Benny Shapira
Global R&D Manager – Phibro Animal Health Corporation
Israel
Email address: Benny.shapira@pahc.com

The Solution For Stress Mitigation – A Technical and Innovation Perspective

Abstract

Aquaculture has been the fastest growing animal production sector for the last four decades, and its importance to secure future global food security is well recognized. Modern aquaculture presents few challenges to our industry which is characterized by the emergence of new pathogens and new diseases. These diseases constitute a major obstacle to further increase aquaculture production.

The development and severity of disease, following exposure to a pathogen involves a number of different factors such as the virulence of the pathogen, the host's physiological and immune condition, cultivation density and stress, of which the latter is often considered to be a major facilitator of disease in aquaculture.

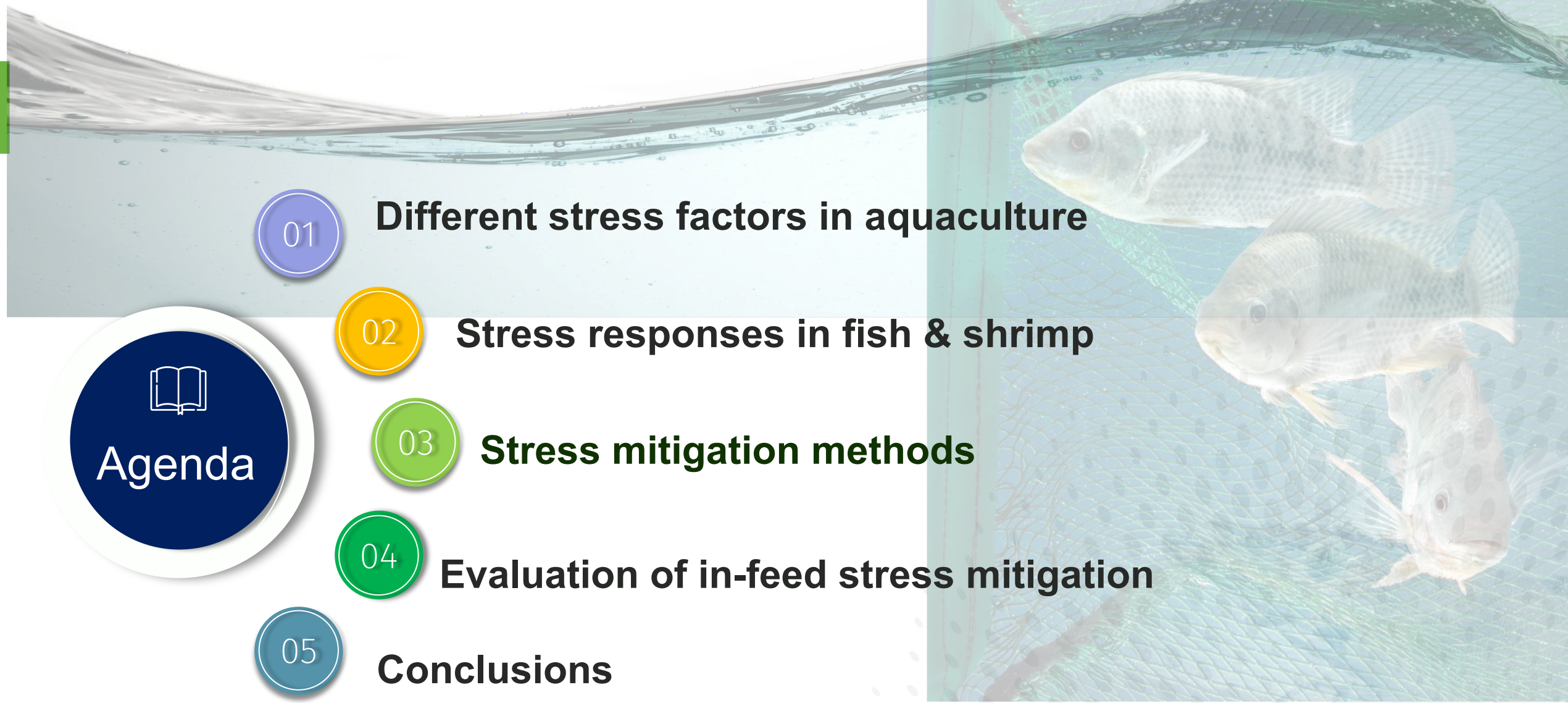
In today's aquaculture there are various stress factors which are constantly present in any cultivation system. Mitigating stress can be a result of several actions taken by farmers. This presentation will discuss the different types of stressors, their influence on productivity and disease resistance and more importantly, how novel feed additives can mitigate those stress factors.

Solutions for Stress Mitigation

A Technical and Innovation Perspective

AQUAFEEDS
A New Equilibrium

Benny Shapira, Global R&D Manager



Agenda

01 Different stress factors in aquaculture

02 Stress responses in fish & shrimp

03 **Stress mitigation methods**

04 Evaluation of in-feed stress mitigation

05 Conclusions

What is stress in aquaculture ?

The sum of all the physiological response by which an animal tries to maintain or re-establish a normal metabolism in the face of physical or chemical force.

Different Stress Factors

- **Physical:**

  Temperature, salinity, light, dissolved oxygen, pH, sound

- **Chemical:**

  Water quality, pollution, diet, metabolic waste

- **Biological:**

  Stocking density, microorganisms (pathogenic and nonpathogenic), macro-organisms (parasites)

- **Procedural:**

  Handling, transportation, stocking, disease treatment, feeding methods

Identifying Stress in Aquaculture

Cellular and molecular stress indicator

- Immediate early genes (IEGs); transcription factor
- Intracellular enzymes: ALT, AST
- Heat shock proteins (HSPs): cellular stress response

Primary and secondary physiological stress indicators

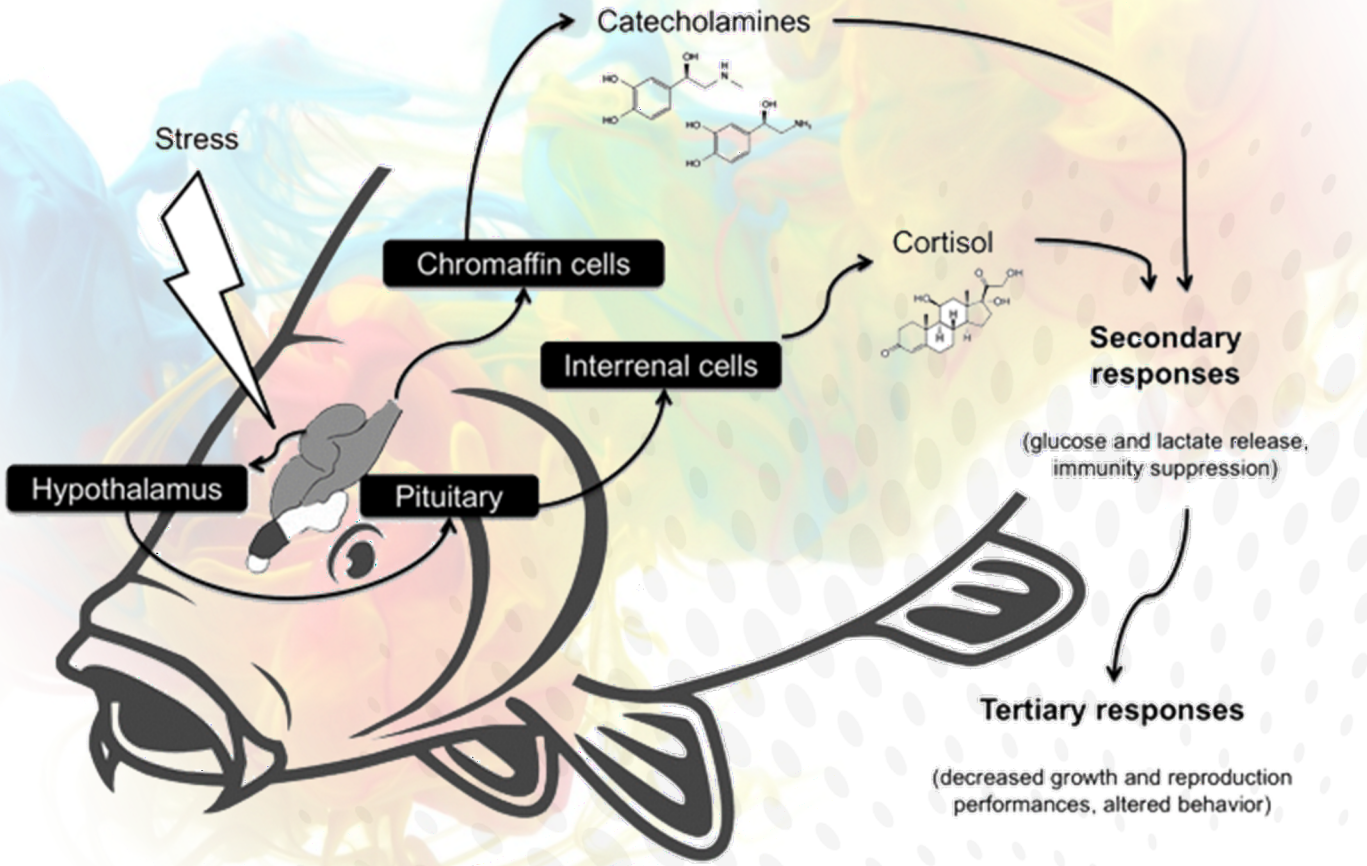
- Catecholamines - epinephrine and norepinephrine
- Steroid hormones - glucocorticoid steroid hormones
- Total hemocytes, hemocyanin

Whole-organism stress indicators

- Behavior
- Swimming performance
- Retarded growth
- Coloration
- Loss of appetite

General Adaptation Syndrome (GAS)

- An alarm reaction in which “stress hormones” (catecholamine and corticosteroids) are released
- A stage of resistance during which adaptation occurs
- A stage of exhaustion in which adaptation is lost because the stress was too severe or long lasting



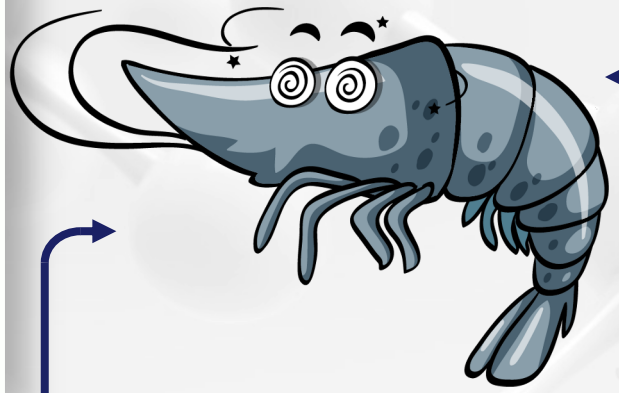
Source: Raposo de Magalhães et al, 2018

Stress Mitigation

Biological Method

Environmental management

- Water quality
- Temperature
- Dissolved oxygen,
- Ammonia, nitrogen, nitrite
- Salinity, pH
- Stocking density



Chemical method

Dietary supplementation

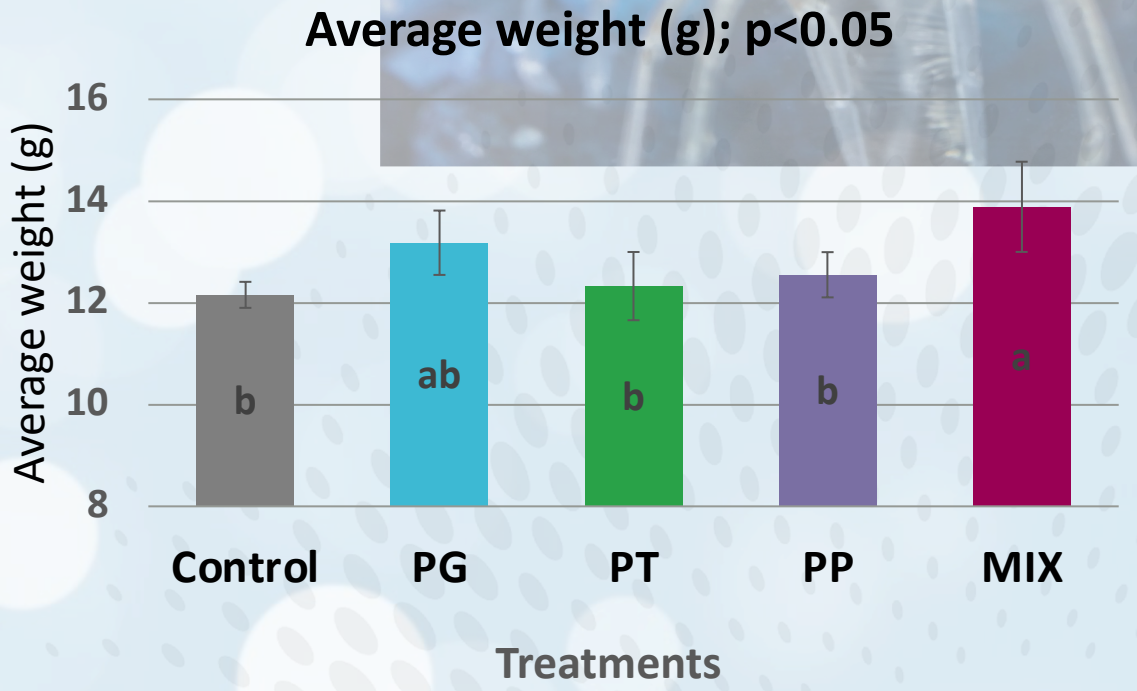
- Vitamins C & E
- Amino Acids
- Lipids and fatty acids
- Prebiotics
- Nucleotides
- Minerals

Evaluation of different feed additive on stress mitigation: Clinical trials

Evaluating the effect of different feed additives on to mitigate stress and improve the overall health status of *Litopenaeus vannamei* culture under normal and challenge conditions

Kasetsart University, Thailand, *2021*

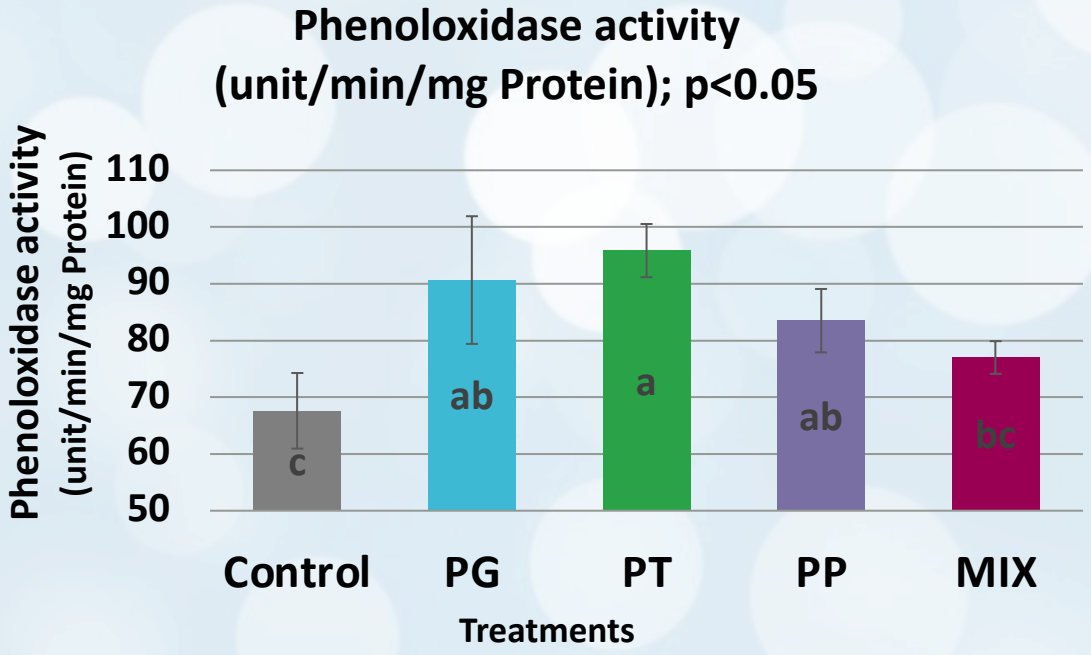
Shrimp *Litopeneaus vannamei*	Species
Commercial feed (control) Commercial feed + Stress modulator Commercial feed + OA blend Commercial feed + Phytogenic Commercial feed + Mix of all three (3-1-1)	**Treatments**
6	**Number Replicas**
60 days	**Duration**
25 shrimp/ 150 l tank	**Stocking**
2g ± 0.05g	**Initial Weight**
Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Thailand.	**Location**

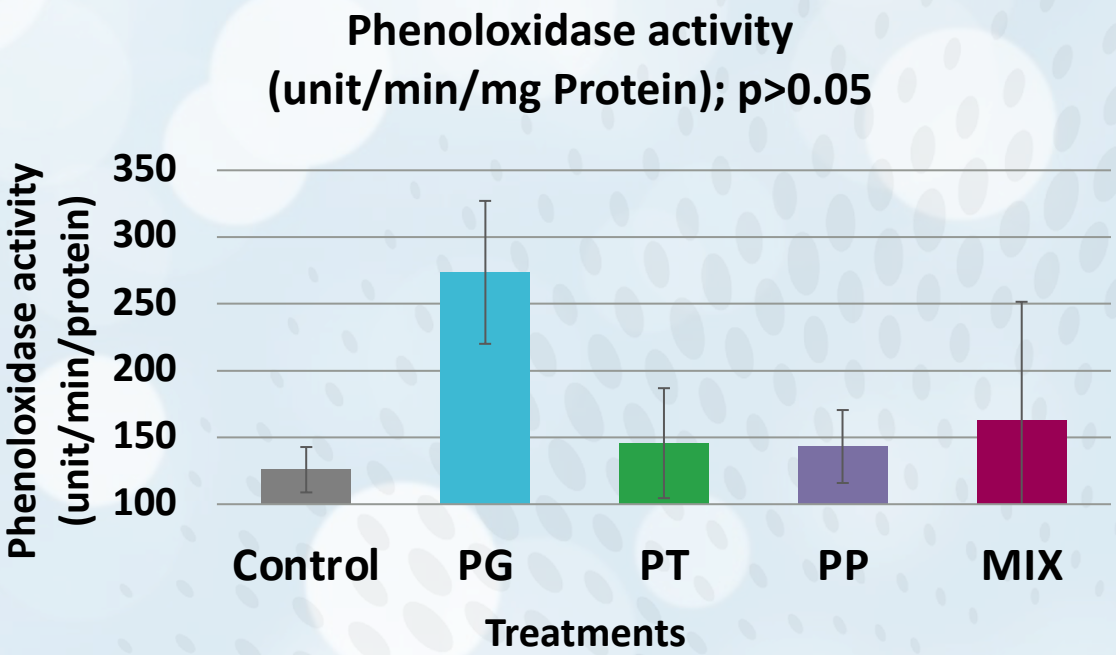


Evaluating the effect of different feed additives on to mitigate stress and improve the overall health status of *Litopenaeus vannamei* culture under normal and challenge conditions

Kasetsart University, Thailand,*2021*

Results - Phenoloxidase activity before and after challenge

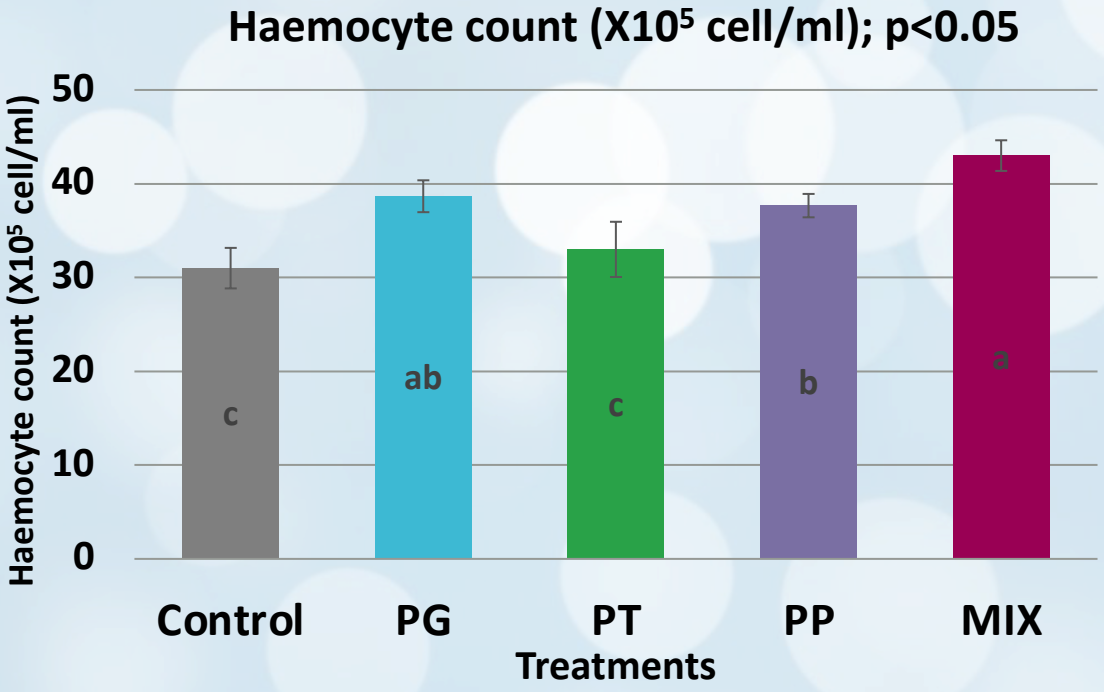


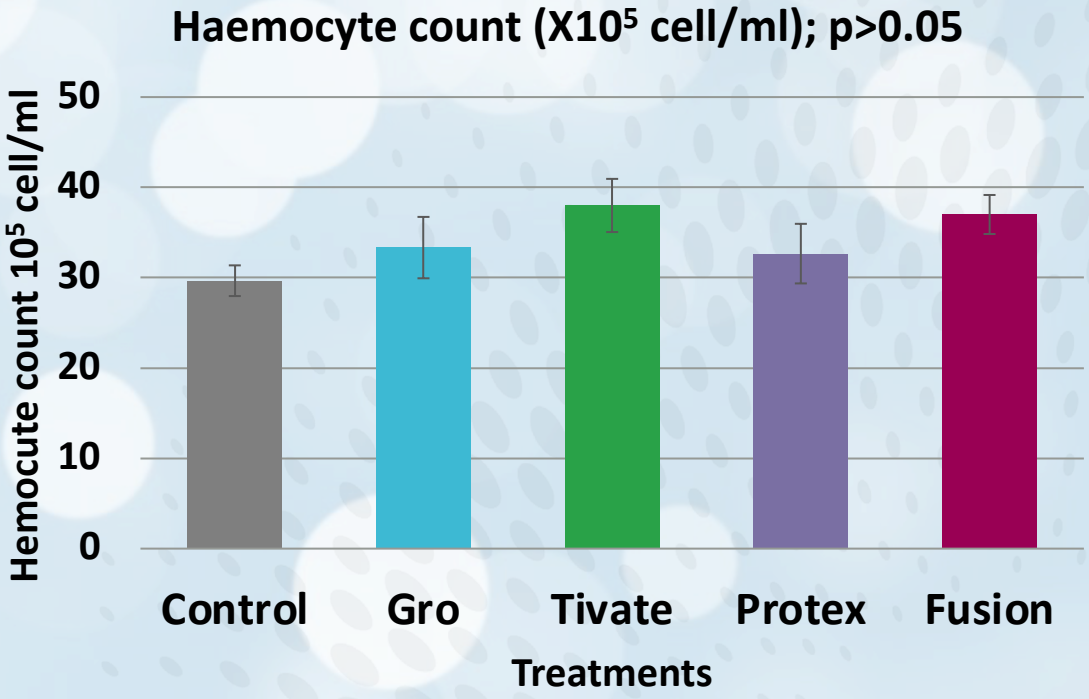


Evaluating the effect of different feed additives on to mitigate stress and improve the overall health status of *Litopenaeus vannamei* culture under normal and challenge conditions

Kasetsart University, Thailand,*2021*

Results – Haemocyte count before and after challenge





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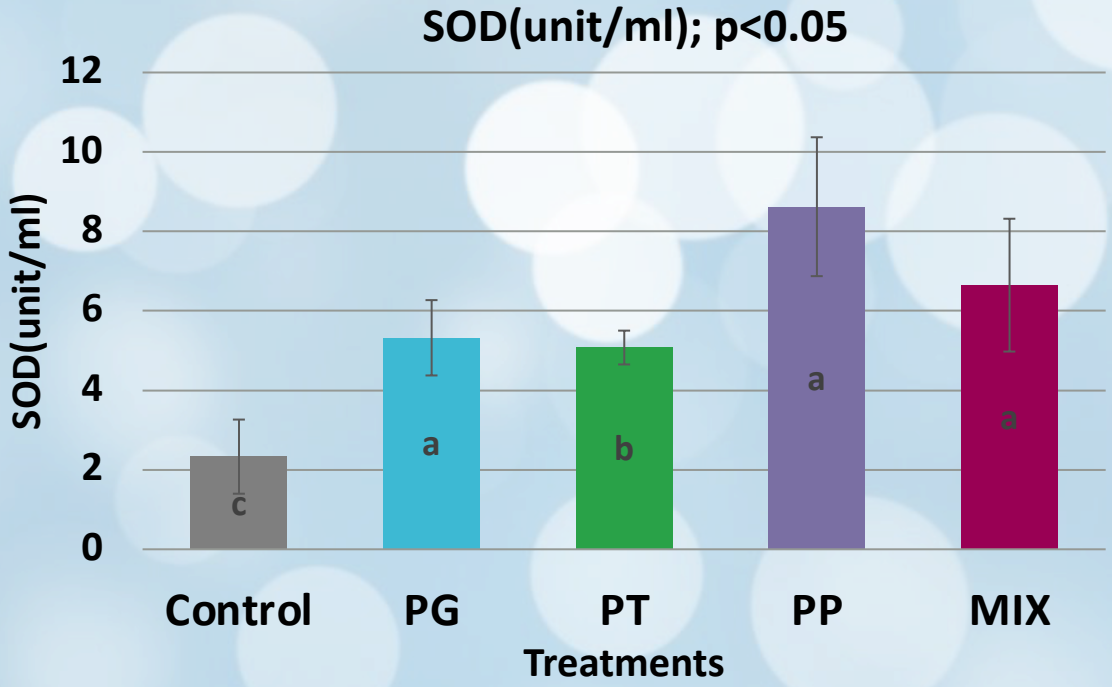
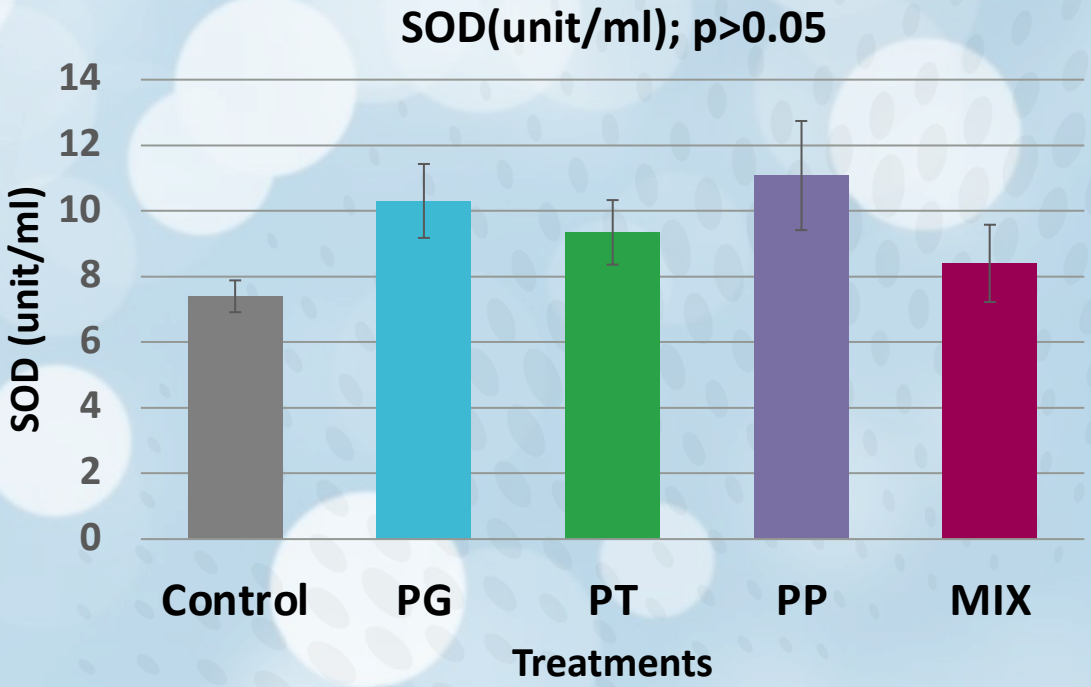
SOD Results before and after challenge

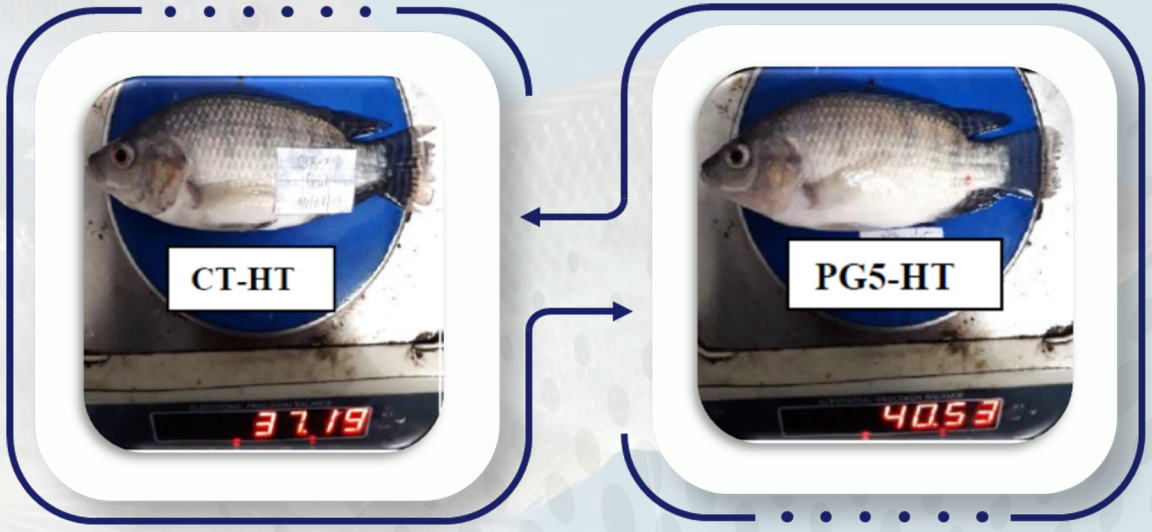




The effect of PAQ-Gro on growth performance and survival of tilapia (*Oreochromis spp.*) under heat stress conditions

NONG LAM University, Vietnam, 2020

Tilapia	Species
Control vs Functional Feed in two doses 2kg/MT and 5kg/MT	**Treatment**
3	**Number Replicas**
42 days	**Duration**
35 fish/tank	**Stocking**
8.8g	**Initial Weight**
Heat stress 36^{0}c for two weeks	**Challenge**



		1 Month				2 Month	
Treatments	CODE	1 week	2 week	3 week	4 week	5 week	6 week
1	CTR-NT						
2	CTR-HT						
3	PG2-NT						
4	PG5-NT						
5	PG2-HT						
6	PG5-HT						

Normal Temperature

High Temperature

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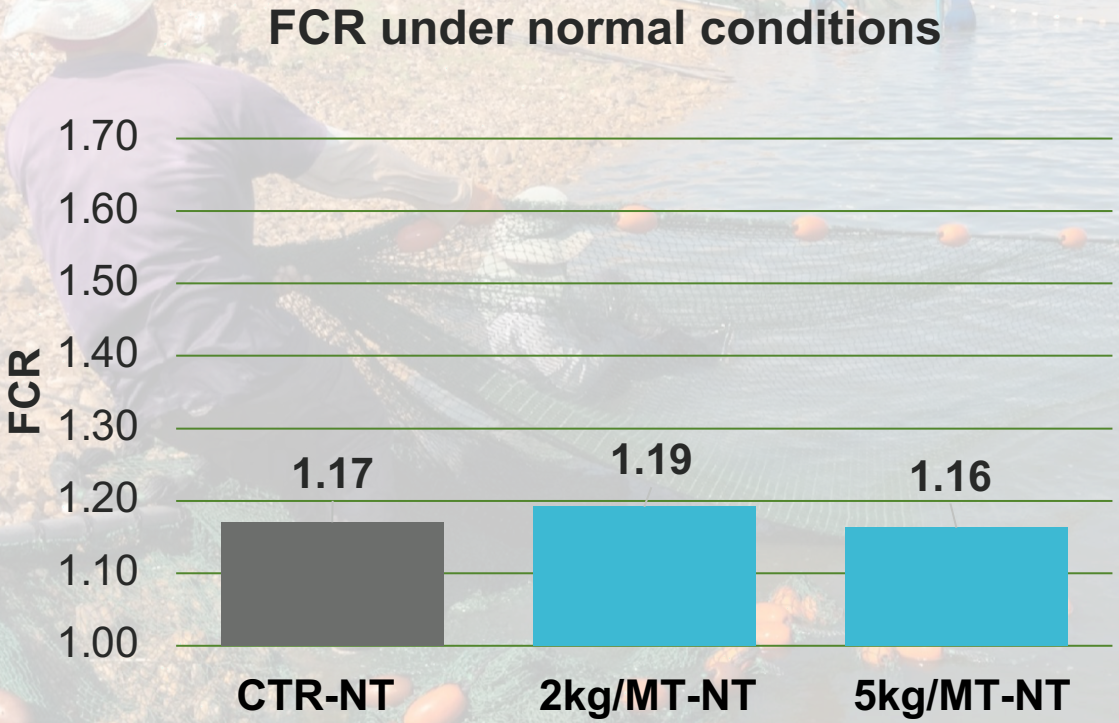
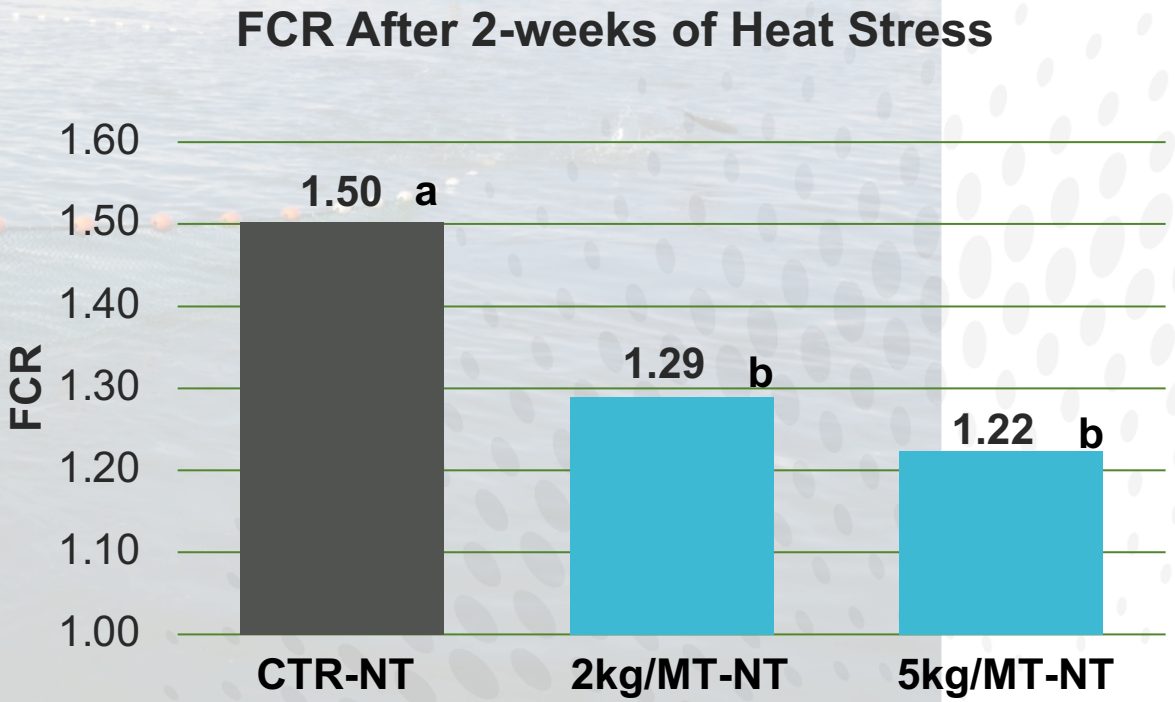
NONG LAM University, Vietnam, 2020





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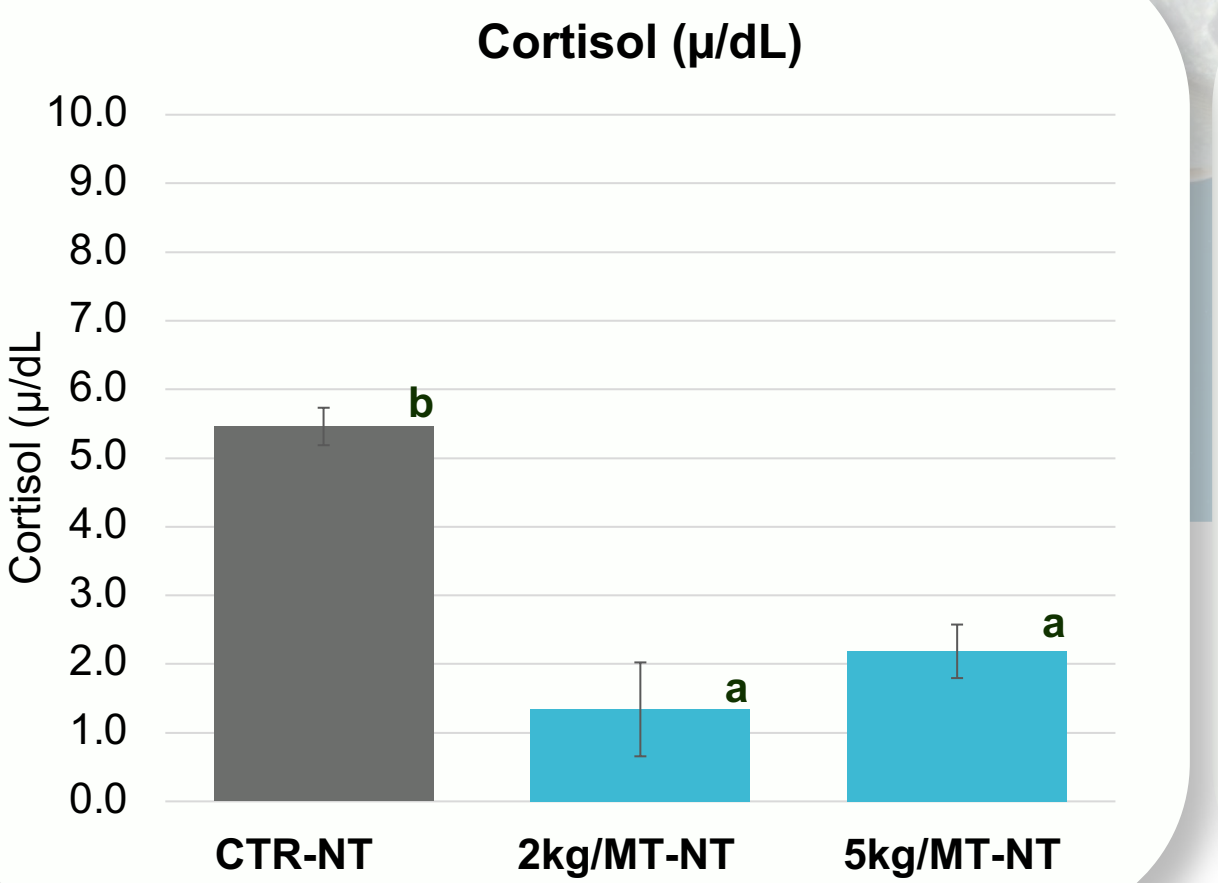
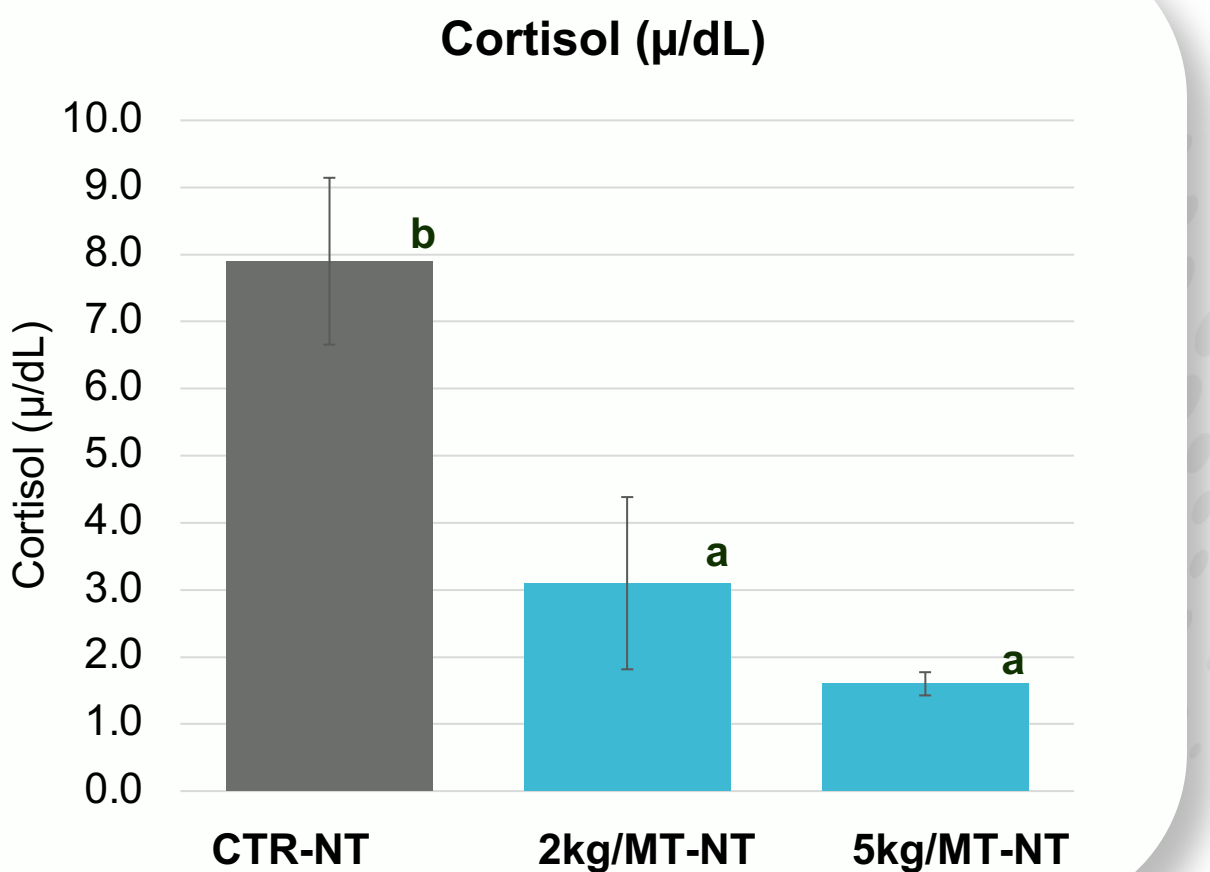
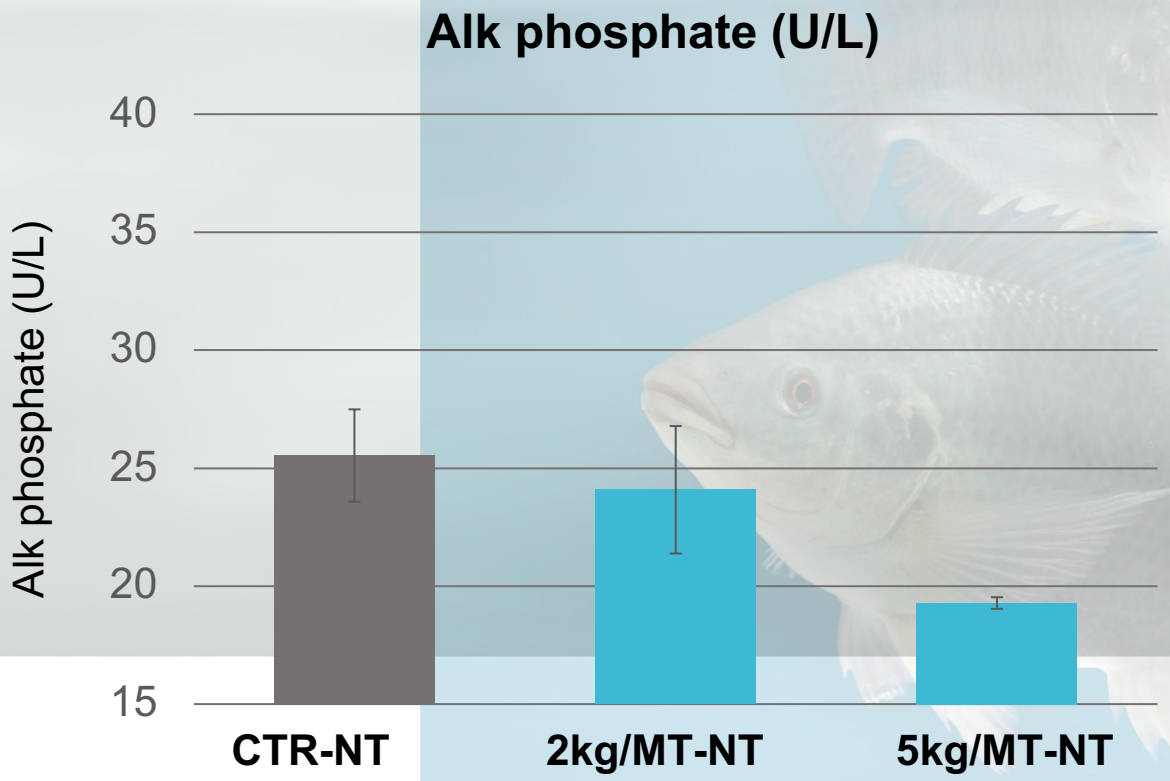
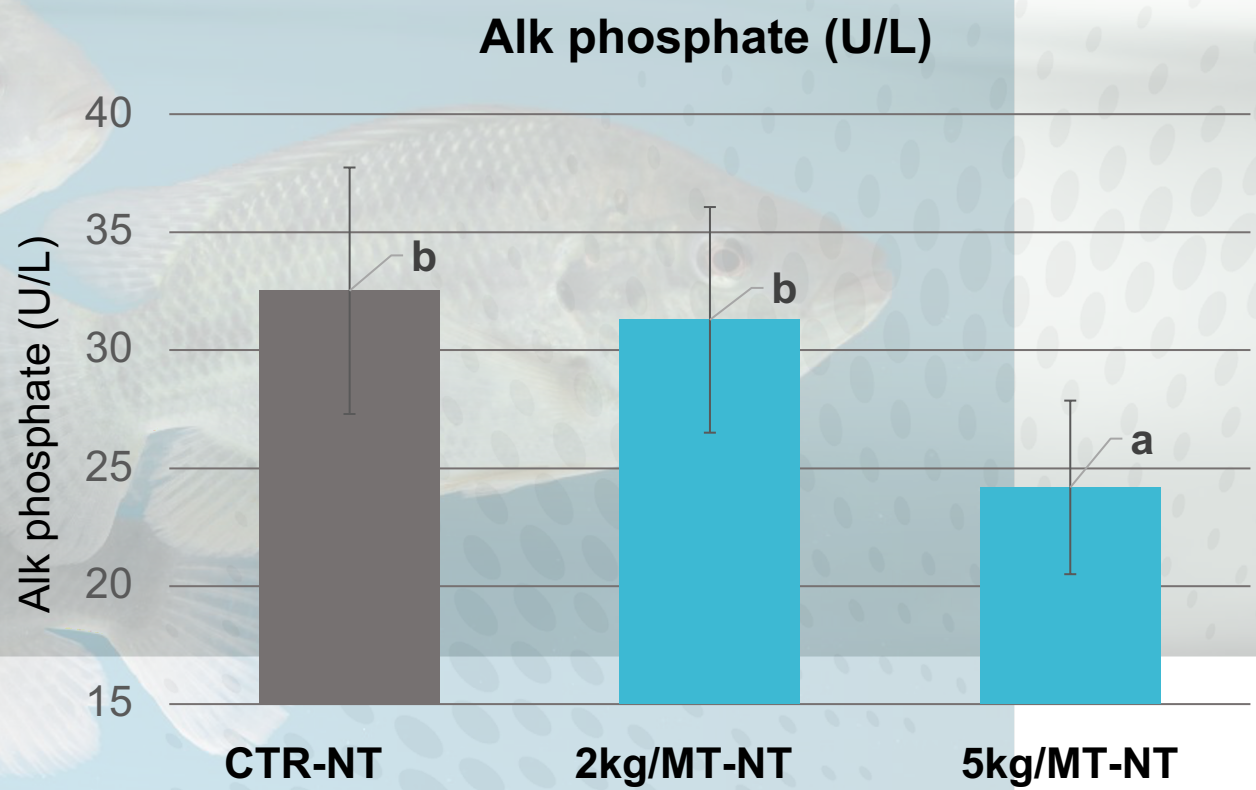




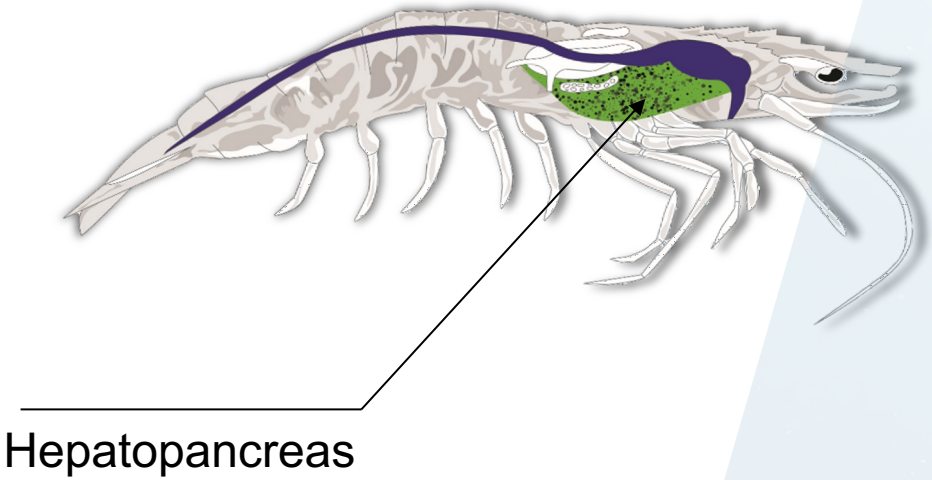
Results

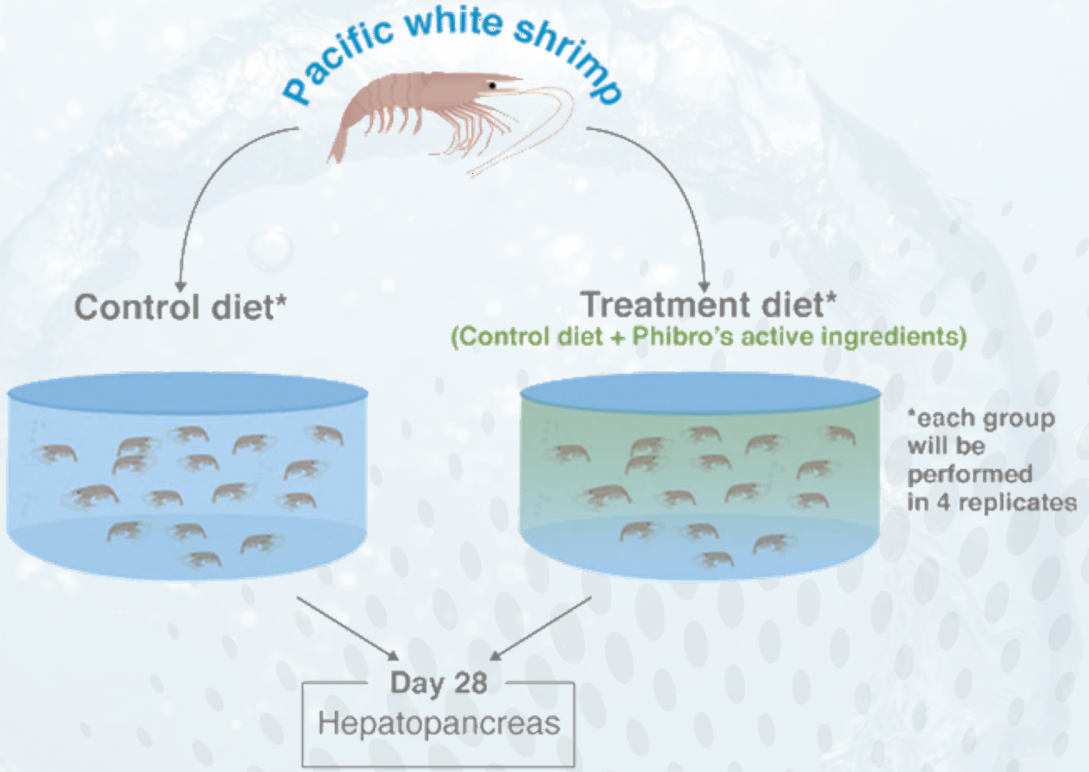
Biochemistry - Alkaline phosphate - week 3 – day 7 within heat stress





Molecular Evaluation of stress modulating by Feed Additive



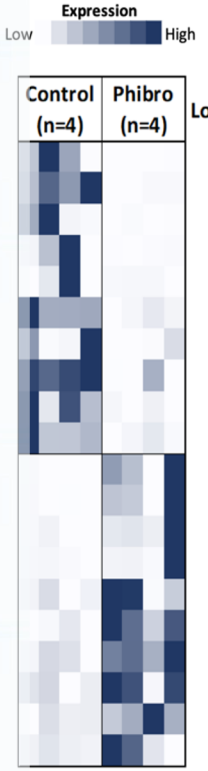


Shrimp hepatopancreas is the main organ for food absorption, transport, secretion of digestive enzymes, and storage of lipids, glycogen, and minerals – microbiota play important roles in this organ

Pacific white shrimp were fed a control diet, or a control diet supplemented with feed additive for 28 days. Hepatopancreas were collected from 4 shrimp fed each diet and analysed for gene expression. After this sampling EMS challenge was performed

Molecular Evaluation of stress modulating by Feed Additive

Top 10 most up- and down-regulated transcripts

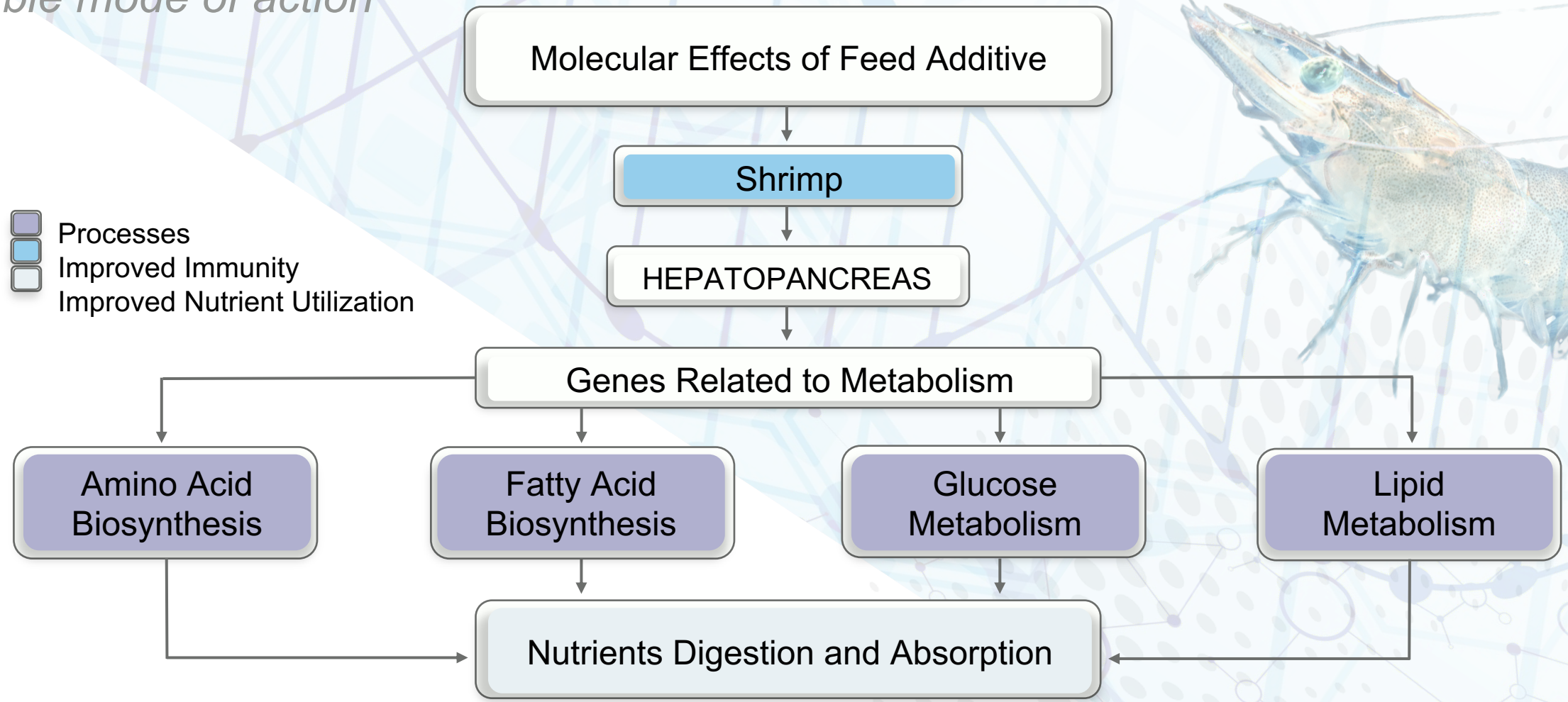


Log_2 foldchange	Description
-7.16	Alpha-2-Macroglobulin-Like Protein 1
-5.46	Endocuticle Structural Glycoprotein Sgabd-8-Like
-5.14	Myosin Regulatory Light Chain 2-Like
-5.05	Betaine--Homocysteine S-Methyltransferase 1-Like
-3.76	Uncharacterized Protein Loc113813285
-3.55	Cyclin-Dependent Kinase 8-Like
-2.77	Canalicular Multispecific Organic Anion Transporter 1-Like
-2.76	Uncharacterized Protein Loc113816666
-2.75	Glutamate Receptor Ionotropic, Nmda 1-Like
-2.55	Chorion Peroxidase-Like
5.89	Gamma-Butyrobetaine Dioxygenase-Like
5.80	Triosephosphate Isomerase B-Like Isoform X2
4.06	Uncharacterized Protein Loc113828607
3.19	Fatty Acid Synthase-Like
3.04	Arylsulfatase B-Like
3.02	Hybrid Signal Transduction Histidine Kinase L-Like
2.90	28S Ribosomal Protein S31, Mitochondrial-Like
2.77	Uncharacterized Protein Loc113802542
2.72	Gamma-Butyrobetaine Dioxygenase-Like
2.46	Uncharacterized Protein Loc113826423

- acts as an antiprotease and is able to inactivate an enormous variety of proteinases
- plays a key components for flexible, soft and hydrated endocuticle
- essential for ATPase activity
- part of the pathway L-methionine biosynthesis
- associate with the mediator complex and regulate transcription by several mechanisms
- involves L-carnitine biosynthesis pathway
- essential for the transport of activated fatty acids
- catalyzes the interconversion between DHAP and G3P in glycolysis and gluconeogenesis
- catalyzes fatty acid synthesis
- converts to 3-oxoalanine of a cysteine residue in eukaryotes, which is critical for catalytic activity
- acts as a receptor histidine kinase for a signal transduction pathway

Molecular Evaluation of stress modulating by Feed Additive

Possible mode of action

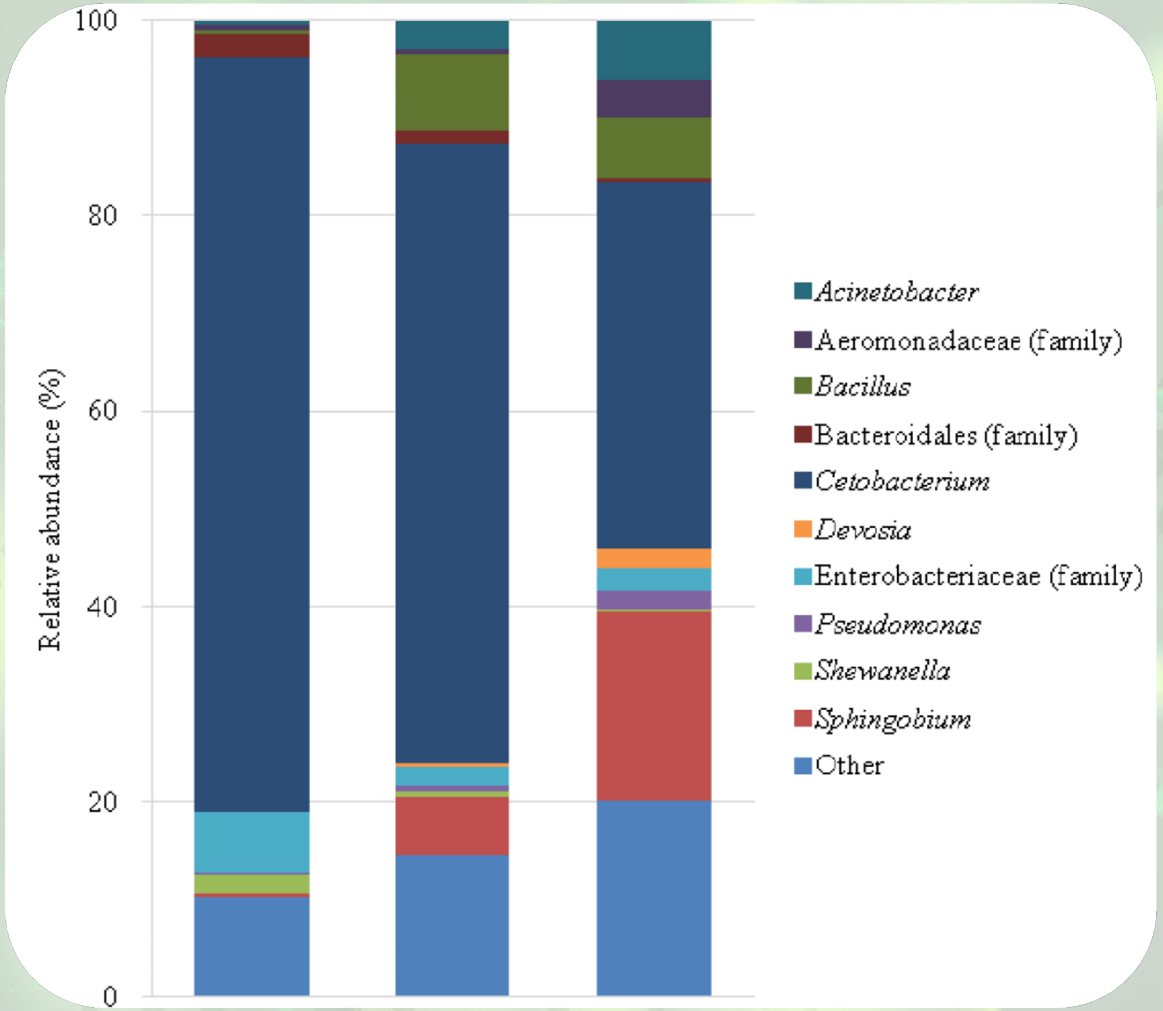


Gut microbiome analysis in shrimp

Modulation of the gut microbiota of Pacific white shrimp (*Penaeus vannamei* Boone, 1931) using feed additive

p-value	1.0% YCW	0.5% YCW	Control	Genus name
0.010*	6.06% [a]	2.79% [ab]	0.37% [b]	**Acitnetobacter**
0.027*	3.86% [a]	0.75% [b]	0.64% [b]	**Aeromonadaceae†**
0.025*	6.37% [a]	7.74% [a]	0.43% [b]	**Bacillus**
<0.001***	0.23% [c]	1.25% [b]	2.30% [a]	**Bacteroidales†**
0.002**	37.46% [b]	63.62% [ab]	77.31% [a]	**Cetobacterium**
0.010*	2.24% [a]	0.32% [b]	0.01% [b]	**Devosia**
0.297	2.28%	1.87%	6.27%	**Enterobacteriacea†**
0.003**	1.77% [a]	0.60% [b]	0.24% [b]	**Pseudomonas**
0.016*	0.26% [b]	0.60% [ab]	1.78% [a]	**Shewanella**
0.005**	19.38% [a]	5.89% [a]	0.37% [b]	**Sphingobium**
-	20.09%	14.55%	10.36%	**Other**

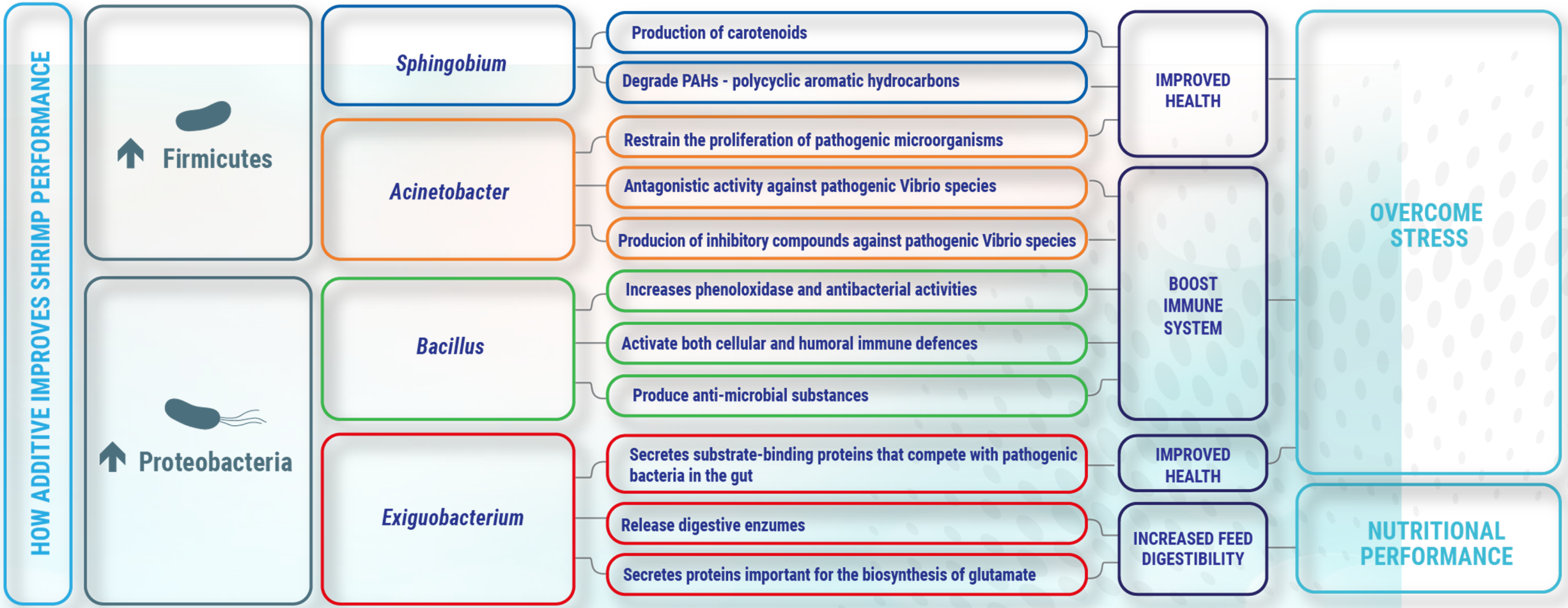
†Family. Different superscript letters indicate significant differences. Data presented as mean (n=7). ANOVA, Post-hoc Tukey test, $p<0.05$.



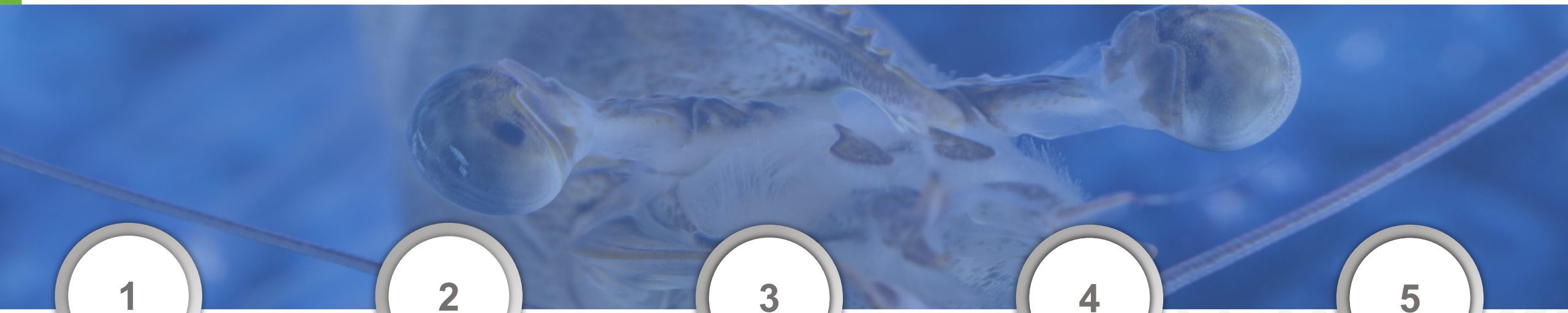
Source: Servin Arce et al 2020

Gut microbiota

Proposed mode of action driven by gut microbiota



Conclusions:



1. Feed additives: Enhances the metabolic function in shrimp
2. Upregulates genes related to immune and metabolic pathways
3. Improve shrimp performance under various stress conditions
4. Manipulates the microbiome resulting in more beneficial bacteria
5. Improves the immune and metabolic response in shrimp to better overcome stress events

Thank You

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E-mail: Benny.Shapira@pahc.com