Thank you both very much for bring me here after 36 years!

Mr. Itamar Rocha, Organizer of both Symposia

Mr. I.T. Guo, NangRong Group., Shanghai

Antioxidant Nutraceuticals in Aquatic Animals: Enhancing Resistance to Environmental and Pathological Stress

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Nutraceutical = Nutritional + Pharmaceutical

Nutrition becomes required at critical conditions, such as under various stress. Under normal conditions, it is not regarded as an requirement or its deficiency may not cause immediate crisis and receive attention. Such nutritional food is usually referred as health food for people.

CONTENTS

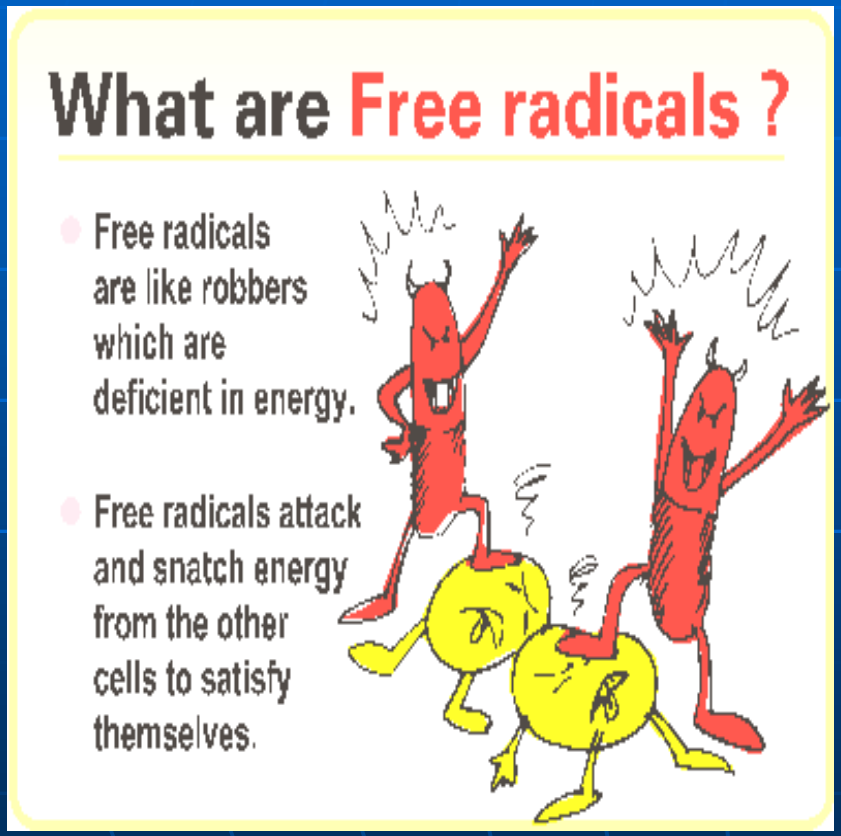
I. Stress, ROS, antioxidants, defense against ROS and resistance to stress.

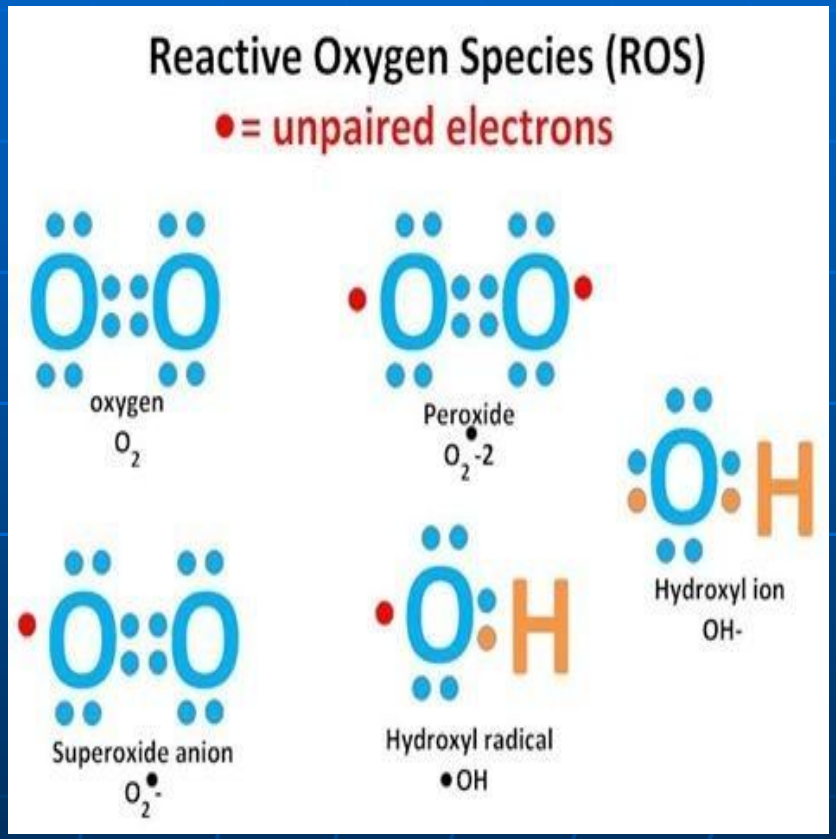
II. More important roles of antioxidant capacity in aquatic animals than terrestrial ones.

III. Expansion of our 20 years' study on I.

IV. Antioxidant capacity building: Aquafeed 4.0 ?!

FREE RADICALS AND ROS





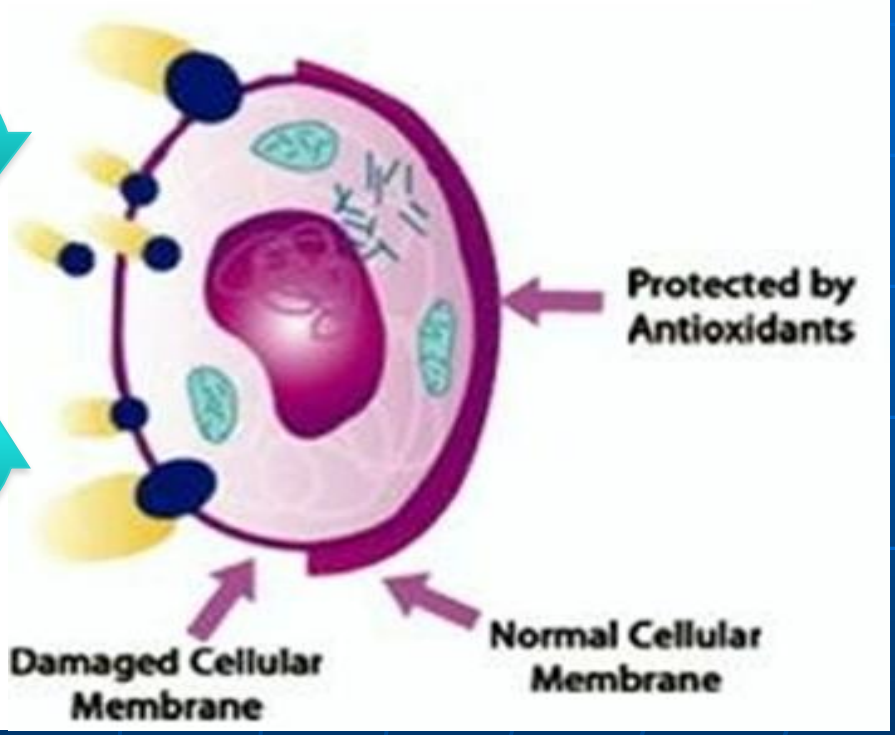
Physical

Chemical

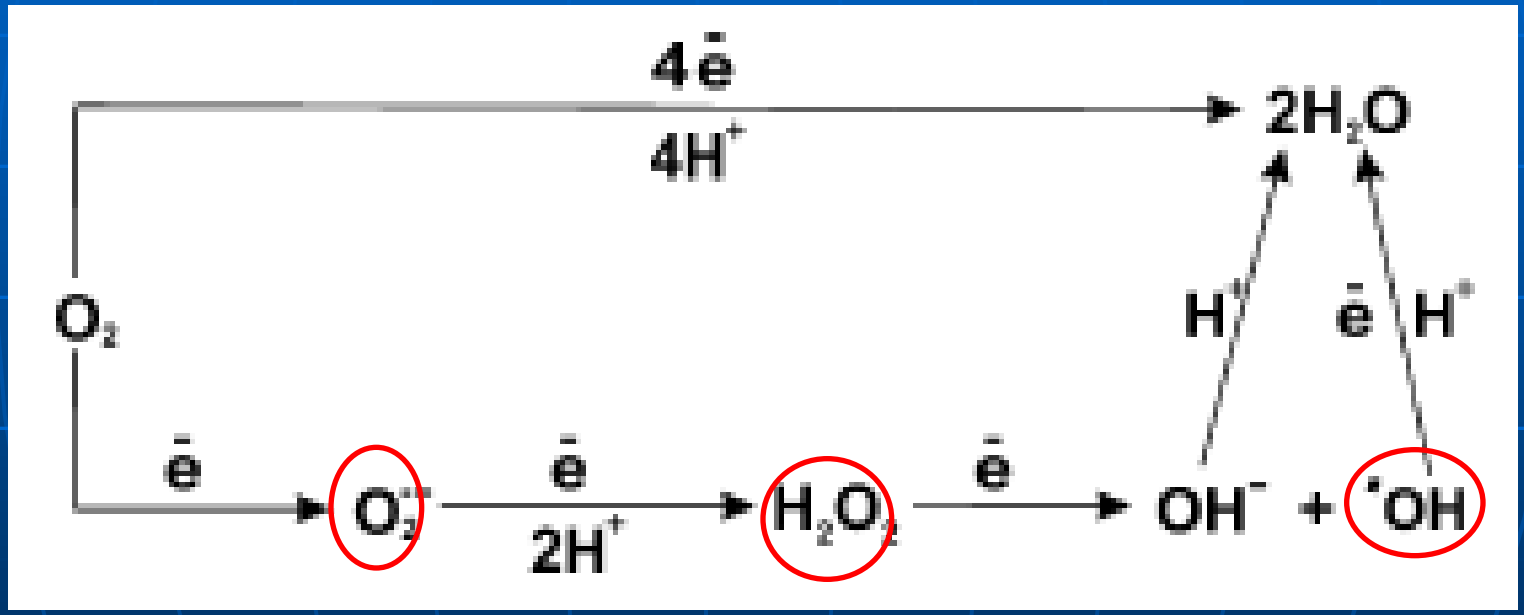
Stress

Biological
(Pathological)
(Nutritional)

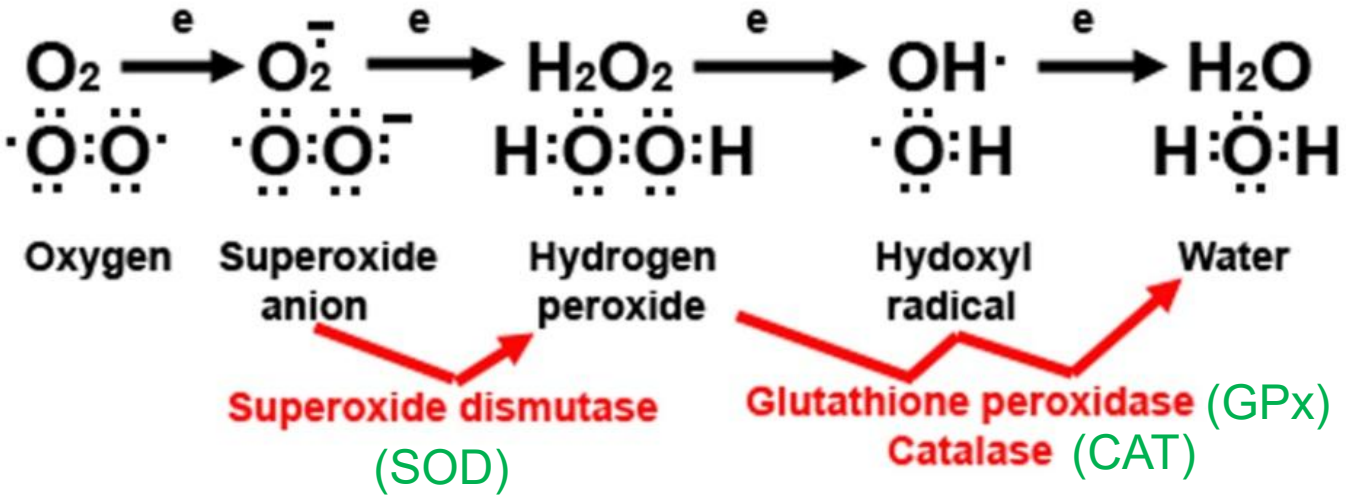
ROS



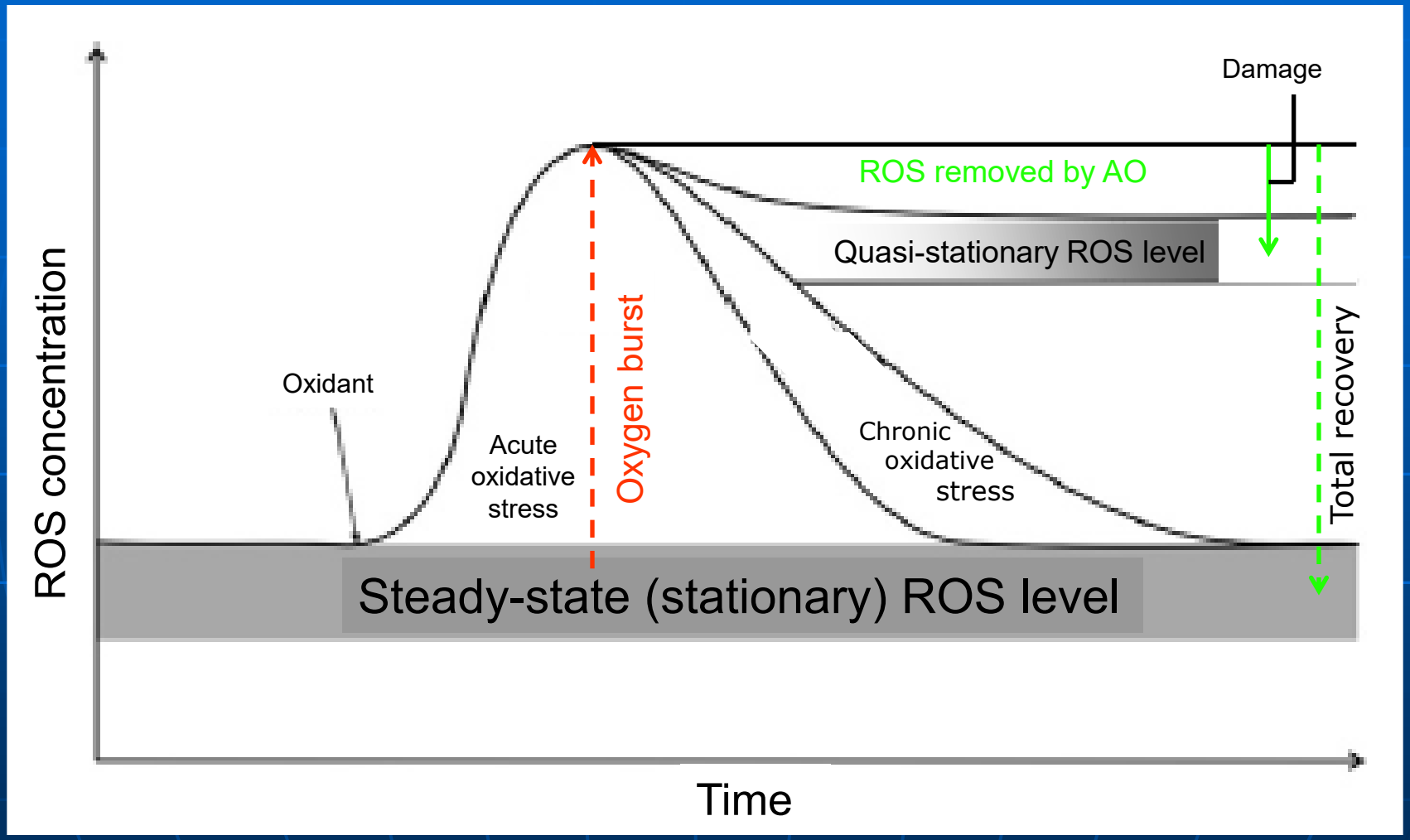
ROUTES OF OXYGEN METABOLISM



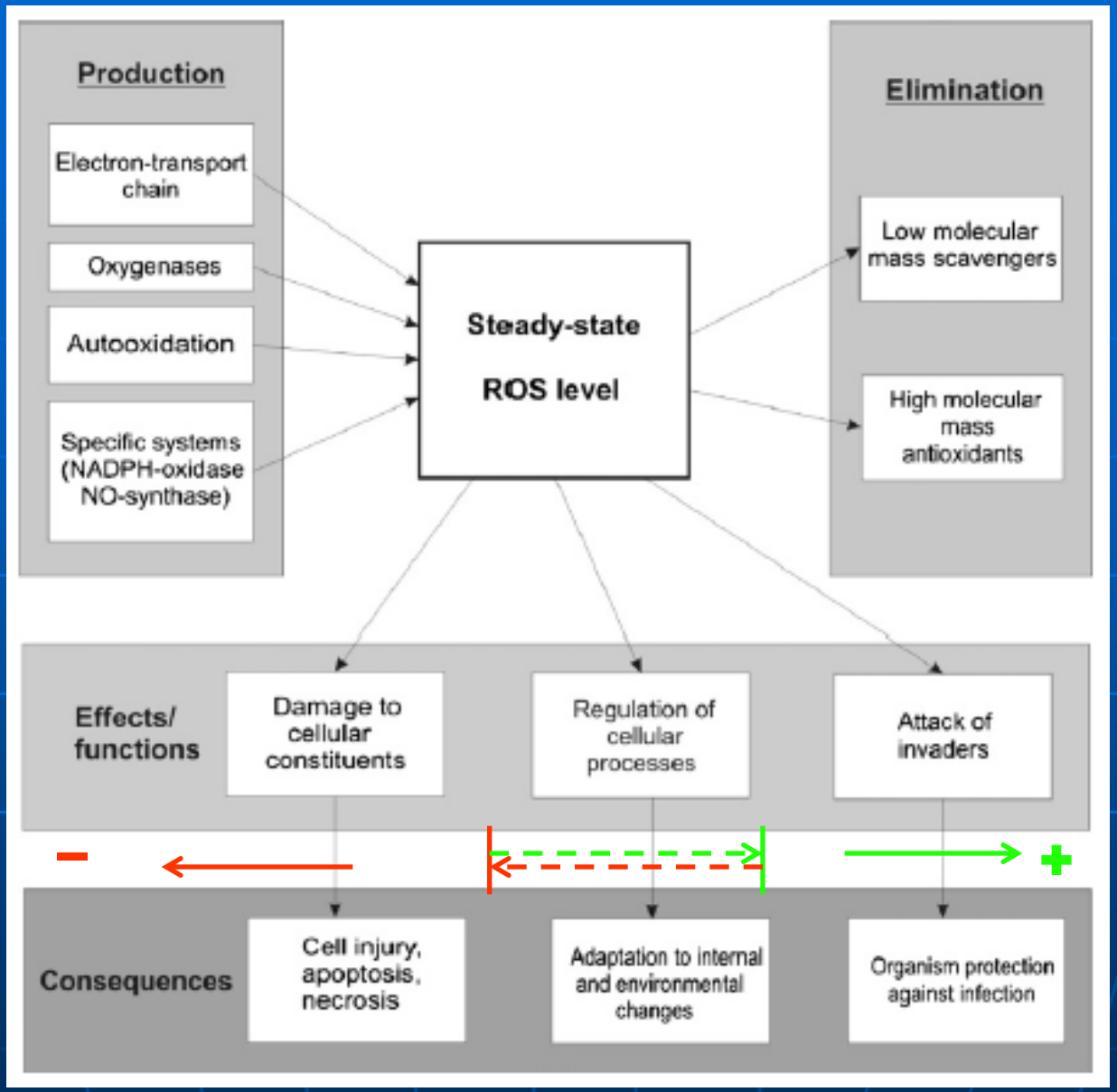
Formation and Elimination of Reactive Oxygen Species (ROS)



Increasing Numbers of electrons



The dynamics of oxidant-induced pertubations of levels of reactive oxygen species in living organisms (Lushchak 2011)



Balance between production and elimination of ROS and their potential biological effects (Lushchak 2011)

ANTIOXIDANTS

Antioxidants are nutrients that help to minimize free-radical damage to the body cells by:

- Reducing their energy,
- Inhibiting their formation or
- Interrupting an oxidizing chain reaction to minimize the damage of reactive oxygen species (ROS).

In short, antioxidants **inhibit oxidation.**

First level of defence:
Prevention of radical formation

Superoxide dismutase, glutathione peroxidase, catalase, glutathione and thioredoxin systems and metal-binding proteins.

Second level of defence:
Prevention and restriction of chain formation and propagation

Vitamins A, E, C, carotenoids, ubiquinols, glutathione, uric acid.

Third level of defence:
Excision and repair of damaged parts of molecules

Lipases, peptidase, proteases, transferases, DNA-repair enzymes etc.

STRESS

Chemical – high ammonia/low DO

Physical – temperature/salinity sudden change

Pathogenic – bacteria/virus attack

CELLULAR PHYSIOLOGICAL RESPONSES

"Burst" – sudden shortage of oxygen

Abnormal oxidative reactions

Singlet oxygen (ROS) & free radicals (ROI)

DAMAGES TO CELL COMPONENTS

Lipids	Membranes
Proteins	Enzymes
Carbohydrates	DNA
Nucleotides	

CONSEQUENCES

Chronicle – growth inhibition

Acute – mortality

Antioxidant Defense System

– Neutralization of the potential ill effect of ROS and ROI

Substances for Primary Defense

Antioxidant compounds:

ascorbic acid (vit.C), α-tocopherol (vit.E), retinal (vit.A), carotenoids, glutathione, and uric acid

-- Antioxidant scavenging enzymes

Carotenoids

β-carotene:

lipid antioxidant- a free radical trap and quencher of ROS; antioxidation ability (AA) 10 times > vit.E

Astaxanthin (Super vit.E):

Inhibit lipid peroxidation

AX 10 times >β-carotene, 100 times > vit.E

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Adaptation: Survive through Eternity

Hypothesis:

Through evolution, aquatic animals may have to develop a more efficient antioxidant defensive system than terrestrial animals to counter-react more oxidative stress in aquatic inhabitation than in terrestrial one. This is because of the relative disadvantage of aquatic animals to conduct aerobic metabolism over terrestrial animals.

Relative disadvantage of aquatic animals to conduct aerobic metabolism over terrestrial animals

1. Extremely poor accessibility for oxygen: <1/5000
   In water: Dissolved oxygen content < 10 part per million(10^{-6})
   In air: Fraction of oxygen = 20 per cent (10^{-2})

2. Carbohydrate not available for energy:
   Nil carbohydrate in natural food sources and hence poor utilization of it for energy generation (not marine but few freshwater finfish).

3. More risk from lipid peroxidation:
   when lipid is used to generate energy and exposed to ROS.

Even more internal and external stress in aquaculture than in animal husbandry

Stress to the cultured animals in captivity (confined space)

* Stress from immediate contact with deteriorated water and sediment where the cultured animals inhabit:

- Stimuli from unsuitable range of physical and chemical parameters, such as temperature, salinity, pH and hardness, low dissolved oxygen, and toxicity derived from metabolic waste.
- Infection by proliferated pathogenic microbes and virus.

* Stress from stocking density:

- Hierarchical pressure (packed order) caused timid feeding.
- Cannibalistic attack.

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Study Realm Expansion

Pigmentation on rainbow trout by crawfish astaxanthin in LSU

→Pigmentation on Kuruma prawn by astaxanthin (AX)

→Resistant against hypoxia in black tiger by AX

→More carotenoids, stress types and responding parameters, also for finfish

→More antioxidants, also prooxidants

→(Modeling for Aquafeed 4.0)

CONCLUSION I

The increase of body astaxanthin content through dietary supplementation in tiger prawn *P. monodon* juvenile could enhance its antioxidant defense capability and resistance to thermal and osmotic stress, ammonia stress, and pathogen challenge. Also hemolymph of the treated shrimp had higher TAS and/or lower SOD, lower AST and/or ALT, and lower *in vitro* activity of lipid peroxide (LPO) than that of the control shrimp, showing that both the antioxidant capability and hepatopancreatic function had been improved by dietary astaxanthin.

Nutrition + pharmaceutical

For *P. monodon*, astaxanthin is a nutraceutical, which can become critical when the animal is under physical and chemical stress and pathogen challenge.

For beauty, ask for astaxanthin!
For protection and insurance do ask for ASTAXANTHIN!

Beauty and protection

Enhancement of Resistance in Characins *Hyphessobrycon callistus* to Physical and Chemical stress by Dietary Supplement of Carotenoids

Effects of dietary supplementation of carotenoids on survival, growth, pigmentation, and antioxidant capacity of characins, *Hyphessobrycon callistus*. Aquaculture 261: 641-648.

The antioxidant capacity response to hypoxia stress during transportation of characins (*Hyphessobrycon callistus* Boulenger) fed diets supplemented with carotenoids. Aquaculture Research 41,973-981

Antioxidant defence to ammonia stress of characins (*Hyphessobrycon eques* Steindachner) fed diets supplemented with carotenoids. Aquaculture Nutrition 17, 258-266

Objectives

Besides achieving better body color, can dietary supplement of various carotenoids at several levels enhance Characins' antioxidant capacity, concomitantly, resistance against different stress that may encounter during live fish shipping?

Experimental Design

Diets

AX, BC, MX

×

10, 20, 40 mg kg^{-1}

Fish

CD

AX

BC

Anti-OX

TAS

SOD

GPx

ALT

AST

Stress

I- High & Low temp.

II- Low DO

III- High TAN

IV- Low pH

Conclusion II

Despite the type of stress varies, the responses in biochemical enzymatic activity remain similar. Only Total Antioxidant Status (TAS) was less sensitive to both stress and dietary carotenoid supplement than the other enzymes. Antioxidant activities in fish varied with dietary carotenoids type and level. Dietary carotenoid increased the antioxidant capacity and protection of the liver. Except TAS, the other four enzyme activities showed decreasing trends with increasing dietary CD level. The activities of TAS, SOD, GPx and AST increased under the stress. Dietary CD reduced serum SOD, GPx, ALT and AST activities. In conclusion, dietary CD increased the resistance of characins to ammonia stress.

Dietary Effects of Various Antioxidant Supplements on Growth, Survival, Antioxidant Capacity, Immune Response, Metabolic Response and Oxidative Stress Status of Pacific White Shrimp (*Litopenaeus vannamei*)

Laila Gallego and Yew-Hu Chien

Objectives

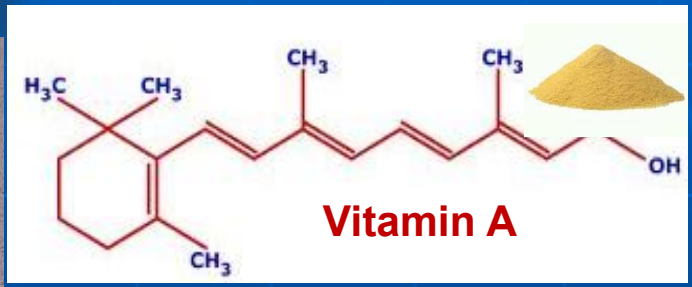
To investigate the effect of antioxidant extracts with the same DPPH antioxidant activity on growth, survival, antioxidant capacity, immune response, metabolic response and oxidative stress status of *Litopenaeus vannamei*

Nitrafito plus

=

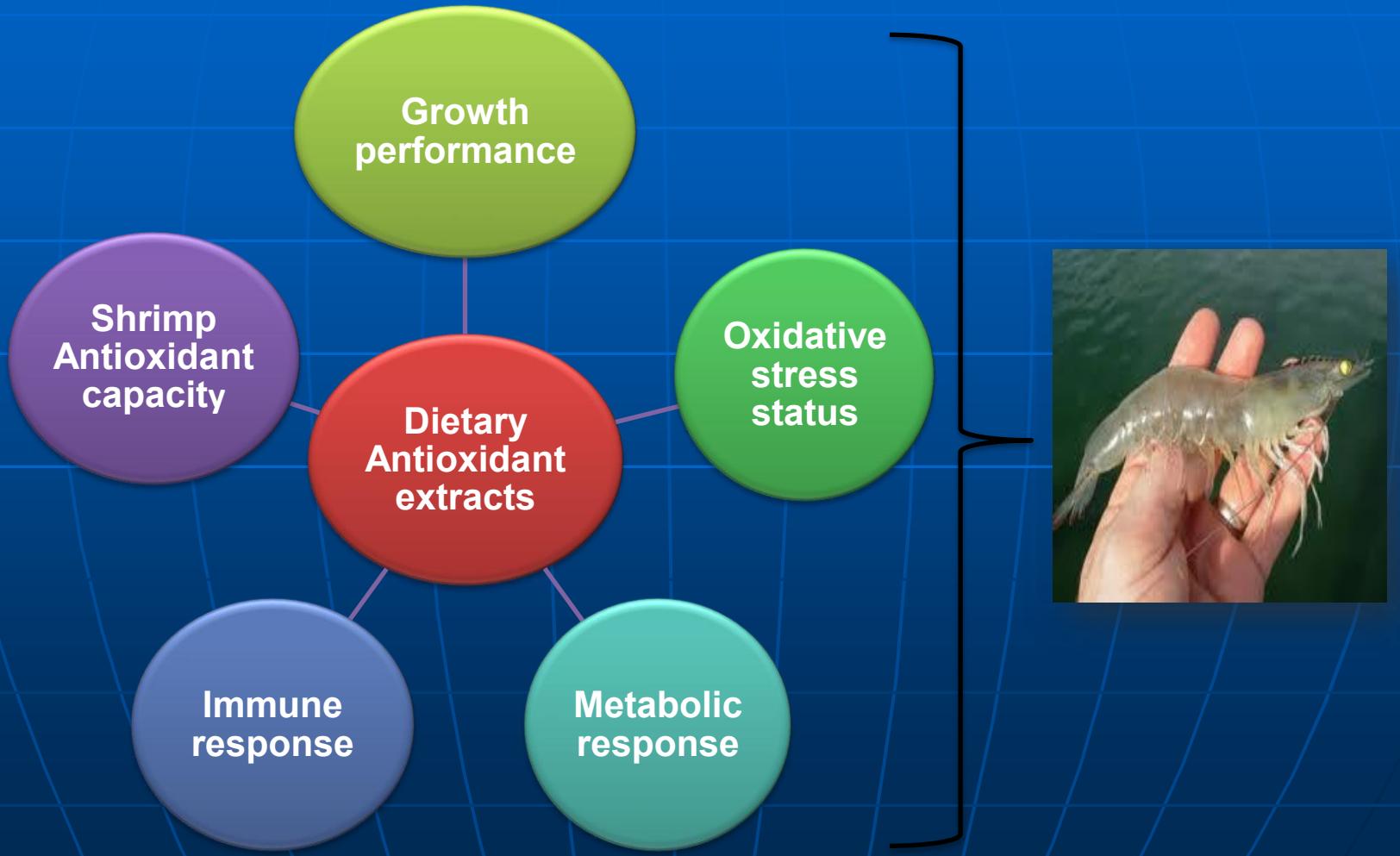
Yucca 20% +

Quillaja 80%



- Astaxanthin
- Vitamin A
- *Quillaja saponaria*
- *Yucca schidigera*
- Nutrafito plus
- Moringa leaves
- Moringa seeds

This is a holistic study to find out the total effects of dietary antioxidant on shrimp's antioxidant capacity, metabolic response, immune response, and oxidative stress status and the relationship among the responding variables. This is also a first study to compare the effects of various dietary antioxidants with the same level of total antioxidant capacity, DPPH, by their responding variables.



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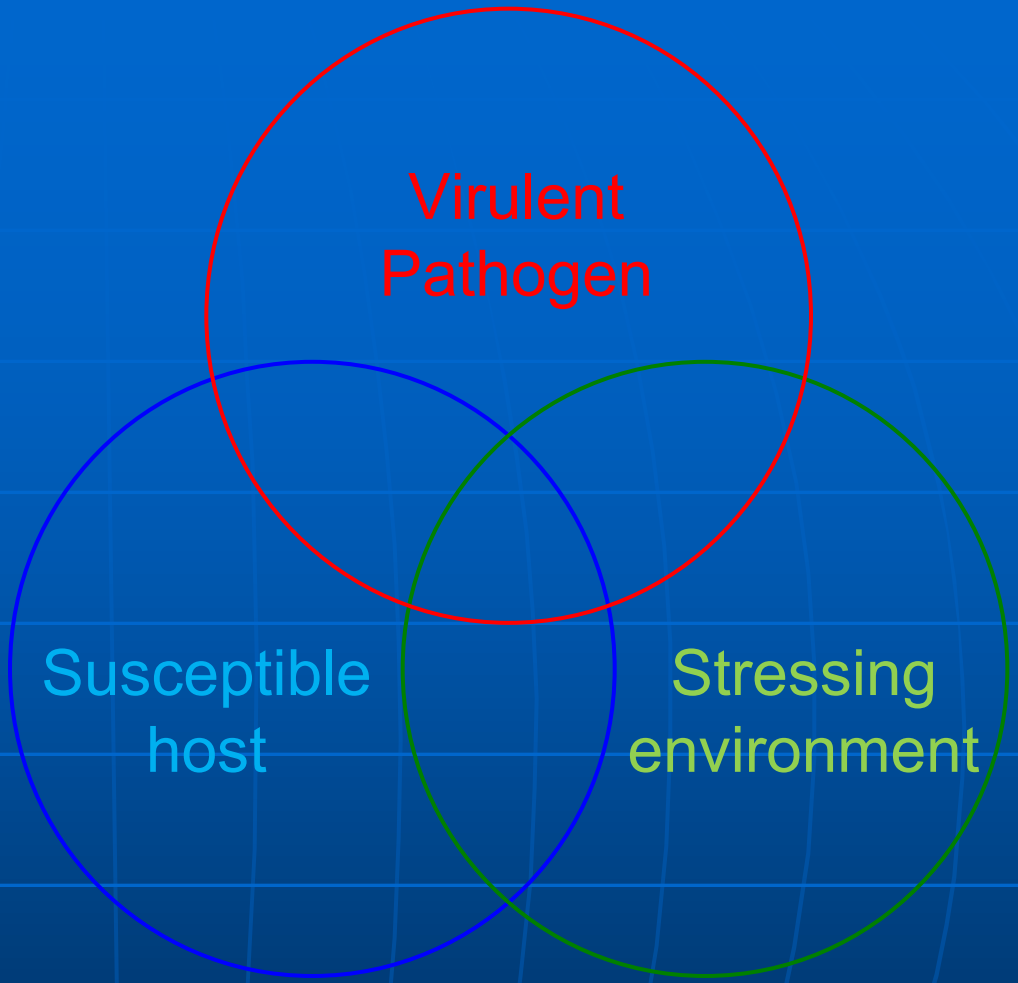
Snieszko (1972): Communicable diseases of fishes occur when susceptible host and virulent pathogen meet in an environmental context, which facilitates such an occurrence.



Snieszko, S.F. (1972) Nutritional fish diseases. In Fish Nutrition, ed. J.E. Halver, pp. 404-37. New York and London: Academic Press.

Snieszko (1974): Infectious diseases of fishes occur when susceptible fishes are exposed to virulent pathogens under certain environmental stress conditions.

Snieszko, S.F. (1974) The effects of environmental stress on outbreaks of infectious diseases of fishes. J. Fish Biology 6, 197-208.

Big Data:
Cause-effect & Interaction

M

M

Diets

Antioxidants
(Anti-Ox)
×
Concentration
(con)
×
cofactors

Fish

Life stages

Target organ or tissue

Anti-Ox assimilation efficiency

Metabolic response

Anti-OX Enzymes

Immune response

M

Stress

Physical
temp, DO…
Chemical
TAN, pH, sal…
Biological
Pathogen
Density, …

Aquafeed 4.0 –
Aquafeed resulted from an integral approach in dietary nutrition to relieve the threats (disease and risk) encountered during culture.

Antioxidant capacity building in cultured animals through nutritional and dietary enhancement.

Next Episode – Approach for Application

Enhancing the antioxidant capacity of aquatic animals brings nutritional (growth,…), nutraceutical (stress resistance,…) and even pharmaceutical (immunity, …) benefits. Consequently, antioxidant (after prooxidant) capacity (overall, specific, or group) can be used as criteria to evaluate the quality of functional aquafeed.

Dietary antioxidant compounds (vitamins, phytochemicals, …) and concentrations, their building (digestibility, bioavailability, assimilation efficiency,…) of various antioxidant capacity (TAS, DPPH, SOD, CAT, GR, GPx, …) in various tissues of aquatic animals (vertebrates, invertebrates) and in various life stages, and before versus after the stress (physical, chemical, biological, behavior, …) form a multi-dimensional matrices. Such big data lays the foundation to be mined, modeled and refined for a virtual antioxidant capacity assessment and antioxidant premix supplement.


Thank you for your attention
Comments and questions are welcome