



LA TECNOLOGÍA DE ACTIVACIÓN MOLECULAR EN DESAFÍOS Y PRODUCTIVIDAD

ESTRÉS OXIDATIVO, INMUNIDAD Y
PRODUCCIÓN ACUÍCOLA

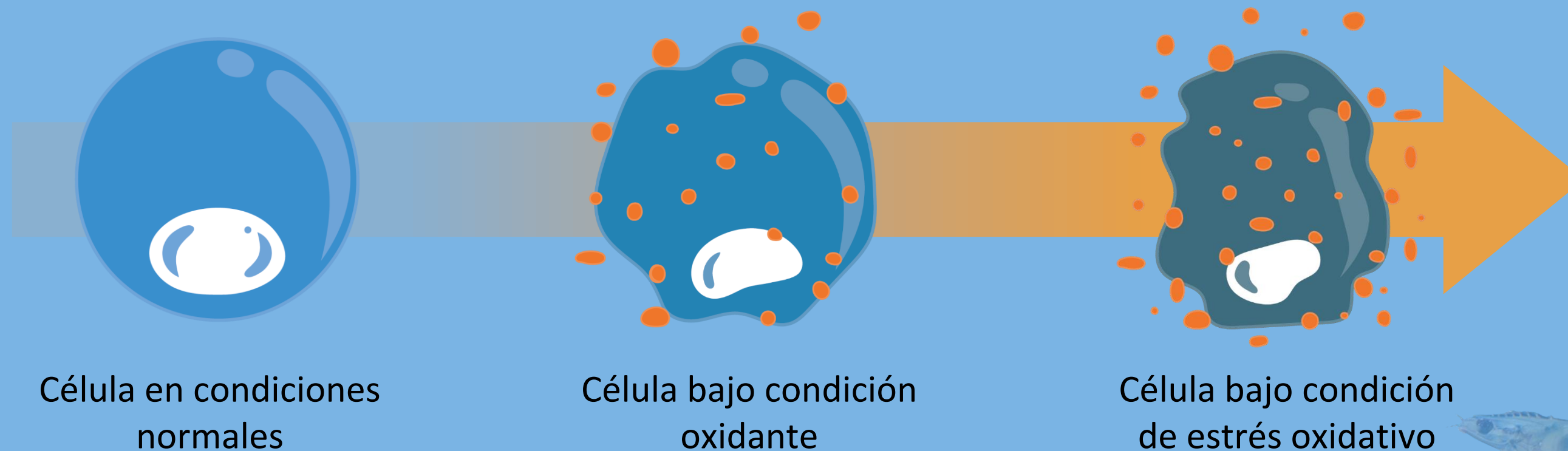
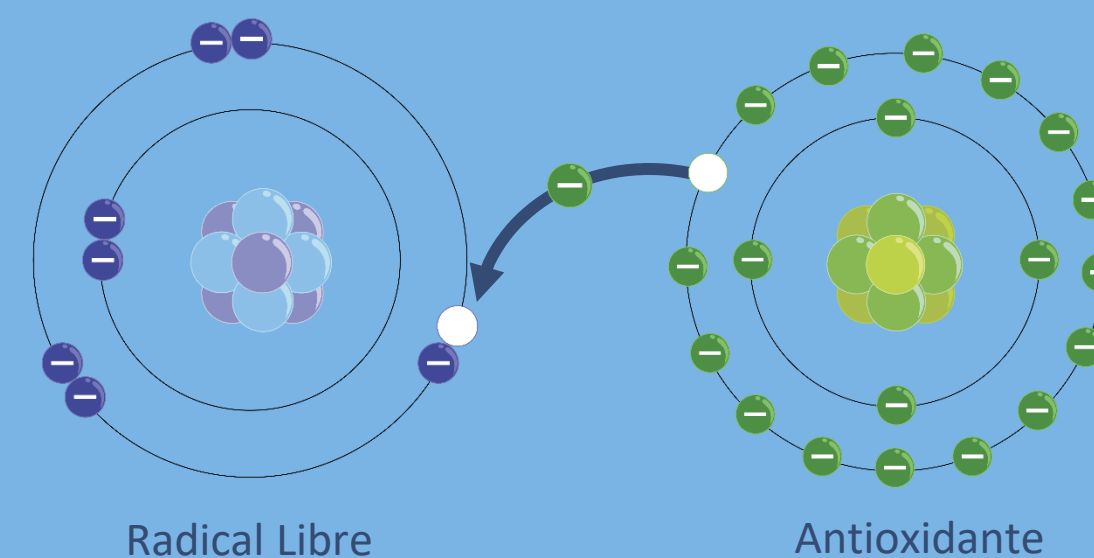
ÍNDICE

- ¿QUÉ ES EL ESTRÉS OXIDATIVO?
- ESTRÉS OXIDATIVO E INMUNIDAD EN CAMARÓN
- PRINCIPALES DESAFÍOS EN CAMARÓN
- FACTORES OXIDANTES EN LA PRODUCCIÓN CAMARONERA
- TECNOLOGÍA DE ACTIVACIÓN MOLECULAR
- IMPLEMENTACIÓN DE ANTIOXIDANTES ACTIVADOS EN PRODUCCIÓN CAMARONERA
- CONCLUSIONES



ESTRÉS OXIDATIVO

El estrés oxidativo es un **proceso que se produce** en los organismos vivos debido a la producción y acumulación en **exceso de radicales libres** y a la **falta de antioxidantes para contrarrestarlos**. El aumento de estos radicales libres y principalmente los de oxígeno en el organismo da lugar a que las células se oxiden, afectando a sus funciones y dañándolas.



ESTRÉS OXIDATIVO E INMUNIDAD EN CAMARÓN

FACTORES QUÍMICOS

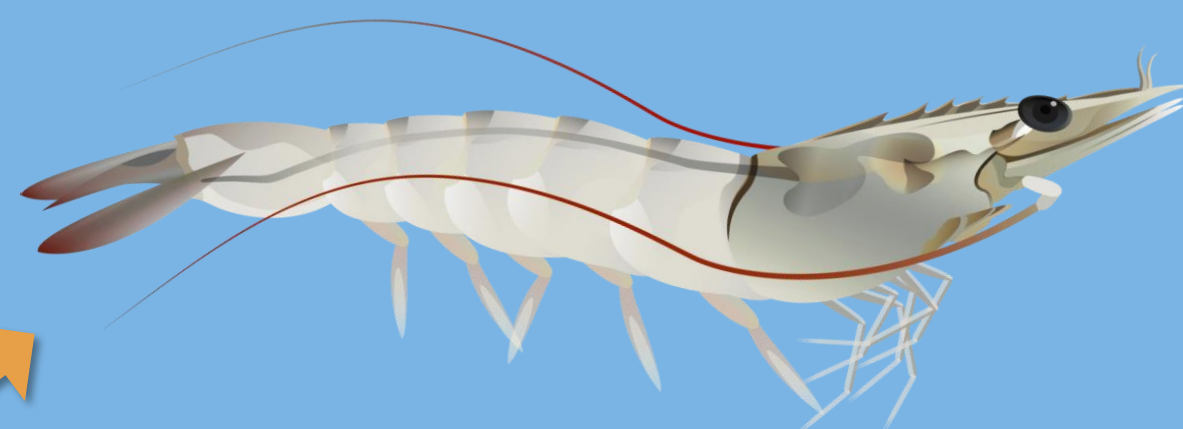
CALIDAD DEL AGUA,
SALINIDAD, NITRITOS, CO₂,
O₂

FACTORES FÍSICOS

pH, TEMPERATURA,
TRANSPORTE, PESAJE,
SEPARACIÓN, VACUNACIÓN...

OTROS FACTORES

ALIMENTACIÓN, RUIDOS,
ILUMINACIÓN



PRIMERA RESPUESTA A ESTRÉS

INCREMENTO DE LA HORMONA DEL ESTRÉS
(CORTISOL)

SEGUNDA RESPUESTA A ESTRÉS

CAMBIOS METABÓLICOS (AUMENTA LA
GLUCOSA, LACTATO, CAMBIOS EN EL SISTEMA
DE DEFENSAS)

TERCERA RESPUESTA A ESTRÉS

CAMBIOS EN EL ESTADO DE SALUD
(CRECIMIENTO, DESARROLLO Y DEFENSAS) Y
COMPORTAMIENTO (PÉRDIDA DE APETITO)

Bajo diferentes condiciones de estrés, la producción de los diferentes animales en acuicultura puede verse afectada de forma negativa, disminuyendo la calidad, la capacidad de crecimiento y de defensa.



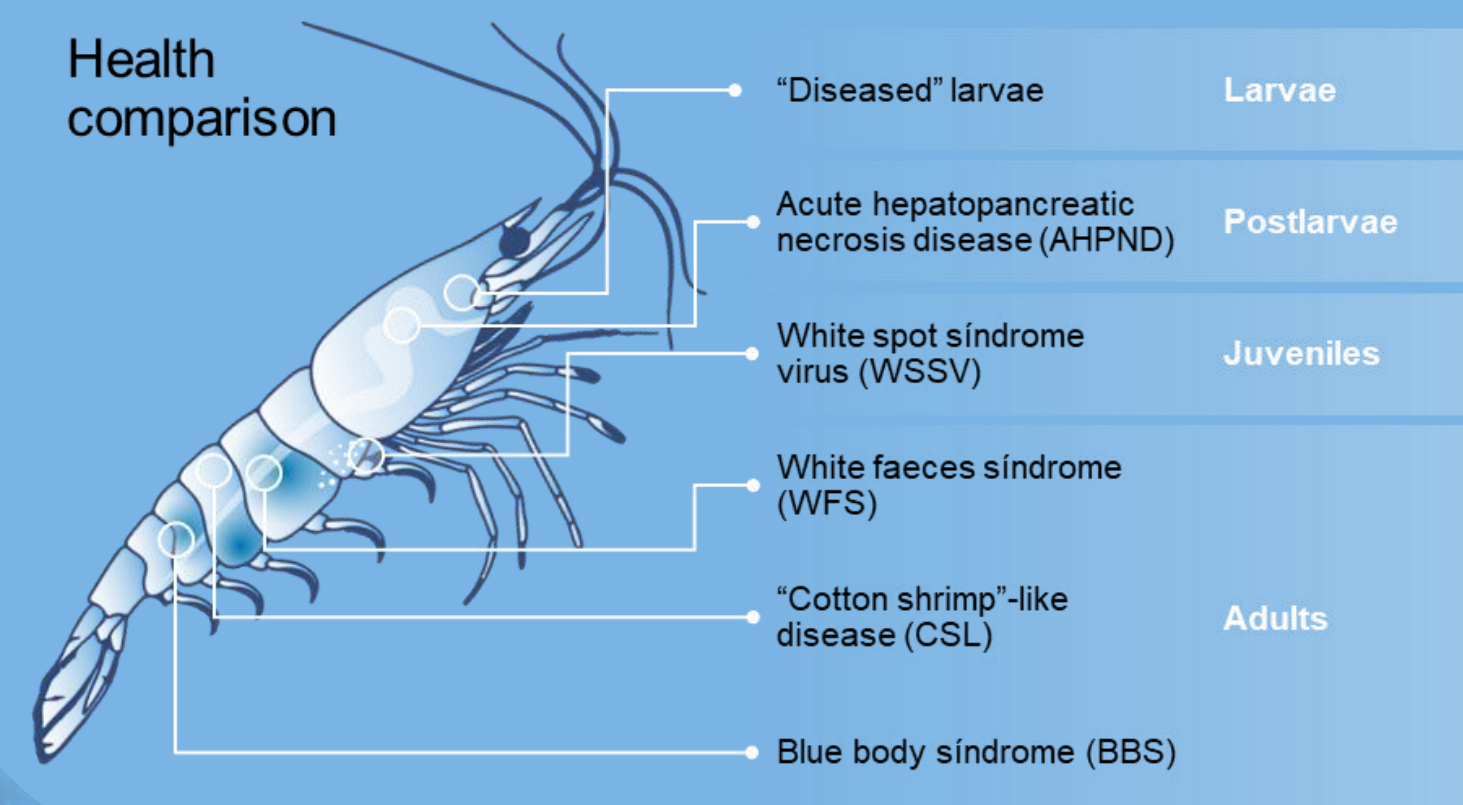
PRINCIPALES DESAFÍOS EN CAMARÓN

Las **enfermedades** y la **calidad de los ingredientes** de los alimentos acuícolas son sin duda los desafíos más importantes que enfrenta actualmente la industria del camarón.

La última década más o menos ha visto la aparición de dos enfermedades nuevas y muy graves, la enfermedad EMS/AHPND (Síndrome de Mortalidad Temprana/Necrosis Hepatopancreática Aguda) causada por una bacteria, *Vibrio parahaemolyticus*; y una nueva enfermedad llamada Microsporidiosis Hepatopancreática (HPM) causada por un parásito microsporidiano, formador de esporas, intracelular (*Enterocytozoon hepatopenaei*, o EHP).

Los principales factores a tener en cuenta en la producción de camarón son: **condiciones del ambiente (contaminantes), la dieta, la flora digestiva y el estrés.**

Su sistema inmune primigenio presenta dificultades en las infecciones ya que carecen de respuesta inmune específico.



PARÁMETRO	VALOR
Temperatura	31 - 33 °C
Oxígeno disuelto	4 - 10 ppm
CO ₂	< 20 ppm
pH	7.0 - 8.5
Amonio no ionizado (NH ₃)	< 0.03 ppm
Nitrito (NO ₂ -)	< 1 ppm
Hierro total	< 1 ppm
Sulfuro de Hidrógeno (H ₂ S)	< 1 ppm

FACTORES OXIDANTES EN LA PRODUCCIÓN CAMARONERA

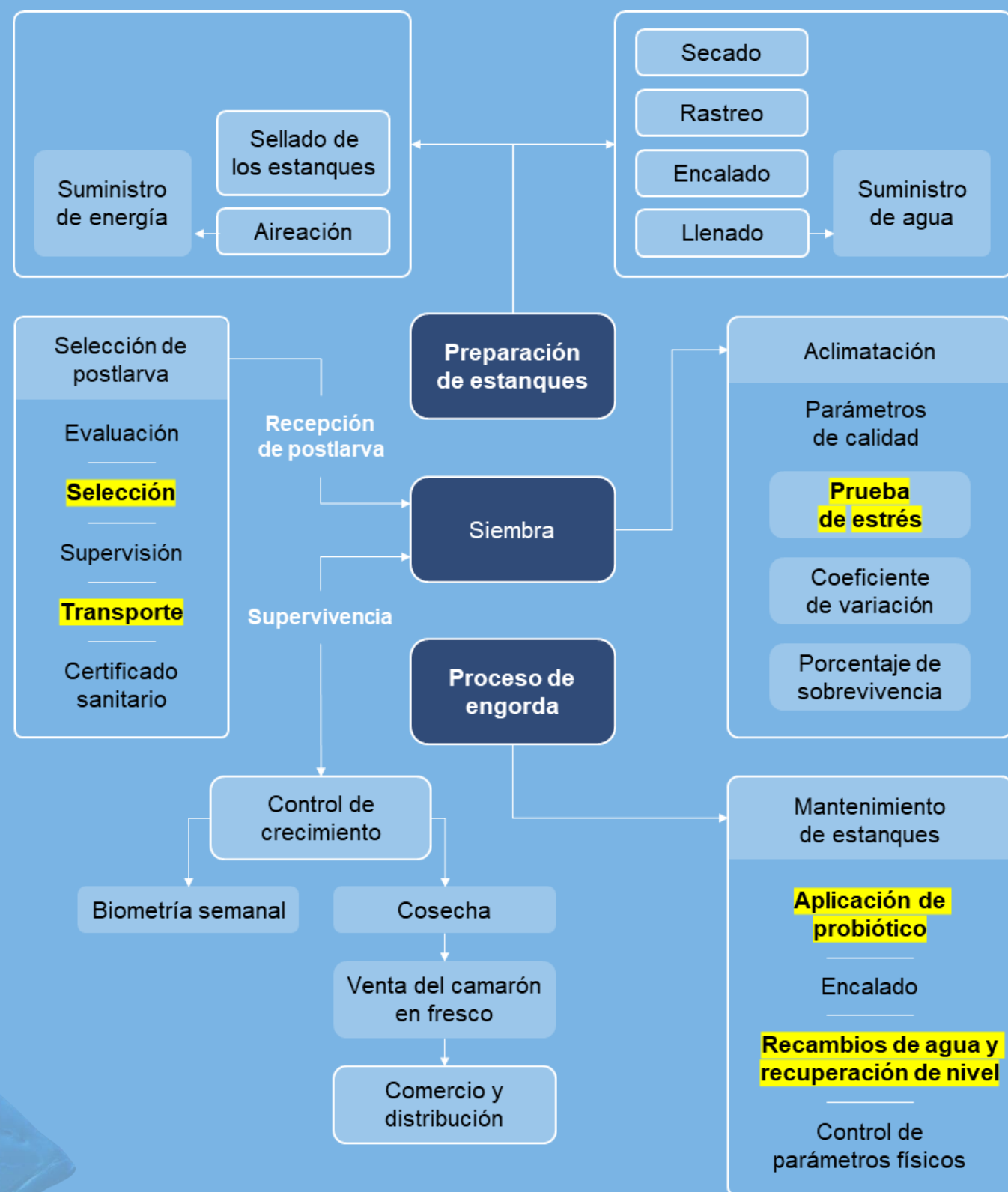
Organism	Species	Size/life stage	Temperature	Factor	Tissue	References
Shrimp	<i>Litopenaeus vannamei</i>	11 g, 4 cm	13°C	SOD, POD, CAT, GSH-Px, T-AOC	Hepatopancreas, haemolymph	Xu et al., 2018
	<i>Litopenaeus vannamei</i>	4.59 ± 0.5 g	13°C	IAP, p53, HSP70	Intestine	Wang et al., 2020
	<i>Litopenaeus vannamei</i>	1.91 ± 0.22 g	15°C	CGL, GSH, TBARS	Hepatopancreas, haemolymph	de Souza et al., 2016
	<i>Litopenaeus vannamei</i>	5.01 ± 0.46 g	12 ± 2°C	MDA	Haemolymph	Qiu et al., 2011
	<i>Litopenaeus vannamei</i>	7.09 ± 3.22 g	13°C	Ser/Thr kinase signal pathway	Muscle	Huang et al., 2017
	<i>Penaeus monodon</i>	16.5 ± 0.6 g	15°C	O ₂ ⁻ , SOD, GSH, NOS, NO	Hepatopancreas	Jiang et al., 2019
	<i>Penaeus monodon</i>	3.96 ± 0.82 g	20°C	SOD, ACP, PO	Haemolymph	Yang et al., 2013
	<i>Marsupenaeus japonicus</i>	13.034 ± 0.88 g	10°C	p53, CYCS, Bax, Bcl2, caspase-3	Hepatopancreas	Ren et al., 2020
	<i>Fenneropenaeus chinensis</i>	P40	4°C	GST, C-type lectin, ASAH,	Whole body	Meng et al., 2019
	<i>Macrobrachium rosenbergii</i>	30.2 ± 4.1 g	22°C	THCs, PO, proPO, RBs, LGBP, PE, a ₂ -M, SOD	Haemolymph	Chang et al., 2015
	<i>Macrobrachium nipponense</i>	0.66 ± 0.03 g	29°C	ALT, SOD, CAT, MDA, INOS	Hepatopancreas, haemolymph	Lv et al., 2021
	<i>Cherax quadricarinatus</i>	22.56 ± 1.25 g	9 ± 2°C	ACP, AKP, LSZ, PO	Hepatopancreas	Wu et al., 2019
	<i>Cherax quadricarinatus</i>	22.56 ± 1.25 g	9°C	HSP21, THC, SOD, T-AOC, GPx, MDA	Hepatopancreas, haemolymph	Wu et al., 2018
Crab	<i>Scylla serrata</i>	145 ± 20 g	4°C	SOD, CAT, GPX, MDA	Gills	Kong et al., 2007
	<i>Portunus trituberculatus</i>	213.8 ± 21.6 g	3°C	SOD, CAT, MDA, PC, caspase-3, HSP70, HSP90	Hepatopancreas, muscle	Meng et al., 2014
	<i>Carcinus aestuarii</i>	4 cm	4°C	CAT, THC	Gills, haemolymph	Matozzo et al., 2011
	<i>Carcinus aestuarii</i>	1–1.7 g	4°C	THC, DCH, NRRT	Haemolymph	Qyli et al., 2020

a₂-M, a₂-macroglobulin; AChE, acetyl cholinesterase; ACP, acid phosphatase; ALP/AKP, alkaline phosphatase; ALT, alanine aminotransferase; Bcl2, B-cell leukemia/lymphoma-2; Bax, Bcl-2-associated X protein; CAT, catalase; CSP, cyclophosphamide; Cu/Zn-SOD, Cu/Zn superoxide dismutase; CYCS, cytochrome C; DCH, differential hemocyte count; GCL, glutamate-cysteine ligase; GSH, glutathione; GSH-Px/GPx, glutathione peroxidase; GST, glutathione s-transferase; HSP21, heat shock protein 21; HSP70, heat shock protein 70; HSP90, heat shock protein 90; IAP, inhibitor of apoptosis protein; INOS, inducible nitric oxide synthase; LDH, lactate dehydrogenase; LGBP, lipopolysaccharide- and b-1,3-glucan binding protein; LSZ, lysozyme; MDA, malondialdehyde; NO, nitric oxide; NOS, nitric oxide synthase; NRRT, neutral red retention time; O₂⁻, negative ions of oxygen; p53, a tumor suppressor gene; PC, protein carbonyl; PE, peroxinectin; PO, polyphenol oxidase; POD, peroxidase; RBs, respiratory bursts; ROS, reactive oxygen species; SeGpx, Selenium containing glutathione peroxidase; SOD, superoxide dismutase, T-AOC, total antioxidant capacity; TBARS, thiobarbituric acid reactive substance; THC, total hemocyte count; Trx, thioredoxin reductase.

La temperatura baja está estrechamente relacionada con el sistema inmunológico y antioxidante de camarones y cangrejos (ver Tabla izquierda), y es el factor de estrés más importante en la acuicultura. La baja temperatura no solo causa un trastorno del metabolismo de los radicales libres, daña la función fisiológica normal y la capacidad de defensa inmunitaria de las células y los tejidos, y afecta directamente el metabolismo de los animales acuáticos, sino que también afecta el oxígeno disuelto y otros factores ambientales, lo que lleva a la susceptibilidad de camarones y cangrejos a patógenos.

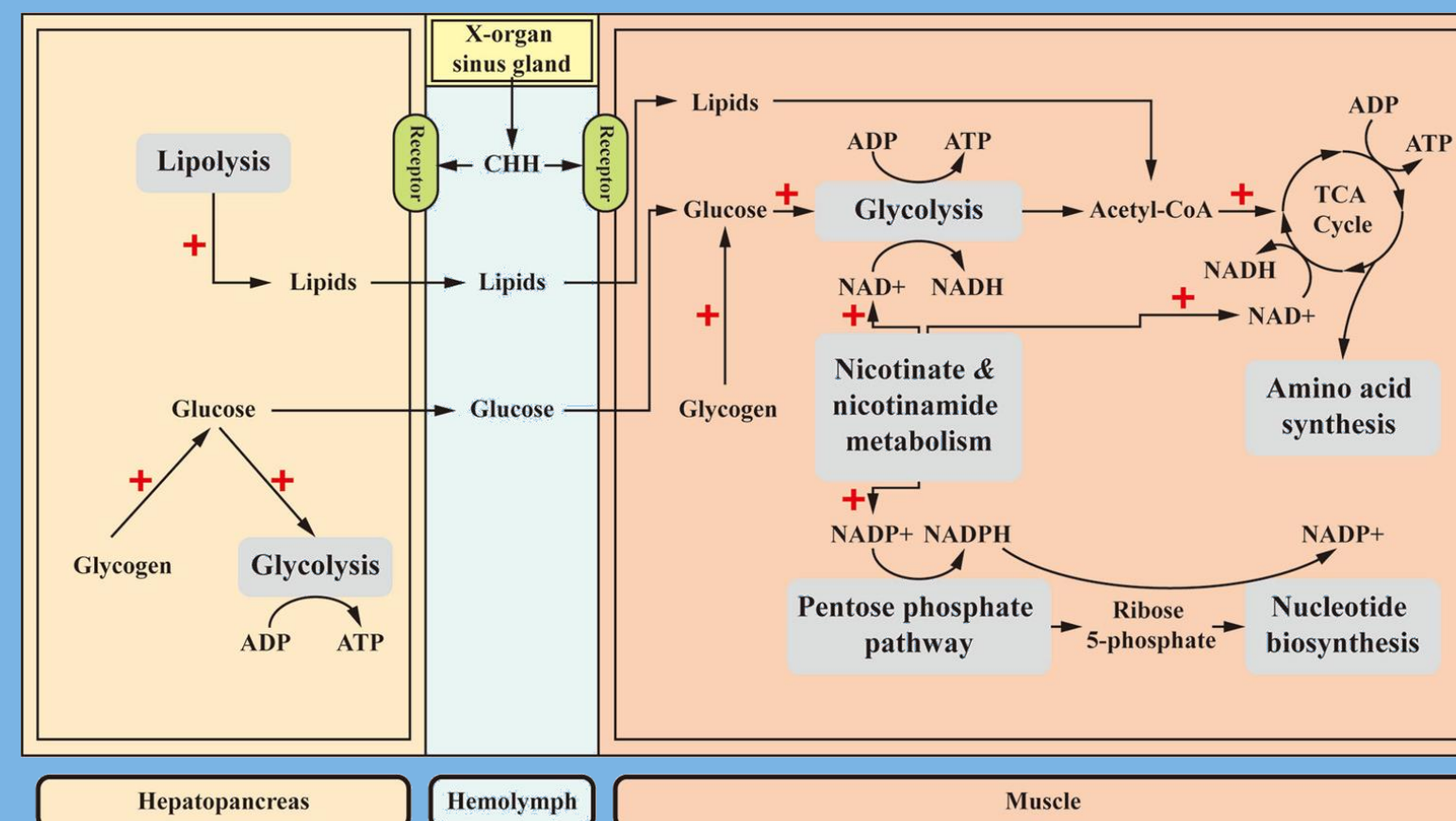


FACTORES OXIDANTES EN LA PRODUCCIÓN CAMARONERA



La osmorregulación en crustáceos está bajo control neuroendócrino por las hormonas hiperglucémicas de crustáceos (CHHs, producida en el complejo órgano X/glándula sinusal (XO/SG) ubicado en cada pedúnculo ocular y liberada en situaciones de estrés), las cuales han mostrado modificar las concentraciones de agua e iones en diversas especies de crustáceos como en *L. vannamei*.

Algunos miembros de la familia CHH también se encuentran expresados en otros tejidos neuronales como el ganglio torácico y el ganglio subesofágico, órgano pericárdico, las branquias, el espermatozoido y el intestino. Además, se ha demostrado que los neuropéptidos de la familia CHH actúan en el **metabolismo** y la **osmorregulación**, así como en la **defensa inmunitaria**.



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UTILIZACIÓN DE ANTIOXIDANTES EN PRODUCCIÓN ACUÍCOLA



antioxidants



Article

An Antioxidant Supplement Function Exploration: Rescue of Intestinal Structure Injury by Mannan Oligosaccharides after *Aeromonas hydrophila* Infection in Grass Carp (*Ctenopharyngodon idella*)

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Abstract: Mannan oligosaccharides (MOS) are a type of functional oligosaccharide which have received increased attention because of their beneficial effects on fish intestinal health. However, intestinal structural integrity is a necessary prerequisite for intestinal health. This study focused on exploring the protective effects of dietary MOS supplementation on the grass carp's (*Ctenopharyngodon idella*) intestinal structural integrity (including tight junction (TJ) and adherent junction (AJ)) and its related signalling molecule mechanism. A total of 540 grass carp (215.85 ± 0.30 g) were fed six diets containing graded levels of dietary MOS supplementation (0, 200, 400, 600, 800 and 1000 mg/kg) for 60 days. Subsequently, a challenge test was conducted by injection of *Aeromonas hydrophila* for 14 days. We used ELISA, spectrophotometry, transmission electron microscope, immunohistochemistry, qRT-PCR and Western blotting to determine the effect of dietary MOS supplementation on intestinal structural integrity and antioxidant capacity. The results revealed that dietary MOS supplementation protected the microvillus of the intestine; reduced serum diamine oxidase and D-lactate levels ($p < 0.05$); enhanced intestinal total antioxidant capacity ($p < 0.01$); up-regulated most intestinal TJ and AJ mRNA levels; and decreased GTP-RhoA protein levels ($p < 0.01$). In addition, we also found several interesting results suggesting that MOS supplementation has no effects on ZO-2 and Claudin-15b. Overall, these findings suggested that dietary MOS supplementation could protect intestinal ultrastructure, reduce intestinal mucosal permeability and maintain intestinal structural integrity via inhibiting MLCK and RhoA/ROCK signalling pathways.

Keywords: permeability; intestine; tight junction; adherent junction; antioxidant capacity



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En general, estos hallazgos sugirieron que la suplementación con MOS en la dieta podría proteger la ultraestructura intestinal, reducir la permeabilidad de la mucosa intestinal y mantener la integridad estructural intestinal mediante la inhibición de las vías de señalización MLCK y RhoA/ROCK.

REVIEWS IN Aquaculture

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Natural antioxidants from sea: a potential industrial perspective in aquafeed formulation

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Abstract

Aquaculture is set to grow amidst threats of new stressors and diseases. The increasing awareness on nutrition and feeding has led a paradigm shift towards therapeutic nutrition, an alternative aquaculture management strategy that can create a balance between productivity and long-term sustainability. The core objective behind this approach was to minimize the impact of stressors via neutralization of free radicals, repair of oxidative damage to biomolecules and membrane systems, immune augmentation and maintenance of normal physiological homeostasis. The eventual shift of balance between oxidants and antioxidants leads to oxidative stress and subsequently immune suppression, pathological symptoms and slow growth. Therefore, in aquaculture the use of supplemental antioxidants and augmentation of endogenous cellular antioxidants becomes essential. Lipid rancidity is the major concern, which determines feed stability and storage time, besides the cellular antioxidant homeostasis. As observed, ethoxyquin (EQ), the widely used synthetic antioxidant in animal feed industry, has growing human health hazard concerns. Efficient and cost-effective natural antioxidants are a real need of time. The most diverse marine ecosystem opens a new horizon for extraction and development of natural antioxidants from sea. The antioxidants such as vitamin E, vitamin C, peptides, amino acids, chitooligosaccharide derivatives (COS), astaxanthin, carotenoids, sulphated polysaccharides (SPs), phlorotannins, phenolic compound and flavones had shown a great potential to be used in feed formulation, as an additive for feed quality maintenance and shelf life. Therefore, new industrial perspectives and novel approaches are required for isolation and development of bioactive substances with antioxidative property for cost-effective feed.

Key words: feed, industrial perspectives, natural antioxidants, oxidative stress, sea.

Introduction

Increasing global population and demand for animal protein have set the course of all animal production system towards intensive zone. Among all meat production systems, aquaculture records the fastest growth rate. Quality feed and ecofriendly management practice make the foundation pillars for aquaculture. But aquaculture industry has been in a boom-and-bust pattern due to its vulnerability to a host pathogen and environment interaction. Initial

residues in environment and potential oxidative stress to the host leading to immunosuppression besides slow growth (Holmstrom *et al.* 2003). The oxidative stress due to various factors such as pesticides, heavy metals, sanitizers and chemotherapeutics, high temperature and hypoxia had been found across the various culture systems. After all, as the industry sets to grow amidst the threat of existing as well as that of new stressors and diseases, there is an increase in awareness on existing culture management practices within the industry. Subsequently, it has proce-

Se requieren nuevas perspectivas industriales y enfoques novedosos para el aislamiento y desarrollo de sustancias bioactivas con propiedades antioxidantes para alimentos rentables.

REVIEWS IN FISHERIES SCIENCE & AQUACULTURE
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REVIEW



Oxidative Stress and Antioxidant Defense in Fish: The Implications of Probiotic, Prebiotic, and Synbiotics

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ABSTRACT

In fish, like other organisms, the lack of balance between the production of reactive oxygen species (ROS) and antioxidant defense system (so-called oxidative stress) can cause DNA hydroxylation, protein denaturation, lipid peroxidation, apoptosis, and ultimately cell damage. To improve the antioxidant defense capability, different approaches such as the administration of synthetic antioxidants were practiced. During the past years, dietary approaches have been suggested as a promising way of increasing the antioxidant defense activity in different fish and shellfish species. Among them, microbial feed additives (including pre-, pro-, and synbiotics) showed promising effects in terms of affecting antioxidant enzymes activities. Their mechanism of action in influencing the antioxidant system is not fully understood. The present review briefly discussed the antioxidant defense activity in fish, influencing factors with special focus on dietary approaches and microbial feed additives. In addition, the proposed mechanism of action of microbial feed additives on the antioxidant system has been discussed.

KEYWORDS

Oxidative stress; antioxidant defense; feed additives; probiotics; synbiotic

1. Introduction

Under physiological processes in the body, the elevated intracellular levels of reactive oxygen species (ROS) related to oxidative stress can cause destructive effects on lipids, proteins, and DNA (Schieber and Chande 2014). In fact, the lack of balance between production of ROS and antioxidant defense that called oxidative stress can cause DNA hydroxylation, protein denaturation, lipid peroxidation, apoptosis, and ultimately cell damage (Martínez-Álvarez *et al.* 2005). The hydroxyl radicals and hydrogen peroxides are among the most important free oxygen radicals within the ROS. To avoid the negative effects of naturally producing ROS, the living organisms developed an antioxidant defense system with two distinct classes: 1) the enzymatic antioxidant system including different enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT); and 2) the non-enzyme antioxidants such as glutathione, thioredoxin, vitamin C and vitamin E (Mishra *et al.* 2015). These elements of antioxidant defense system are capable of providing

the balance between production and removal of ROS under normal physiological conditions (Martínez-Álvarez *et al.* 2005). The presence of such system is of high importance because malfunctioning can cause an imbalance in the system of homeostasis and oxidative stress (Winston and Di Giulio 1991; Livingstone 2001; Zenteno-Savín *et al.* 2006). Also, it has been reported that there is a clear relation between physiological status and organism antioxidant defense (Martínez-Álvarez *et al.* 2005). The increase of efficiency and levels of antioxidant defense has an effective role in mediating host benefits (Figure 1). This has led to extensive research attempts to increase antioxidant enzyme activities of fish and shellfish. For many years, synthetic antioxidants (e.g. butylated hydroxyl anisole and butylated hydroxyl toluene) have been used to increase the antioxidant defense levels and inhibiting lipid peroxidation, but the use of synthetic compounds is becoming more restricted since they present numerous side effects for the environment and health safety includes the liver damage and cancer (Williams *et al.* 1999). During the past years, there were extensive research attempts aimed at

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Estos resultados destacaron los efectos potencialmente beneficiosos de los simbióticos dietéticos sobre la defensa antioxidante en los peces. Los resultados obtenidos alientan más intentos de investigación sobre varios aspectos de la administración de simbióticos para la modulación de las actividades de las enzimas antioxidantes.

UTILIZACIÓN DE PLANTAS EN PRODUCCIÓN ACUÍCOLA



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Effects of Dietary Glycyrrhizin on Growth and Nonspecific Immunity of White Shrimp, *Litopenaeus vannamei*

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Abstract

The growth response, total hemocyte count (THC), respiratory burst (release of superoxide anion), phenoloxidase (PO) activity, superoxide dismutase (SOD) activity, nitric oxide synthase (NOS) activity as well as resistance to the pathogen *Vibrio alginolyticus* were measured in *Litopenaeus vannamei*, which had been fed diets supplemented with glycyrrhizin (the aqueous extract of licorice, *Glycyrrhiza glabra*, roots) at 0, 50, 100, 150, and 200 mg/kg of feed for 8 wk. In the feeding trial, there was no significant difference in survival rate ($P > 0.05$). Significant higher specific growth rate was observed in treatments with dietary glycyrrhizin than that in the control group ($P < 0.05$). Shrimp fed a diet with 200 mg/kg of glycyrrhizin had significant higher THC, PO activity, respiratory burst activity and SOD activity than that in the control group ($P < 0.05$). Increased THC, respiratory burst activity and NOS activity were also noticed in shrimp fed glycyrrhizin at 100 or 150 mg/kg ($P < 0.05$). There were significantly lower cumulative mortalities after the disease challenge in shrimp fed glycyrrhizin diets (8.33–16.67%) than that in the control group (36.67%) ($P < 0.05$). These results indicated that glycyrrhizin may enhance the nonspecific immune system, increase the resistance of *L. vannamei* as well as improve production in shrimp farming.

During the past several years, penaeid shrimps have suffered problems of infectious diseases due to vibriosis such as *Vibrio harveyi* (Liu et al. 1996), *Vibrio damsela* (Song et al. 1993), and *Vibrio alginolyticus* (Lee et al. 1996), in addition to viruses such as monodon baculovirus (Lightner et al. 1987), white spot syndrome virus (Lo and Kou 1998), Taura syndrome virus (Yu and Song 2000), and infectious hypodermal and hematopoietic necrosis virus (Lo et al. 2003). In addition, these infectious diseases in shrimp culture have reduced production levels during the last decade. The increase in production in the areas that have recovered

from diseases has not been able to compensate for the decrease in production in the areas that are still suffering from disease outbreaks (Gullian et al. 2004). Thus, many measures have been taken to control these diseases, such as the routine use of antibiotics. However, the excessive and inappropriate use of antibiotics has resulted in the presence of resistant strains of bacteria in shrimp culture. A number of other preventive approaches, such as the use of vaccines (Subasinghe 1997), immunostimulants (Sakai 1999), and probiotics (Rengpipat et al. 2000; Gullian et al. 2004), have been explored in order to reduce the losses due to diseases and mortality of cultured stock.

It is well known that crustaceans lack a truly adaptive immune response system and appear to rely on a variety of innate immune response

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Research Paper

EFFECTS OF MYRISTICA FRAGRANS, GLYCYRRHIZA GLABRA AND QUERCUS INFECTORIA ON GROWTH PROMOTION IN THE PRAWN *MACROBRACHIUM ROSENBERGII*

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Objective: To understand whether the medicinal herbs, *Myristica fragrans* (nutmeg) *Glycyrrhiza glabra* (liquorice) and *Quercus infectoria* (gallnut) have effects on growth promotion in the post larvae (PL) of the prawn, *Macrobrachium rosenbergii*. **Methods:** *M. fragrans* (seed powder), *G. glabra* (stem powder) and *Q. infectoria* (fruit powder) were incorporated with basal diet at three different concentrations (1%, 3%, and 5%) individually and fed to the PL of *M. rosenbergii* (1.56±0.18 cm; 0.074±0.02 g) for a period of 60 days under triplicate experimental set-up. **Results:** Significant elevations ($P < 0.05$) in weight gain, survival rate, activities of digestive enzymes (protease, amylase and lipase), concentrations of total protein, non-enzymatic antioxidants (vitamins C and E) and minerals (Na⁺ and K⁺) were recorded in *M. fragrans* incorporated feed followed by *G. glabra* and *Q. infectoria* when compared with control. Nine polypeptide bands of molecular weight between 116-14 kDa were resolved in the muscle tissue of PL. These bands were stained more intensely in experimental PL when compared with control. **Conclusion:** These herbs have the ability to induce secretion of protease, amylase and lipase in *M. rosenbergii* PL, which lead to increased food consumption and absorption of nutrients, and resulted in elevation of total protein, vitamins and minerals.

Keywords: Prawn, Nutmeg, Liquorice, Gallnut, Growth, Digestive enzymes, Vitamins, Protein

INTRODUCTION

Aquaculture is the production of aquatic plants and animals under the controlled or semi controlled conditions (Stickney et al., 2000). It is one of the fastest growing animal food sectors and provides over 13% of the animal protein for the human consumption (WHO, 2003; FAO,

2010). In addition to contributing to global food production, aquaculture is a major economic activity and an important source of foreign exchange for several developing countries. Currently aquaculture supplies about 50% of the global demand for fish and fishery products with about 90% of the products coming from the Asian region (FAO, 2009). The production of carp and

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ORIGINAL ARTICLE



WILEY

Dietary licorice (*Glycyrrhiza glabra*) improves growth, lipid metabolism, antioxidant and immune responses, and resistance to crowding stress in common carp, *Cyprinus carpio*

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Abstract

This study aimed to investigate the effects of dietary licorice (*Glycyrrhiza glabra*) on growth, lipid metabolism, antioxidant and immune response, and stress resistance of common carp. The fish (12.14 ± 0.39 g) were fed diets containing 0 (control), 10 (G1), 20 (G2) and 30 (G3) g/kg licorice root powder for 60 days and then subjected to 3-hr crowding stress (40 kg/m³). The highest final weight and catalase activity and the lowest FCR and cholesterol levels were observed in fish fed diets 10-30 g/kg licorice inclusions. The highest lipase activity and the lowest LDL and triglyceride levels were recorded in G2 and G3 groups. Serum glutathione peroxidase (GPx) and complement (ACH50) activities and total immunoglobulin (Ig) levels were significantly higher and malondialdehyde levels were lower in G2 and G3 groups than the control. Serum lysozyme and ALP activities significantly increased in 10-30 g/kg licorice groups before and after stress. Crowding stress increased cortisol levels in all groups; however, its levels significantly reduced in G2 and G3 groups at 24 hr after stress. Furthermore, dietary licorice significantly decreased ALT activity in the G2 group at 8 hr after stress. These findings demonstrate the hepatoprotective, antioxidant, immunoregulatory, anti-stress and growth-promoting effects of *G. glabra* in common carp.

KEYWORDS

common carp, crowding stress, *Glycyrrhiza glabra*, health, immunity

1 | INTRODUCTION

Common carp (*Cyprinus carpio*) is widely reared in the polyculture system in Iran. In recent years, this species has reared in concrete ponds under high culture densities. Various aquaculture practices such as high stocking densities and crowding conditions can stimulate stress responses in cultured fish (Adineh et al., 2019; Xie et al., 2008). The stress response can elevate reactive oxygen species (ROS) and cause oxidative stress, immunosuppression and susceptibility to infectious diseases (Naderi et al., 2019; Tort, 2011). Also, aquaculture is under pressure to decrease the application of

antibiotics due to the spread of antibiotic-resistant pathogens and food security problems (Chakraborty & Hancz, 2011). Under these conditions, herbal products have been used as immunostimulants in aquaculture to avoid the careless use of dangerous antibiotics. Herbal products can be used in fish farming as alternatives to vaccines, chemicals and antibiotics to prevent and control diseases in fish (Citarasu, 2010; Galina et al., 2009). Thus, the potential use of herbal products as immunostimulants in aquaculture is a promising novel strategy and further studies are beneficial.

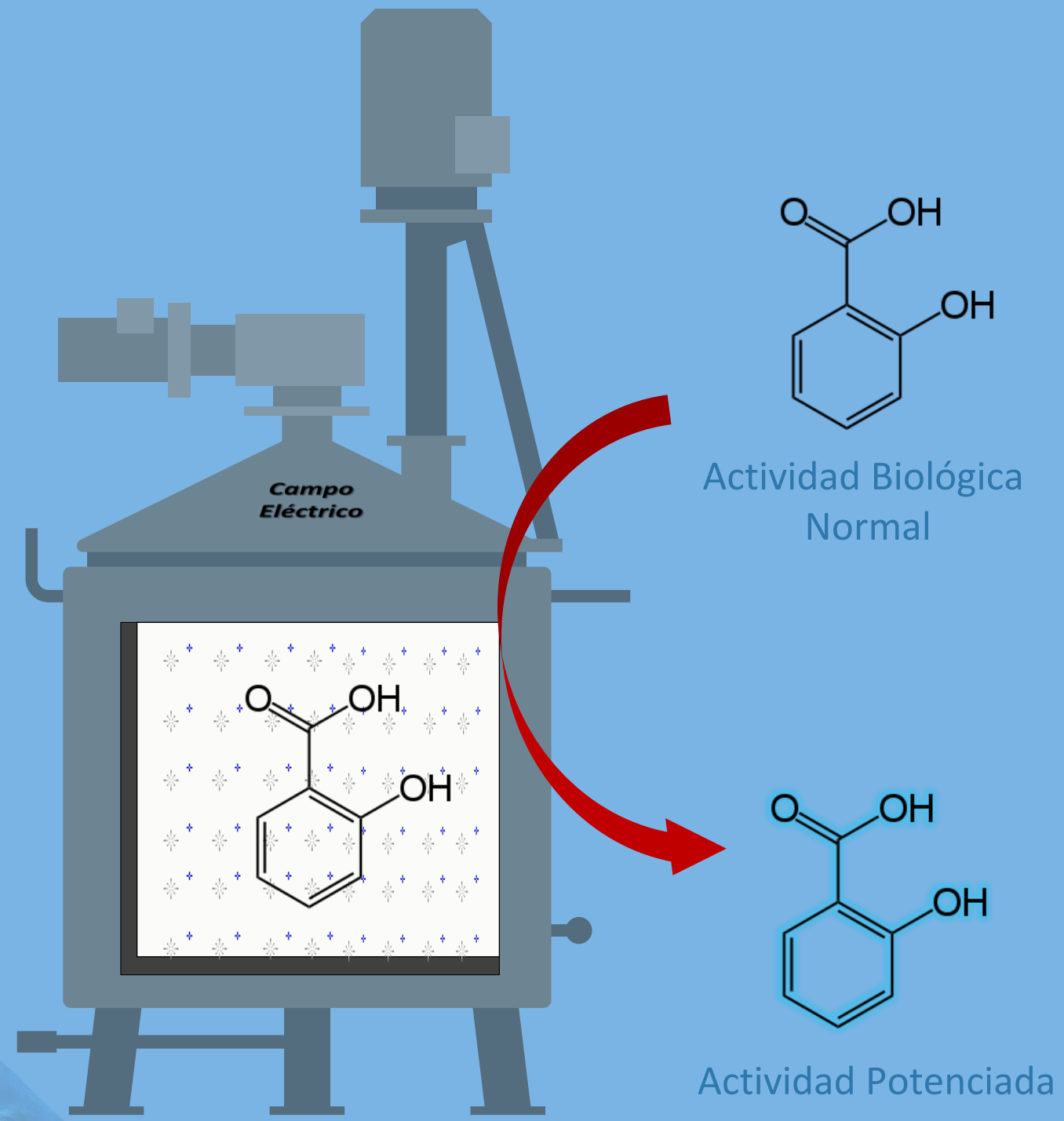
Many herbal compounds as natural antioxidants inhibit the production of ROS and scavenge free radicals (Chakraborty &

El regaliz dietético disminuyó significativamente la actividad de ALT en el grupo G2 a las 8 horas después del estrés. Estos hallazgos demuestran los efectos hepatoprotectores, antioxidantes, inmunorreguladores, antiestrés y promotores del crecimiento de *G. glabra* en la carpa común.

Estos resultados indicaron que la glicirricina puede mejorar el sistema inmunitario no específico, aumentar la resistencia de *L. vannamei* y mejorar la producción en la cría de camarones.

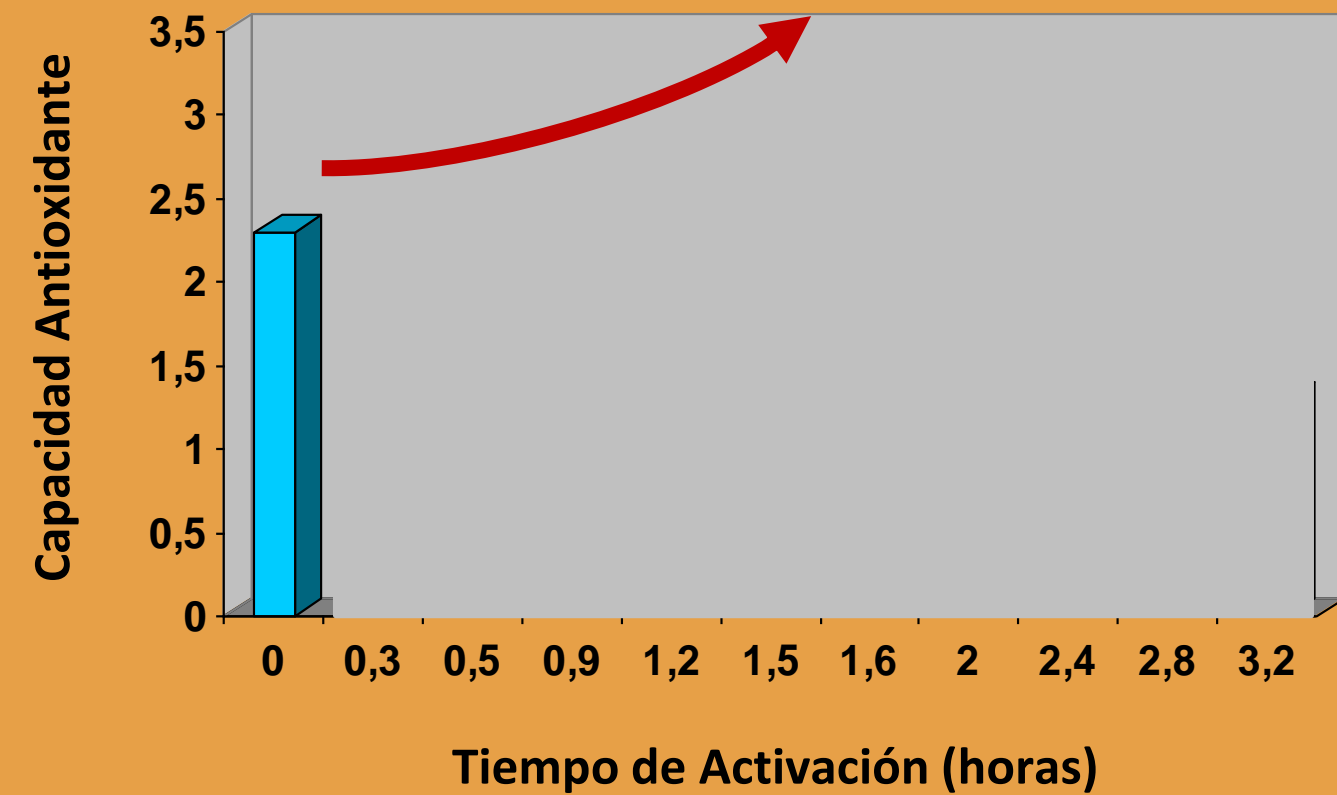
Aumento de la ganancia de peso, tasa de supervivencia, actividades de las enzimas digestivas (proteasa, amilasa y lipasa), concentraciones de proteína total, antioxidantes no enzimáticos, minerales (Na⁺ y K⁺) e induce la secreción de proteasa, amilasa y lipasa en *M. rosenbergii*.

TECNOLOGÍA DE ACTIVACIÓN MOLECULAR



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IMPLEMENTACIÓN DE ANTIOXIDANTES ACTIVADOS EN PRODUCCIÓN ACUÍCOLA



Efficacy of a pharmaceutical preparation based on glycyrrhizic acid in a challenge study of white spot syndrome in white shrimp (*Litopenaeus vannamei*)

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ABSTRACT

There is a lack of preventive and therapeutic drug-based treatments for the shrimp viral disease known as white spot syndrome (WSSV). Thus a challenge study inducing WSSV in juvenile white shrimp (*Litopenaeus vannamei*) was established, setting 4 groups: challenged – not treated and unchallenged, untreated control groups and two experimental ones (E1 and E2) both treated with diammonium glycyrrhizic acid, extracted from licorice with added vitamins and oligoelements, and as in-feed medication. Group E1 received diammonium glycyrrhizic acid included in their daily feed, starting 17 days before challenge with WSSV and maintaining the treatment for further 5 days after the end of the trial, which was set on day 18. Group E2 received this medication as group E1 throughout the trial, but starting 1 day before the challenge with WSSV. The group with highest surviving median values was E1, amounting two times the survival median in comparison with the control groups ($P = 0.007$). Also a statistical difference was found in terms of survival means in favor of group E1 as compared to group E2. Macroscopic and histopathological findings revealed lesions compatible with WSSV and similar mortality in the challenged untreated group. These findings were highly reduced or inexistent in mortality analyzed from groups E1 as well as in the unchallenged – untreated control group and greatly reduced in group E2. Considering the apparent high efficacy observed and that glycyrrhizic acid and mineral and vitamin components added as treatment, and taking as an advantage that this preparation has been regarded as nutraceuticals, it is here proposed that large scale trials should be conducted to evaluate the effects here observed in commercial and larger scale shrimp farms.

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1. Introduction

In spite of difficulties, shrimp production is an important and profitable food producing industry. Even though, in many countries cultured shrimp production has been severely hindered by various viral diseases i.e., white spot syndrome – WSSV (Inouye et al., 1994; Rosenberry, 2001). First signs of this disease, such as sudden reduction in food consumption and red discoloration, are followed by a sharp increase in mortality in shrimp farms over the next 3–10 days, even reaching 100% mortality (Peinado-Guevara and López-Meyer, 2006). First description of WSSV appears to have been from an outbreak in Taiwan in 1992 (Chen, 1995; Chou et al., 1995). This disease seems to have spread world-wide, except perhaps to Australia. It has been suggested that world weather changes have contributed to the dissemination of this disease (Sonnenholzer et al., 2002). As the name of the disease suggests, the main signs of WSSV are 0.5–2.0 mm white spots in the

interior part of the shell, appendices, uropods, telson, pereopods, pleopods and cuticle of the abdominal segments (Takahashi et al., 1994). The color of shrimps becomes pale red and the lymphoid organ becomes turgid (Takahashi et al., 1994), and it has been described as hypertrophic (Vidal et al., 2001). Diseased shrimps become lethargic, and they show erratic swimming and lack of appetite and die during the next three days.

Viusid® (from Catalysis, S.A. de C.V., Mexico) is the proprietary name preparation based on diammonium glycyrrhizic acid, extracted from licorice with added vitamins and oligoelements. It has been claimed that this drug preparation stimulates production of gamma interferon in human beings (Sugawara, 1986). Glycyrrhizic acid possesses antiviral activity in vitro and in vivo interfering with both DNA and RNA replications, hence interfering with replication of a wide range of viruses, including herpes, influenza A and B, hepatitis B, coronavirus, and SARS (Badam, 1994; Chen, 1995; Durand et al., 1997; Lee et al., 2007; Lin, 2003; Pompei et al., 2009). Glycyrrhizic acid has also demonstrated to be capable of impeding virion eclosion from its capsid (Pompei et al., 2009), apparently due to a dose-dependant inhibition

Estudio de desafío para inducir WSSV en juveniles de camarón blanco (*Litopenaeus vannamei*), estableciendo 4 grupos: desafiados, no tratados y no desafiados, grupos de control sin tratar y dos experimentales (E1 y E2) ambos tratados con ácido glicirrónico activado, con vitaminas y oligoelementos añadidos, y como medicación en el pienso. El grupo E1 recibió ácido glicirrónico activado incluido en su alimentación diaria, comenzando 17 días antes del desafío con WSSV y manteniendo el tratamiento durante 5 días más después del final del ensayo, que se fijó en el día 18. El grupo E2 recibió este tratamiento como el grupo E1 durante todo el ensayo, pero comenzando 1 día antes del desafío con WSSV. **El grupo con valores de mediana de supervivencia más altos fue E1, que asciende a dos veces la mediana de supervivencia en comparación con los grupos de control (P = 0,007). También se encontró una diferencia estadística en términos de medias de supervivencia a favor del grupo E1 en comparación con el grupo E2.** Los hallazgos macroscópicos e histopatológicos revelaron lesiones compatibles con WSSV y una mortalidad similar en el grupo desafiado no tratado. **Estos hallazgos fueron muy reducidos o inexistentes en la mortalidad analizada de los grupos E1, así como en el grupo de control no desafiado, no tratado y muy reducido en el grupo E2.**



ANTIVIRALES

- Ácido glicirrónico
- Glucosamina
- Ácido málico
- Zinc

ANTIOXIDANTES

- Ácido ascórbico
- Ácido málico
- Zinc
- Arginina

AGENTES ANTIANÉMICOS

- Ácido fólico
- Cianocobalamina
- Piridoxina

INMUNOMODULADORES

- Arginina
- Glucosamina
- Ácido glicirrónico
- Zinc

BIOCATALÍTICOS

- Zinc
- Pantotenato de calcio



IMPLEMENTACIÓN DE ANTIOXIDANTES ACTIVADOS EN PRODUCCIÓN ACUÍCOLA

CRUSTACEANS

Study on the impact of an antiviral and immune modulator for sustainable health management and seed production of *Macrobrachium rosenbergii*

Hiranmoy Bhattacharjee, Mohammed Tarique Sarker, Mohammad Sohel Miah, Md. Masud Rana, Bangladesh Shrimp & Fish Foundation, Bulent Kukurtcu Targotay, Laboratorios Catalysis



No suitable immune modulator and/or antiviral therapeutic has been found to use in *Macrobrachium rosenbergii* hatcheries to help achieve the sustainable production of PLs in Bangladesh. There is a remarkable crisis regarding the production of PL due to the inability to prevent and/or control the observed and

suspected protozoan, bacterial, and possible but yet unidentified viral diseases occurring in the hatchery phase due to the unavailability of any suitable immune-enhancing and/or antiviral remedial product. Presently, available approved aquaculture medicinal products, protozoacides, probiotics, etc. have not been found

VIUSID® Aqua ofrece estimulación inmunológica e influencias antivirales en la fase larvaria de camarones de agua dulce en cautiverio de criadero. Se descubrió que contribuye a aumentar la supervivencia, el crecimiento y la producción general de larvas al aumentar su apetito, mejorar la eficiencia de alimentación y hacer que las larvas y las PL sean resistentes a las enfermedades, lo que ayuda a desarrollar un protocolo sostenible de gestión de criaderos de camarones para combatir la posible aparición de enfermedades críticas. problemas de enfermedades en el sector de criadero de camarones de agua dulce



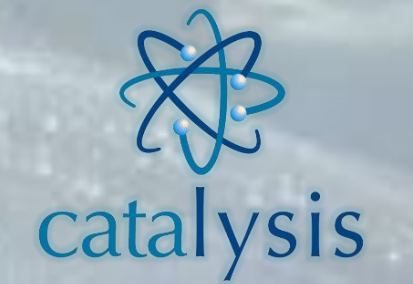
	With VIUSID® Aqua	Without VIUSID® Aqua
Pigmentation	Good and glowing pigmentation	Some grayish, not glowing pigmentation
Hepatopancreas	Bright and brownish	Brownish gray to grayish
Protozoan parasitic infestation	Did not occur	Occurred
Bacterial necrosis	Did not occur	Occurred during 9-10 days
Viral disease symptoms seen	No	No
Larval movement	Strong, speedy, good movement	Sluggish or poorer movement than trial tanks with VIUSID
Feeding performance	Excellent all throughout the cycle	Sometimes poorer throughout the cycle
Metamorphosis by molting	Usual, as expected	More delayed than the trial tanks
Larval stages at 23-35 days	Stage PL2-PL13	--
Larval stages at 29-35 days	--	Stage PL2 -PL8
Survival (%)	85	68
Average harvested PL/tank (million)	0.255	0.204
Total harvested PL (million)	2.295	0.612
PL production/liter (Pcs./L)	102.0	81.6
PL3 average body length (mm)	10.0	08.5
PL3 average body weight (mg)	13.0	11.3
Growth rate	Satisfactory and as desired	Slower than the trial tanks
Size variation of larvae	Minor	Major
Cannibalism	Absent	Observed throughout
1st day of appearance of PL (days)	22	28
Completion of metamorphosis to PL (days)	26	35

CONCLUSIONES

- Las fitomoléculas son herramientas eficaces en la acuicultura.
- La combinación de antioxidantes e inmunomoduladores aportan un beneficio adicional debido a los efectos sinérgicos, especialmente en las situaciones de estrés abiótico y biótico.
- La Tecnología de Activación Molecular se ha aplicado para lograr la máxima eficiencia de estos principios activos.

“Es fundamental satisfacer las crecientes expectativas de los consumidores con respecto a la salubridad, la sostenibilidad y la responsabilidad, y la certificación adecuada es una herramienta cada vez más valiosa para lograr estos objetivos”





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