as feed additive for salmon and trout. *EFSA J*. 2010; 8(1):1428. doi:10.2903/j.efsa.2010.1428
114. EFSA. Scientific opinion on the safety and efficacy of β-carotene as a feed additive for all animal species and categories. *EFSA J*. 2012; 10(6):2737. doi:10.2903/j.efsa.2012.2737
115. EFSA. Scientific opinion on the safety and efficacy of canthaxanthin as a feed additive for poultry and for ornamental birds and ornamental fish. *EFSA J*. 2014;12(1):3527. doi:10.2903/j.efsa.2014.3527
116. EFSA. Scientific opinion on the safety and efficacy of synthetic astaxanthin as feed additive for salmon and trout, other fish, ornamental fish, crustaceans and ornamental birds. *EFSA J*. 2014;12(6):3724. doi:10.2903/j.efsa.2014.3724
117. EFSA. Scientific opinion on the safety and efficacy of astaxanthin (CAROPHYLL® pink 10% CWS) for salmonids and ornamental fish. *EFSA J*. 2014;12(6):3725. doi:10.2903/j.efsa.2014.3725
118. Wang W, Ishikawa M, Koshio S, et al. Interactive effects of dietary astaxanthin and cholesterol on the growth, pigmentation, fatty acid analysis, immune response and stress resistance of kuruma shrimp (*Marsupenaeus japonicus*). *Aquacult Nutr*. 2019;25(4):946-958. doi:10.1111/anu.12913
119. Wang W, Ishikawa M, Koshio S, et al. Effects of dietary astaxanthin and vitamin E and their interactions on the growth performance, pigmentation, and digestive enzyme activity of kuruma shrimp (*Marsupenaeus japonicus*). *Aquacult Res*. 2019;50(4):1186-1197. doi:10.1111/are.13993
120. Ettefaghdoost M, Haghighi H. Impact of different dietary lutein levels on growth performance, biochemical and immuno-physiological parameters of oriental river prawn (*Macrobrachium nipponense*). *Fish Shellfish Immunol*. 2021;115:86-94. doi:10.1016/j.fsi.2021.05.024
121. Zhang C, Jin Y, Yu Y, Xiang J, Li F. Effects of natural astaxanthin from microalgae and chemically synthetic astaxanthin supplementation on two different varieties of the ridgetail white prawn (*Exopalaemon carinicauda*). *Algal Res*. 2021;57(2):102347. doi:10.1016/j.algal.2021.102347
122. Hansen OJ, Puvanendran V, Bangera R. Broodstock diet with water and astaxanthin improve condition and egg output of brood fish and

larval survival in Atlantic cod, *Gadus Morhua* L. *Aquac Res*. 2016;47(3):819-829. doi:10.1111/are.12540
123. Li F, Huang S, Lu X, et al. Effects of dietary supplementation with algal astaxanthin on growth, pigmentation, and antioxidant capacity of the blood parrot (*Cichlasoma citrinellum* x *Cichlasoma synspilum*). *J Oceannol Limnol*. 2018;36(5):1851-1859. doi:10.1007/s00343-019-7172-7
124. Torres Sanchez EG, Fuenmayor CA, Vasquez Mejia SM, Diaz-Moreno C, Suarez MH. Effect of bee pollen extract as a source of natural carotenoids on the growth performance and pigmentation of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 2020;514:734490. doi:10.1016/j.aquaculture.2019.734490
125. Kalinowski CT, Socorro J, Robaina LE. Effect of dietary canthaxanthin on the growth and lipid composition of red porgy (*Pagrus pagrus*). *Aquacult Res*. 2015;46(4):893-900. doi:10.1111/are.12245
126. Nogueira N, Canada P, Caboz J, Andrade C, Cordeiro N. Effect of different levels of synthetic astaxanthin on growth, skin color and lipid metabolism of commercial sized red porgy (*Pagrus pagrus*). *Anim Feed Sci Technol*. 2021;276(1):114916. doi:10.1016/j.anifeedsci.2021.114916
127. Brizio P, Benedetto A, Righetti M, et al. Astaxanthin and canthaxanthin (xanthophyll) as supplements in rainbow trout diet: in vivo assessment of residual levels and contributions to human health. *J Agric Food Chem*. 2013;61(46):10954-10959. doi:10.1021/jf4012664
128. da Silva FO, Tramonte VLCG, Parisenti J, Lima-Garcia JF, Maraschin M, da Silva EL. *Litopenaeus vannamei* muscle carotenoids versus astaxanthin: a comparison of antioxidant activity and in vitro protective effects against lipid peroxidation. *Food Biosci*. 2015;9:12-19. doi:10.1016/j.fbio.2014.11.001
129. Zhang C, Su F, Li S, et al. Isolation and identification of the main carotenoid pigment from a new variety of the ridgetail white prawn *Exopalaemon carinicauda*. *Food Chem*. 2018;269:450-454. doi:10.1016/j.foodchem.2018.06.143
130. Xue F, Li C, Pan S. Subacute toxicity assessments of carotenoids extracted from citrus peel (Nanfengmiju, *Citrus reticulata* Blanco) in rats. *Regul Toxicol Pharmacol*. 2012;62(1):16-22. doi:10.1016/j.yrtph.2011.12.003
131. Grenfell-Lee D, Zeller S, Cardoso R, Pucaj K. The safety of β-carotene from *Yarrowia lipolytica*. *Food Chem Toxicol*. 2014;65:1-11. doi:10.1016/j.fct.2013.12.010
132. Ravi H, Arunkumar R, Baskaran V. Chitosan-glycolipid nanogels loaded with anti-obese marine carotenoid fucoxanthin: acute and sub-acute toxicity evaluation in rodent model. *J Biomater Appl*. 2015;30(4):420-434. doi:10.1177/0885328215590753
133. Lin YJ, Lin JY, Wang DS, Chen CH, Chiou MH. Safety assessment of astaxanthin derived from engineered *Escherichia coli* K-12 using a 13-week repeated dose oral toxicity study and a prenatal development toxicity study in rats. *Regul Toxicol Pharmacol*. 2017;87:95-105. doi:10.1016/j.yrtph.2017.05.003
134. Niu T, Zhou J, Wang F, et al. Safety assessment of astaxanthin from *Haematococcus pluvialis*: acute toxicity, genotoxicity, distribution and repeat dose toxicity studies in gestation mice. *Regul Toxicol Pharmacol*. 2020;115:104695. doi:10.1016/j.yrtph.2020.104695
135. Wang PT, Sudirman S, Hsieh MC, Hu JY, Kong ZL. Oral supplementation of fucoxanthin-rich brown algae extract ameliorates cisplatin-induced testicular damage in hamsters. *Biomed Pharmacother*. 2020;125:109992. doi:10.1016/j.biopha.2020.109992
136. Ravi KB, Reddy KRR, Shankaranarayanan J, Deshpande JV, Juturu V, Soni MG. Safety evaluation of zeaxanthin concentrate (OmniXan™): acute, subchronic toxicity and mutagenicity studies. *Food Chem Toxicol*. 2014;72:30-39. doi:10.1016/j.fct.2014.06.015
137. Yamashita E. Let astaxanthin be thy medicine. *PharmaNutrition*. 2015;3(4):115-122. doi:10.1016/j.phanu.2015.09.001
138. Baswan SM, Klosner AE, Weir C, et al. Role of ingestible carotenoids in skin protection: a review of clinical evidence. *Photodermatol Photoimmunol Photomed*. 2021;37(6):490-504. doi:10.1111/phpp.12690
139. Bohm V, Lietz G, Olmedilla-Alonso B, et al. From carotenoid intake to carotenoid blood and tissue concentrations—implications for dietary intake recommendations. *Nutr Rev*. 2021;79(5):544-573. doi:10.1093/nutrit/nuaa008
140. Brendler T, Williamson EM. Astaxanthin: how much is too much? A safety review. *Phytother Res*. 2019;33(12):3090-3111. doi:10.1002/ptr.6514
141. Feng L, Nie K, Fan W. Effects of lutein supplementation in age-related macular degeneration. *PLoS One*. 2019;14(12):e0227048. doi:10.1371/journal.pone.0227048
142. Nouri M, Amani R, Nasr-Esfahani M, Tarrahi MJ. The effects of lycopene supplement on the spermatogram and seminal oxidative stress in infertile men: a randomized, double-blind, placebo-controlled clinical trial. *Phytother Res*. 2019;33(12):3203-3211. doi:10.1002/ptr.6493
143. Przybylska S. Lycopene—a bioactive carotenoid offering multiple health benefits: a review. *Int J Food Sci Technol*. 2019;55(1):11-32. doi:10.1111/ijfs.14260
144. Rodriguez-Amaya DB. Update on natural food pigments—a mini-review on carotenoids, anthocyanins, and betalains. *Food Res Int*. 2019;124:200-205. doi:10.1016/j.foodres.2018.05.028
145. Fakhri S, Nouri Z, Moradi SZ, Farzaei MH. Astaxanthin, COVID-19 and immune response: focus on oxidative stress, apoptosis and autophagy. *Phytother Res*. 2020;34(11):2790-2792. doi:10.1002/ptr.6797
146. Yim SK, Kim I, Warren B, Kim J, Jung K, Ku B. Antiviral activity of two marine carotenoids against SARS-CoV-2 virus entry in silico and in vitro. *Int J Mol Sci*. 2021;22(12):6481. doi:10.3390/ijms22126481
147. Shen Y, Li J, Gu R, et al. Carotenoid and superoxide dismutase are the most effective antioxidants participating in ROS scavenging in phenanthrene accumulated wheat leaf. *Chemosphere*. 2018;197:513-525. doi:10.1016/j.chemosphere.2018.01.036
148. Melendez-Martinez AJ. An overview of carotenoids, apocarotenoids, and vitamin a in agro-food, nutrition, health, and disease. *Mol Nutr Food Res*. 2019;64(12):1801045. doi:10.1002/mnfr.20180105
149. Nabi F, Arain MA, Rajput N, et al. Health benefits of carotenoids and potential application in poultry industry: a review. *J Anim Physiol Anim Nutr*. 2020;104(6):1809-1818. doi:10.1111/jpn.13375
150. Nishino A, Yasui H, Maoka T. Reaction and scavenging mechanism of β-carotene and zeaxanthin with reactive oxygen species. *J Oleo Sci*. 2017;66(1):77-84. doi:10.5650/jos.ess16107
151. Yahia EM, Ornelas-Paz J, Emanuelli T, Jacob-Lopes E, Zepka LQ, Cervantes-Pas B. Chemistry, stability, and biological actions of carotenoids. In: Yahia EM, ed. *Fruit and Vegetable Phytochemicals: Chemistry and Human Health*. Blackwell Publishing; 2018:285-346.
152. Browne D, Williams MA, Maxwell AP, et al. Serum xanthophyll carotenoids are associated with estimated glomerular filtration rate in an aged cohort. *Sci Rep*. 2019;9(1):17068. doi:10.1038/s41598-019-53674-5
153. Rutz JK, Borges CD, Zambiazi RC, da Rosa CG, da Silva MM. Elaboration of microparticles of carotenoids from natural and synthetic sources for applications in food. *Food Chem*. 2016;202:324-333. doi:10.1016/j.foodchem.2016.01.140
154. Riaz M, Zia-Ul-Haq M, Dou D. Chemistry of carotenoids. In: Zia-Ul-Haq M, Dewanjee S, Riaz M, eds. *Carotenoids: Structure and Function in the Human Body*. Springer Nature; 2021:43-76.
155. Sandmann G. Antioxidant protection from UV- and light-stress related to carotenoid structures. *Antioxidants*. 2019;8(7):219. doi:10.3390/antiox8070219
156. Kiokias S, Proestos C, Varzakas TH. A review of the structure, biosynthesis, absorption of carotenoid analysis and properties of their

common natural extract. *Curr Res Nutr Food Sci*. 2016;4(1):25-37. doi:10.12944/CRNFSJ.4.Special-Issue1.03
157. Widomska J, Welc R, Gruszecki WI. The effect of carotenoids on the concentration of singlet oxygen in lipid membranes. *Biochim Biophys Acta Biomembr*. 2019;1861(4):845-851. doi:10.1016/j.bbamem.2019.01.012
158. Tamura H, Ishikita H. Quenching of singlet oxygen by carotenoids via ultrafast superexchange dynamics. *J Phys Chem A*. 2020;124(25):5081-5088. doi:10.1021/acs.jpca.0c02228
159. Dogra V, Kim C. Singlet oxygen metabolism: from genesis to signaling. *Front Plant Sci*. 2020;10:1640. doi:10.3389/fpls.2019.01640
160. Kruk J, Szymanska R. Singlet oxygen oxidation products of carotenoids, fatty acids and phenolic prenyllipids. *J Photochem Photobiol B, Biol*. 2021;216:112148. doi:10.1016/j.jphotobiol.2021.112148
161. Focsan AL, Kispert LD. Radicals formed from proton loss of carotenoid radical cations: a special form of carotenoid neutral radical occurring in photoprotection. *J Photochem Photobiol B, Biol*. 2017;166:148-157. doi:10.1016/j.jphotobiol.2016.11.015
162. Milani A, Basirnejad M, Shahbazi S, Bolhassani A. Carotenoids: biochemistry, pharmacology and treatment. *Br J Pharmacol*. 2017;174(11):1290-1324. doi:10.1111/bph.13625
163. Edge R, Truscott TG. Singlet oxygen and free radical reactions of retinoids and carotenoids—a review. *Antioxidants*. 2018;7(1):5. doi:10.3390/antiox7010005
164. Focsan AL, Polyakov NE, Kispert LD. Carotenoids: importance in daily life—insight gained from EPR and ENDOR. *Appl Magn Reson*. 2021;52(8):1093-1112. doi:10.1007/s00723-021-01311-8
165. Lai TT, Yang CM, Yang CH. Astaxanthin protects retinal photoreceptor cells against high glucose-induced oxidative stress by induction of antioxidant enzymes via the PI3K/Akt/Nrf2 pathway. *Antioxidants*. 2020;9(9):729. doi:10.3390/antiox9080729
166. Sharifi-Rad M, Anil Kumar NV, Zucca P. Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. *Front Physiol*. 2020;2:11. doi:10.3389/fphys.2020.00694
167. Brotosudarmo THP, Limantara L, Setiyono E, Heriyanto H. Structures of astaxanthin and their consequences for therapeutic application. *Int J Food Sci*. 2020;2020:2156582. doi:10.1155/2020/2156582
168. Polyakov NE, Focsan AL, Bowman MK, Kispert LD. Free radical formation in novel carotenoid metal ion complexes of astaxanthin. *J Phys Chem B*. 2010;114(50):16968-16977. doi:10.1021/jp109039v
169. Hernandez-Marin E, Barbosa A, Martinez A. The metal cation chelating capacity of astaxanthin. Does this have any influence on antiradical activity? *Molecules*. 2012;17(1):1039-1054. doi:10.3390/molecules17011039
170. Focsan AL, Polyakov NE, Kispert LD. Photo protection of *Haematococcus pluvialis* algae by astaxanthin: unique properties of astaxanthin deduced by EPR, optical and electrochemical studies. *Antioxidants*. 2017;6(4):80. doi:10.3390/antiox6040080
171. Britton G. Carotenoid research: history and new perspectives for chemistry in biological systems. *Biochim Biophys Acta Mol Cell Biol Lipids*. 2020;1865(11):158699. doi:10.1016/j.bbalip.2020.158699
172. Zhai S, Xia X, He Z. Carotenoids in staple cereals: metabolism, regulation, and genetic manipulation. *Front Plant Sci*. 2016;7:1197. doi:10.3389/fpls.2016.01197
173. Enfissi EMA, Nogueira M, Bramley PM, Fraser PD. The regulation of carotenoid formation in tomato fruit. *Plant J*. 2017;89(4):774-788. doi:10.1111/tpj.13428
174. Stanley L, Yuan Y. Transcriptional regulation of carotenoid biosynthesis in plants: so many regulators, so little consensus. *Front Plant Sci*. 2019;10(5):1017. doi:10.3389/fpls.2019.01017
175. Karniel U, Koch A, Zamir D, Hirschberg J. Development of zeaxanthin-rich tomato fruit through genetic manipulations of carotenoid biosynthesis. *Plant Biotechnol J*. 2020;18(11):2292-2303. doi:10.1111/pbi.13387
176. Tran NT, Kaldenhoff R. Metabolic engineering of ketocarotenoids biosynthetic pathway in *Chlamydomonas reinhardtii* strain CC-4102. *Sci Rep*. 2020;10(1):10688. doi:10.1038/s41598-020-67756-2
177. Shi H, Yan KK, Ding L, Qian C, Chi H, Yu J. Network approaches for dissecting the immune system. *iScience*. 2020;23(8):101354. doi:10.1016/j.isci.2020.101354
178. dos Reis EF, Viney M, Masuda N. Network analysis of the immune state of mice. *Sci Rep*. 2021;11(1):4306. doi:10.1038/s41598-021-83139-7
179. Semple SL, Dixon B. Salmonid antibacterial immunity: an aquaculture perspective. *Biology*. 2020;9(10):331. doi:10.3390/biology9100331
180. Yu Y, Wang Q, Huang Z, Ding L, Xu Z. Immunoglobulins, mucosal immunity and vaccination in teleost fish. *Front Immunol*. 2020;11:567941. doi:10.3389/fimmu.2020.567941
181. Wu L, Li L, Gao A, Ye J, Li J. Antimicrobial roles of phagocytosis in teleost fish: phagocytic B cells vs professional phagocytes. *Aquac Fish*. 2022. doi:10.1016/j.aaf.2021.12.008
182. Amparyup P, Charoensapsri W, Samaluka N, Chumtong P, Yocawibun P, Imjongjirak C. Transcriptome analysis identifies immune-related genes and antimicrobial peptides in Siamese fighting fish (*Betta splendens*). *Fish Shellfish Immunol*. 2020;99(2):403-413. doi:10.1016/j.fsi.2020.02.030
183. Islam MA, Rony SA, Rahman MB, et al. Improvement of disease resistance in livestock: application of immunogenomics and CRISPR/Cas9 technology. *Animals*. 2020;10(12):2236. doi:10.3390/ani10122236
184. You X, Shan X, Shi Q. Research advances in the genomics and application for molecular breeding of aquaculture animals. *Aquaculture*. 2020;526:735357. doi:10.1016/j.aquaculture.2020.735357
185. Diaz-Rosales P, Munoz-Atienza E, Tafalla C. Roles of teleost B cells in viral immunity. *Fish Shellfish Immunol*. 2019;86:135-142. doi:10.1016/j.fsi.2018.11.039
186. Smith NC, Rise ML, Christian SL. A comparison of the innate and adaptive immune systems in cartilaginous fish, ray-finned fish, and lobe-finned fish. *Front Immunol*. 2019;10:2292. doi:10.3389/fimmu.2019.02292
187. Netea MG, Schlitzer A, Placek K, Joosten L, Schultze JL. Innate and adaptive immune memory: an evolutionary continuum in the host's response to pathogens. *Cell Host Microbe*. 2019;25(1):13-26. doi:10.1016/j.chom.2018.12.006
188. Sun L, Wang X, Saredy J, Yuan Z, Yang X, Wang H. Innate-adaptive immunity interplay and redox regulation in immune response. *Redox Biol*. 2020;37:101759. doi:10.1016/j.redox.2020.101759
189. Tafalla C, Leal E, Yamaguchi T, Fischer U. T cell immunity in teleost digestive tract. *Dev Comp Immunol*. 2016;64:166-177. doi:10.1016/j.dci.2016.02.019
190. Rodrigues MV, Zanuzzo FS, Koch JFA, de Oliveira CAF, Sima P, Vetvicka V. Development of fish immunity and the role of β-glucan in immune responses. *Molecules*. 2020;25(22):5378. doi:10.3390/molecules25225378
191. Zhang Z, Chi H, Dalmo RA. Trained innate immunity of fish is a viable approach in larval aquaculture. *Front Immunol*. 2019;10:42. doi:10.3389/fimmu.2019.00042
192. Low CF, Chong CM. Peculiarities of innate immune memory in crustaceans. *Fish Shellfish Immunol*. 2020;104:605-612. doi:10.1016/j.fsi.2020.06.047
193. Netea MG, Dominguez-Andres J, Barreiro LB, et al. Defining trained immunity and its role in health and disease. *Nat Rev Immunol*. 2020;20(6):375-388. doi:10.1038/s41577-020-0285-6
194. Ming J, Ye J, Zhang Y, et al. Optimal dietary curcumin improved growth performance, and modulated innate immunity, antioxidant capacity and related genes expression of NF-κB and Nrf2 signaling pathways in grass carp (*Ctenopharyngodon idella*) after infection with *Aeromonas hydrophila*. *Fish Shellfish Immunol*. 2020;97:540-553. doi:10.1016/j.fsi.2019.12.074

195. Wu P, Liu XW, Feng L, et al. (2-Carboxyethyl) dimethylsulfonium bromide supplementation in non-fish meal diets for on-growing grass carp (*Ctenopharyngodon idella*): beneficial effects on immune function of the immune organs via modulation of NF-κB and TOR signalling pathway. *Fish Shellfish Immunol*. 2020;107A:309-323. doi:10.1016/j.fsi.2020.08.032
196. Ballarin L, Karahan A, Salvetti A, et al. Stem cells and innate immunity in aquatic invertebrates: bridging two seemingly disparate disciplines for new discoveries in biology. *Front Immunol*. 2021;12: 688106. doi:10.3389/fimmu.2021.688106
197. Chu Q, Xu T. MicroRNA regulation of toll-like receptor, RIG-I-like receptor and nod-like receptor pathways in teleost fish. *Rev Aquac*. 2020;12(4):2177-2193. doi:10.1111/raq.12428
198. Zheng J, Wang P, Mao Y, Su Y, Wang J. Full-length transcriptome analysis provides new insights into the innate immune system of *Marsupenaeus japonicus*. *Fish Shellfish Immunol*. 2020;106:283-295. doi:10.1016/j.fsi.2020.07.018
199. Natnan ME, Low CF, Chong CM, Bunawan H, Baharum SN. Integration of omics tools for understanding the fish immune response due to microbial challenge. *Front Mar Sci*. 2021;8:668771. doi:10.3389/fmars.2021.668771
200. Ravi V, Venkatesh B. The divergent genome of teleosts. *Annu Rev Anim Biosci*. 2018;6:47-68. doi:10.1146/annurev-animal-030117-014821
201. Daane JM, Detrich WH III. Adaptations and diversity of Antarctic fishes: a genomic perspective. *Annu Rev Anim Biosci*. 2021;10:39-62. doi:10.1146/annurev-animal-081221-064325
202. Wu L, Qin Z, Liu H, Lin L, Ye J, Li J. Recent advances on phagocytic B cells in teleost fish. *Front Immunol*. 2020;11:824. doi:10.3389/fimmu.2020.00824
203. Bjorgen H, Koppang EO. Anatomy of teleost fish immune structures and organs. *Immunogenetics*. 2021;73(7):53-63. doi:10.1007/s00251-020-01196-0
204. Romo MR, Perez-Martinez D, Ferrer CC. Innate immunity in vertebrates: an overview. *Immunology*. 2016;148(2):125-139. doi:10.1111/imm.12597
205. Tongsri P, Meng K, Liu X, et al. The predominant role of mucosal immunoglobulin IgT in the gills of rainbow trout (*Oncorhynchus mykiss*) after infection with *Flavobacterium columnare*. *Fish Shellfish Immunol*. 2020;99(2):654-662. doi:10.1016/j.fsi.2020.01.044
206. Lu XJ, Chen J. Specific functions and modulation of teleost monocytes/macrophages: polarization and phagocytosis. *Zool Res*. 2019; 40(3):146-150. doi:10.24272/j.issn.2095-8137.2019.035
207. Wang L, Zhang H, Wang M, et al. The transcriptomic expression of pattern recognition receptors: insight into molecular recognition pattern of various invading pathogens in oyster *Crassostrea gigas*. *Dev Comp Immunol*. 2019;91:1-7. doi:10.1016/j.dci.2018.09.021
208. Wang X, Zhang R, Liu S, Zhang B, Tang L, Lin Q. Characterization and expression analysis of key genes of pathogen-associated molecular pattern-triggered toll-like receptor 5 signaling in the lined seahorse responses to bacterial and parasitic infection. *Aquaculture*. 2022;549:737777. doi:10.1016/j.aquaculture.2021.737777
209. Rauta PR, Nayak B, Das S. Immune system and immune responses in fish and their role in comparative immunity study: a model for higher organisms. *Immunol Lett*. 2012;148(1):23-33. doi:10.1016/j.imlet.2012.08.003
210. Ashfaq H, Soliman H, Saleh M, El-Matbouli M. CD4: a vital player in the teleost fish immune system. *Vet Res*. 2019;50(1):1. doi:10.1186/s13567-018-0620-0
211. Huang Y, Ren Q. Research progress in innate immunity of freshwater crustaceans. *Dev Comp Immunol*. 2020;104:103569. doi:10.1016/j.dci.2019.103569
212. Perazzolo LM, Li C, Somboonwiwat K. Editorial: aquatic invertebrate immunity against infectious diseases. *Front Immunol*. 2021;12: 762082. doi:10.3389/fimmu.2021.762082
213. Roy S, Bossier P, Norouzitallab P, Vanrompay D. Trained immunity and perspectives for shrimp aquaculture. *Rev Aquac*. 2020;12(4): 2351-2370. doi:10.1111/raq.12438
214. Bouallegui Y. A comprehensive review on crustaceans' immune system with a focus on freshwater crayfish in relation to crayfish plague disease. *Front Immunol*. 2021;12:667787. doi:10.3389/fimmu.2021.667787
215. Yang W, Tran NT, Zhu CH, et al. Immune priming in shellfish: a review and an updating mechanistic insight focused on cellular and humoral responses. *Aquaculture*. 2021;530:735831. doi:10.1016/j.aquaculture.2020.735831
216. Cubillo-Martinez AA, Pereyra MA, Garfias Y, Guluarte C, Zenteno E, Sanchez-Salgado JL. Extracellular traps involved in invertebrate immune mechanisms. *Fish Shellfish Immunol*. 2022;121:380-386. doi:10.1016/j.fsi.2022.01.024
217. Sandamalika WMG, Priyathilaka TT, Lee S, Yang H, Lee J. Immune and xenobiotic responses of glutathione S-transferase theta (GST-θ) from marine invertebrate disk abalone (*Haliotis discus discus*): with molecular characterization and functional analysis. *Fish Shellfish Immunol*. 2019;91:159-171. doi:10.1016/j.fsi.2019.04.004
218. Wang Z, Liang X, Li G, et al. Molecular characterization of complement component 3 (C3) in the pearl oyster *Pinctada fucata* improves our understanding of the primitive complement system in bivalve. *Front Immunol*. 2021;12:652805. doi:10.3389/fimmu.2021.652805
219. Auguste M, Balbi T, Ciacci C, Canesi L. Conservation of cell communication systems in invertebrate host-defence mechanisms: possible role in immunity and diseases. *Biology*. 2020;9(8):234. doi:10.3390/biology9080234
220. Lanz-Mendoza H, Contreras-Garduno J. Innate immune memory in invertebrates: concept and potential mechanisms. *Dev Comp Immunol*. 2022;127:104285. doi:10.1016/j.dci.2021.104285
221. Hoseinifar SH, Yousefi S, Doan HV, et al. Oxidative stress and antioxidant defense in fish: the implications of probiotic, prebiotic, and synbiotics. *Rev Fish Sci Aquac*. 2020;29(2):198-217. doi:10.1080/23308249.2020.1795616
222. Silvestre F. Signaling pathways of oxidative stress in aquatic organisms exposed to xenobiotics. *J Exp Zool A Ecol Integr Physiol*. 2020; 333(6):436-448. doi:10.1002/jez.2356
223. Gao Q, Liu B, Shan F, et al. Effects of oxidized fish oil on digestive enzyme activity and antioxidant system in *Macrobrachium rosenbergii* post-larvae. *Aquac Rep*. 2022;23:101062. doi:10.1016/j.aqrep.2022.101062
224. Wang X, Yao Q, Zhang D, et al. Effects of acute salinity stress on osmoregulation, antioxidant capacity and physiological metabolism of female Chinese mitten crabs (*Eriocheir sinensis*). *Aquaculture*. 2022;552:737989. doi:10.1016/j.aquaculture.2022.737989
225. Santana MS, de Melo GD, Sandrini-Neto L, Di Domenico M, Prodocimo MM. A meta-analytic review of fish antioxidant defense and biotransformation systems following pesticide exposure. *Chemosphere*. 2022;291(1):132730. doi:10.1016/j.chemosphere.2021.132730
226. Sies H, Belousov VV, Chandel NS, et al. Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. *Nat Rev Mol Cell Biol*. 2022;23(7):499-515. doi:10.1038/s41580-022-00456-z
227. Dong S, Wang C, Nie H, et al. Type II ice structuring protein (ISP II) gene and its potential role in low temperature tolerance in Manila clam, *Ruditapes philippinarum*. *Aquaculture*. 2022;549:7377723. doi:10.1016/j.aquaculture.2021.737723
228. Rahman M, Khosravi S, Chang KH, Lee S. Effects of dietary inclusion of astaxanthin on growth, muscle pigmentation and antioxidant capacity of juvenile rainbow trout (*Oncorhynchus mykiss*). *Prev Nutr Food Sci*. 2016;21(3):281-288. doi:10.3746/pnf.2016.21.3.281
229. Yi X, Shen H, Li J, et al. Effects of dietary vitamin E and astaxanthin on growth, skin colour and antioxidative capacity of large yellow

croaker *Larimichthys crocea*. *Aquacult Nutr*. 2018;24(1):472-480. doi:10.1111/anu.12580
230. Li MY, Guo WQ, Guo GL, et al. Effects of dietary astaxanthin on lipopolysaccharide-induced oxidative stress, immune responses and glucocorticoid receptor (GR)-related gene expression in *Channa argus*. *Aquaculture*. 2020;517:734816. doi:10.1016/j.aquaculture.2019.734816
231. Zhu X, Hao R, Zhang J, et al. Dietary astaxanthin improves the antioxidant capacity, immunity and disease resistance of coral trout (*Plectropomus leopardus*). *Fish Shellfish Immunol*. 2022;122(4):38-47. doi:10.1016/j.fsi.2022.01.037
232. Han T, Li X, Wang J, Wang C, Yang M, Zheng P. Effects of dietary astaxanthin (AX) supplementation on pigmentation, antioxidant capacity and nutritional value of swimming crab, *Portunus trituberculatus*. *Aquaculture*. 2018;490:169-177. doi:10.1016/j.aquaculture.2018.02.030
233. Wang Z, Cai C, Cao X, et al. Supplementation of dietary astaxanthin alleviated oxidative damage induced by chronic high pH stress, and enhanced carapace astaxanthin concentration of Chinese mitten crab *Eriocheir sinensis*. *Aquaculture*. 2018;483:230-237. doi:10.1016/j.aquaculture.2017.10.006
234. Liu L, Li J, Cai X, et al. Dietary supplementation of astaxanthin is superior to its combination with *Lactococcus lactis* in improving the growth performance, antioxidant capacity, immunity and disease resistance of white shrimp (*Litopenaeus vannamei*). *Aquac Rep*. 2022;24(1):101124. doi:10.1016/j.aqrep.2022.101124
235. Bacchetta C, Rossi AS, Cian RE, Drago SR, Cazenave J. Dietary β-carotene improves growth performance and antioxidant status of juvenile *Piaractus mesopotamicus*. *Aquacult Nutr*. 2019;25(4):761-769. doi:10.1111/anu.12893
236. Fawzy S, Wang W, Zhou Y, et al. Can dietary β-carotene supplementation provide an alternative to astaxanthin on the performance of growth, pigmentation, biochemical, and immuno-physiological parameters of *Litopenaeus vannamei*? *Aquac Rep*. 2022;23:101054. doi:10.1016/j.aqrep.2022.101054
237. Wang P, Zhou X, Xiong G, et al. Effect of dietary canthaxanthin and xanthophyll on growth, antioxidant capacity, body colour, and BCO2, CAT and SOD2 gene expression in Chinese soft-shelled turtle (*Pelodiscus sinensis*). *Aquacult Nutr*. 2021;27(6):2365-2377. doi:10.1111/anu.13369
238. Fawzy S, Wang W, Wu M, Yi G, Huang X. Effects of dietary different canthaxanthin levels on growth performance, antioxidant capacity, biochemical and immune-physiological parameters of white shrimp (*Litopenaeus vannamei*). *Aquaculture*. 2022;556:738276. doi:10.1016/j.aquaculture.2022.738276
239. Mansour AT, El-feky MMM, El-Beltagi HS, Sallam AE. Synergism of dietary co-supplementation with lutein and bile salts improved the growth performance, carotenoid content, antioxidant capacity, lipid metabolism, and lipase activity of the marbled spinefoot rabbitfish, *Siganus rivulatus*. *Animals*. 2020;10(9):1643. doi:10.3390/ani10091643
240. Ramin KI, Allison SD. Bacterial tradeoffs in growth rate and extracellular enzymes. *Front Microbiol*. 2019;10:2956. doi:10.3389/fmicb.2019.02956
241. Bullon N, Seyfoddin A, Alfaro AC. The roles of aquafeeds in abalone nutrition and health: a comprehensive review. *J World Aquac Soc*. 2022. doi:10.1111/jwas.12883
242. Ciji A, Akhtar MS. Stress management in aquaculture: a review of dietary interventions. *Rev Aquac*. 2021;13(4):2190-2247. doi:10.1111/raq.12565
243. Arechavala-Lopez P, Cabrera-Alvarez MJ, Maia CM, Saraiva JL. Environmental enrichment in fish aquaculture: a review of fundamental and practical aspects. *Rev Aquac*. 2022;14(2):704-728. doi:10.1111/raq.12620
244. Liu F, Shi HZ, Guo QS, et al. Effects of astaxanthin and emodin on the growth, stress resistance and disease resistance of yellow catfish (*Pelteobagrus fulvidraco*). *Fish Shellfish Immunol*. 2016;51:125-135. doi:10.1016/j.fsi.2016.02.020
245. Wang W, Ishikawa M, Koshio S, Yokoyama S, Hossain MS, Moss AS. Effects of dietary astaxanthin supplementation on juvenile kuruma shrimp, *Marsupenaeus japonicus*. *Aquaculture*. 2018;491(2):197-204. doi:10.1016/j.aquaculture.2018.03.025
246. Wang W, Ishikawa M, Koshio S, Yokoyama S, Dawood MA, Zhang Y. Effects of dietary astaxanthin supplementation on survival, growth and stress resistance in larval and post-larval kuruma shrimp, *Marsupenaeus japonicus*. *Aquac Res*. 2018;49(6):2225-2232. doi:10.1111/are.13679
247. Liu M, Jiang X, Chen A, Chen T, Cheng Y, Wu X. Transcriptome analysis reveals the potential mechanism of dietary carotenoids improving antioxidative capability and immunity of juvenile Chinese mitten crabs *Eriocheir sinensis*. *Fish Shellfish Immunol*. 2020;104:359-373. doi:10.1016/j.fsi.2020.06.033
248. Haddad NF, Teodoro AJ, de Oliveira FL, et al. Lycopene and beta-carotene induce growth inhibition and proapoptotic effects on ACTH-secreting pituitary adenoma cells. *PLoS One*. 2013;8(5):e62773. doi:10.1371/journal.pone.0062773
249. Yada T, Tort L. Stress and disease resistance: immune system and immunoendocrine interactions. *Fish Physiol*. 2016;35:365-403. doi:10.1016/B978-0-12-802728-8.00010-2
250. Mateus AP, Power DM, Canario AVM. Stress and disease in fish. In: Jeney G, ed. *Fish Diseases – Prevention and Control Strategies*. Academic Press; 2017:187-220.
251. Pisoschi AM, Pop A, Iordache F, Stanca L, Predoi G, Serban AI. Oxidative stress mitigation by antioxidants - an overview on their chemistry and influences on health status. *Eur J Med Chem*. 2021;209:112891. doi:10.1016/j.ejmech.2020.112891
252. Martos-Sitcha JA, Mancera JM, Prunet P, Magnoni LJ. Editorial: welfare and stressors in fish: challenges facing aquaculture. *Front Physiol*. 2020;11:162. doi:10.3389/fphys.2020.00162
253. Bendich A, Shapiro SS. Effect of β-carotene and canthaxanthin on the immune responses of the rat. *J Nutr*. 1986;116(11):2254-2262. doi:10.1093/jn/116.11.2254
254. Cheng Y, Wu S. Effect of dietary astaxanthin on the growth performance and nonspecific immunity of red swamp crayfish *Procambarus clarkii*. *Aquaculture*. 2019;512:734341. doi:10.1016/j.aquaculture.2019.734341
255. Eldessouki EAA, Diab AM, Selema TAMA, et al. Dietary astaxanthin modulated the performance, gastrointestinal histology, and antioxidant and immune responses and enhanced the resistance of *Litopenaeus vannamei* against *Vibrio harveyi* infection. *Aquac Int*. 2022;30(1):1869-1887. doi:10.1007/s10499-022-00876-w
256. Grayfer L, Kerimoglu B, Yaparla A, Hodgkinson JW, Xie J, Belosevic M. Mechanisms of fish macrophage antimicrobial immunity. *Front Immunol*. 2018;9:1105. doi:10.3389/fimmu.2018.01105
257. Uribe-Querol E, Rosales C. Phagocytosis: our current understanding of a universal biological process. *Front Immunol*. 2020;11:1066. doi:10.3389/fimmu.2020.01066
258. Young AJ, Lowe GM. 2001. Antioxidant and prooxidant properties of carotenoids. *Arch Biochem Biophys*. 2001;385(1):20-27. doi:10.1006/abbi.2000.2149
259. Halliwell B. Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and in vivo studies? *Arch Biochem Biophys*. 2008;476(2):107-112. doi:10.1016/j.abb.2008.01.028
260. Ivanova IP, Trofimova SV, Piskarev IM. Evaluation of prooxidant properties of ascorbic acid. *Biofizika*. 2013;58(4):582-586. doi:10.1134/S0006350913040076
261. Rahal A, Kumar A, Singh V, et al. Oxidative stress, prooxidants, and antioxidants: the interplay. *Biomed Res Int*. 2014;2014:761264. doi:10.1155/2014/761264
262. Ribeiro D, Freitas M, Silva AMS, Carvalho F, Fernandes E. Antioxidant and pro-oxidant activities of carotenoids and their oxidation products. *Food Chem Toxicol*. 2018;120:681-699. doi:10.1016/j.fct.2018.07.060

263. Arias A, Feijoo G, Moreira MT. Exploring the potential of antioxidants from fruits and vegetables and strategies for their recovery. *IFSET*. 2022;77(10):102974. doi:10.1016/j.ifset.2022.102974
264. Jyonouchi H, Sun S, Gross M. Effect of carotenoids on in vitro immunoglobulin production by human peripheral blood mononuclear cells: astaxanthin, a carotenoid without vitamin a activity, enhances in vitro immunoglobulin production in response to a T-dependent stimulant and antigen. *Nutr Cancer*. 1995;23(2):171-183. doi:10.1080/01635589509514373
265. Park JS, Chyun JH, Kim YK, Line LL, Chew BP. Astaxanthin decreased oxidative stress and inflammation and enhanced immune response in humans. *Nutr Metab (Lond)*. 2010;7:18. doi:10.1186/1743-7075-7-18
266. Lyu Y, Wu L, Wang F, Shen X, Lin D. Carotenoid supplementation and retinoic acid in immunoglobulin a regulation of the gut microbiota dysbiosis. *Exp Biol Med*. 2018;243(7):613-620. doi:10.1177/1535370218763760
267. Gong HZ, Wu M, Lang WY, et al. Effects of laying breeder hens dietary β-carotene, curcumin, allicin, and sodium butyrate supplementation on the growth performance, immunity, and jejunum morphology of their offspring chicks. *Poult Sci*. 2020;99(1):151-162. doi:10.3382/ps/pez584
268. Chen J, Chen J, Zhang Y, et al. Effects of maternal supplementation with fully oxidised β-carotene on the reproductive performance and immune response of sows, as well as the growth performance of nursing piglets. *Brit J Nutr*. 2021;125(1):62-70. doi:10.1017/S0007114520002652
269. Fan Q, Chen Z, Wu Y, Zhu J, Yu Z. Study on the enhancement of immune function of astaxanthin from *Haematococcus pluvialis*. *Foods*. 2021;10(8):1847. doi:10.3390/foods10081847
270. Durge SM, Das A, Saha SK. Dietary lutein supplementation improves immunity and antioxidant status of captive Indian leopards (*Panthera fusca*). *Zoo Biol*. 2022;41(4):328-339. doi:10.1002/zoo.21671
271. Pohlenz C, Gatlin DM. Interrelationships between fish nutrition and health. *Aquaculture*. 2014;431:111-117. doi:10.1016/j.aquaculture.2014.02.008
272. Kumar S, Choubey AK, Srivastava PK. The effects of dietary immunostimulants on the innate immune response of Indian major carp: a review. *Fish Shellfish Immunol*. 2022;123:36-49. doi:10.1016/j.fsi.2022.02.039
273. Kumar S, Verma AK, Singh SP, Awasthi A. Immunostimulants for shrimp aquaculture: paving pathway towards shrimp sustainability. *Environ Sci Pollut Res*. 2022. doi:10.1007/s11356-021-18433-y
274. Sudhakaran G, Guru A, Haridevamuthu B, Murugan R, Arshad A, Arockiaraj J. Molecular properties of postbiotics and their role in controlling aquaculture diseases. *Aquacult Res*. 2022;53(9):3257-3273. doi:10.1111/are.15846
275. Lee CL, Chang CC, Kuo HW, Cheng W. Pectin of cacao pod husk, an efficient immunostimulant for white shrimp, *Litopenaeus vannamei*. *Fish Shellfish Immunol*. 2020;107:357-366. doi:10.1016/j.fsi.2020.10.026
276. Roy AL. Transcriptional regulation in the immune system: one cell at a time. *Front Immunol*. 2019;10:1355. doi:10.3389/fimmu.2019.01355
277. Devenish LP, Mhlanga MM, Negishi Y. Immune regulation in time and space: the role of local- and long-range genomic interactions in regulating immune responses. *Front Immunol*. 2021;12:662565. doi:10.3389/fimmu.2021.662565
278. Duque GA, Descoteaux A. Macrophage cytokines: involvement in immunity and infectious diseases. *Front Immunol*. 2014;5:491. doi:10.3389/fimmu.2014.00491
279. Abdel-Latif HMR, Shukry M, Abd-elaziz RA. Clinico-pathological findings and expression profile of inflammatory cytokines, apoptosis, and oxidative stress-related genes draw mechanistic insights in Nile tilapia reared under ammonia-N exposure and *Aeromonas hydrophila* challenge. *Fish Shellfish Immunol*. 2022;127(2):1-12. doi:10.1016/j.fsi.2022.06.001
280. Li S, Yang Z, Tian H, Ren S, Zhang W, Wang A. Effects of dietary carbohydrate/lipid ratios on non-specific immune responses, antioxidant capacity, hepatopancreas and intestines histology, and expression of TLR-MAPK/NF-κB signaling pathway-related genes of *Procambarus clarkii*. *Fish Shellfish Immunol*. 2022;124:219-229. doi:10.1016/j.fsi.2022.04.005
281. Li MY, Liu XY, Xia CG, Wang GQ, Zhang DM. Astaxanthin enhances hematology, antioxidant and immunological parameters, immune-related gene expression, and disease resistance against in *Channa argus*. *Aquacult Int*. 2019;27(3):735-746. doi:10.1007/s10499-019-00362-w
282. Yu Z, Quan YN, Huang ZQ, Wang HH, Wu LF. Monitoring oxidative stress, immune response, Nrf2/NF-κB signaling molecules of *Rhynchocypris lagowski* living in BFT system and exposed to waterborne ammonia. *Ecotoxicol Environ Saf*. 2020;205:111161. doi:10.1016/j.ecoenv.2020.111161
283. Petit J, David L, Dirks R, Wiegertjes GF. Genomic and transcriptomic approaches to study immunology in cyprinids: what is next? *Dev Comp Immunol*. 2017;75:48-62. doi:10.1016/j.dci.2017.02.022
284. Yang B, Zhai S, Zhang F, et al. Genome-wide association study toward efficient selection breeding of resistance to *Vibrio alginolyticus* in Pacific oyster, *Crassostrea gigas*. *Aquaculture*. 2022;548:737592. doi:10.1016/aquaculture.2021.737592
285. Mordi RC, Ademosun OT, Ajanaku CO, Olanrewaju IO, Walton JC. Free radical mediated oxidative degradation of carotenes and xanthophylls. *Molecules*. 2020;25(5):1038. doi:10.3390/molecules25051038

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