# Antimicrobial peptides from egg white:

## Antibiotic alternatives for aquaculture

*เปปไทด์ต้านจุลชีพจากไข่ขาว*

*ทางเลือกทดแทนยาปฏิชีวนะสำหรับอุตสาหกรรมสัตว์น้ำ*

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**Functional Ingredients and Food Innovation Research Group**
**National Center for Genetic Engineering and Biotechnology (BIOTEC)**

## Other than productivity (yield), **FOOD SAFETY** is everyone's business in aquaculture.

Food and Agriculture Organization of the United Nations | World Health Organization | SUSTAINABLE DEVELOPMENT GOALS

# Food safety is everyone's business in aquaculture

## The importance of food safety

Aquaculture products are an important source of nutritious food, contributing with 88 million tonnes (49 percent of the global production of aquatic animals) to food security and are expected to continue their expansion. These products provide nutrients for millions of people all over the world, including landlocked regions. Food safety is a key component of good aquaculture governance, which is necessary to enhance the sector's contribution to the achievement of related Sustainable Development Goals (SDGs).

Hazards associated with aquaculture products are broadly the same as those occurring in wild-caught fish and seafood varieties. However, the inputs to aquaculture, such as feed, medication, stock and water and the practices and production environment, for example, proximity to other farming systems, all have the potential to introduce new hazards. For instance, the misuse of antimicrobials during aquaculture production can lead to the presence of antimicrobial residues in food and water bodies, as well as for the selection and spread of antimicrobial resistance (AMR).

For World Food Safety Day (7 June), reveal an insider's perspective by making a video of the food safety practices used on your fish farm.

the misuse of antimicrobials during aquaculture production can lead to the presence of antimicrobial residues in food and water bodies, as well as for the selection and spread of antimicrobial resistance (AMR).

Source: https://iris.who.int/bitstream/handle/10665/367492/WHO-HEP-NFS-AFS-2023.4-eng.pdf?sequence=1

# Our (animals') needs …

**Protect**
from bacterial infection

Source: freepik

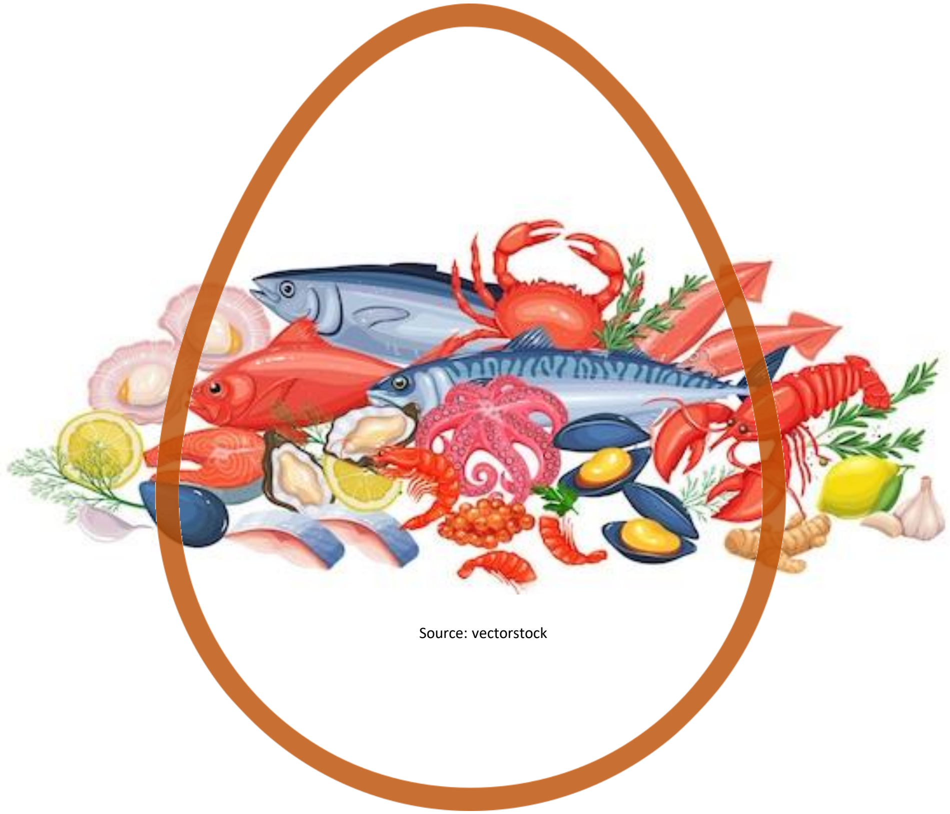
**Treat**
when infected by pathogens

**Nurture**
for their growth and well-being

# … egg may address.

## Protect

from bacterial infection
(high viscous albumen, protease inhibitors, nutrient sequesters, lytic enzyme, antibodies)



Source: vectorstock

## Treat

when infected by pathogens
(protease inhibitors, nutrient sequesters, lytic enzyme, antibodies)

## Nurture

for their growth and well-being
(nutrients)

# Chickens' eggs are for them, not you.

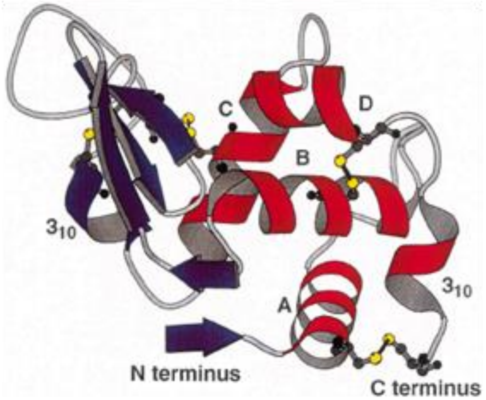
Source: vectorstock

Eggs are naturally designed/revolutionized to contain elements for chicken's growth and microbial protection.

Egg white is an alkaline solution (pH 8.0 – 9.0) and contains up to 148 proteins.

| Function | Protein | Abundance |
|---|---|---|
| | Ovalbumin | 54% |
| Di- and tri-valent cations binder – antimicrobial protein | Ovotransferrin | 12% |
| Trypsin inhibitor – inhibits microbial enzyme | Ovomucoid | 11% |
| | Ovoglobulin G2 | 4% |
| | Ovoglobulin G3 | 4% |
| | Ovomucin | 3.5% |
| **Antimicrobial enzyme – lyses bacterial cell wall** | Lysozyme | 3.4% |
| Trypsin inhibitor – inhibits microbial enzyme | Ovoinhibitor | 1.5% |
| | Ovoglycoprotein | 1% |
| | Flavoprotein | 0.8% |
| | Ovomacroglobulin | 0.5% |
| Biotin binding protein - insecticide, antimicrobial protein | Avidin | 0.05% |
| | Cystatin | 0.05% |

# What is lysozyme ?

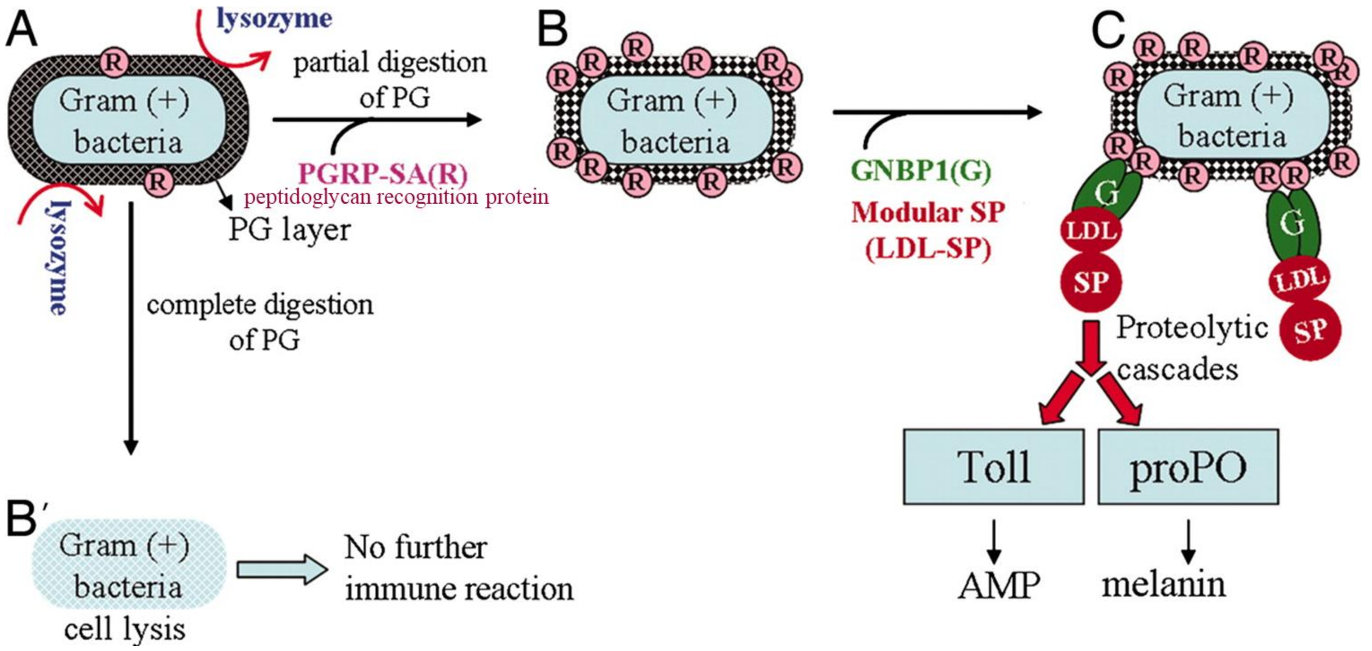



**Lysozyme**, enzyme found in the secretions (tears) of the lacrimal glands of animals and in nasal mucus, gastric secretions, and **egg white**.

Discovered in 1921 by Sir Alexander Fleming, lysozyme catalyzes the **breakdown of certain carbohydrates found in the cell walls of certain bacteria.**

Source: https://www.britannica.com/science/lysozyme

# Lysozyme's action




Lysozyme **activated toll and proPO pathways**.

Source: Park et al. (2007). Clustering of peptidoglycan recognition protein-SA is required for sensing lysine-type peptidoglycan in insects.

# Lysozyme's action

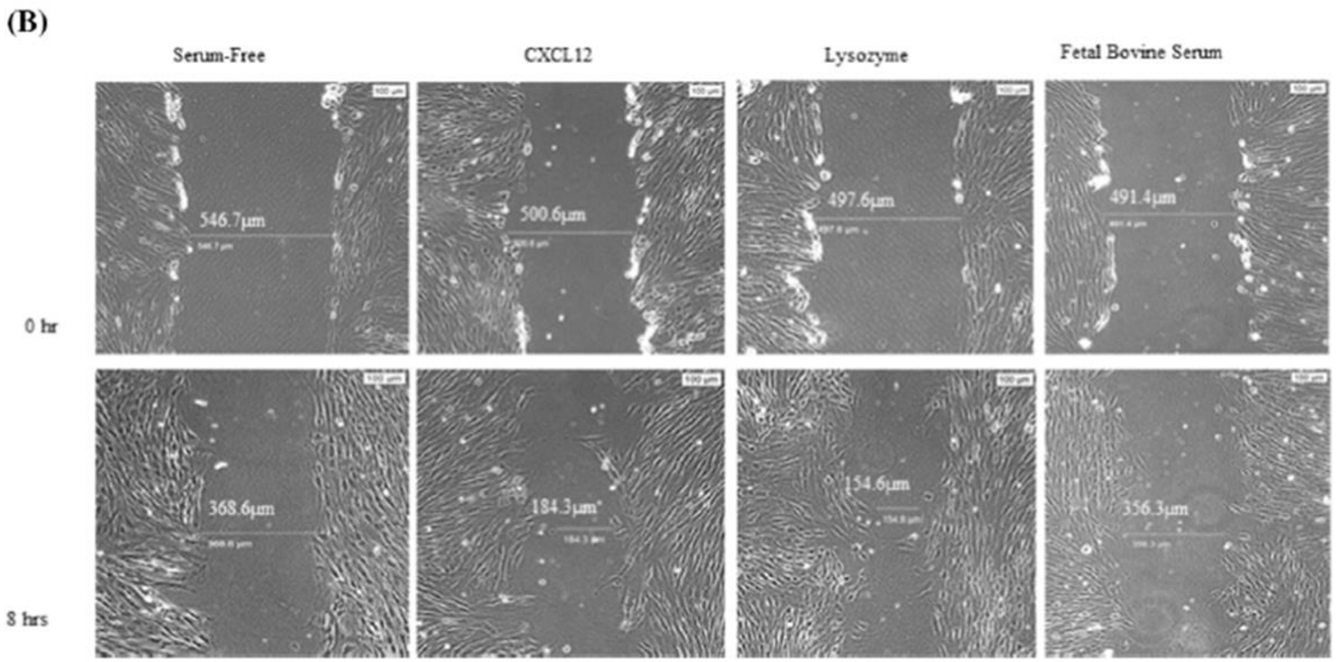
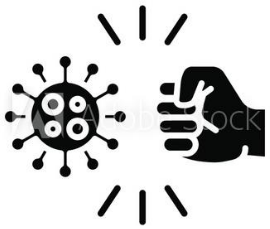



**Fig. 3.** (A) Human fetal colon epithelial cells CRL-1790 migrated towards lysozyme-containing media. Negative control was serum-free media ("Serum-Free"). Positive control was CXCL12-containing media. Fluorescent index values are means ± standard deviations from three biological replicates. (B) Scratch wound assay: Following an overnight incubation in serum-free media (serum starvation), CRL-1790 cells were scratch-wounded at time 0 h followed by incubation in serum-free media containing lysozyme, CXCL12, or 10% fetal bovine serum. Negative control was serum-free media. Images were taken and wound gaps were measured at 0 h and 8 h post-wounding.

Lysozyme induced migration and **repaired scratch wound** of intestinal epithelial.

Source: Abey et al. (2017). Lysozyme association with circulating RNA, extracellular vesicles, and chronic stress.

# Lysozyme’s action



## Attacks bacteria
by hydrolyzing bacterial cell wall

## Improves innate immunity
by up-regulating immune- and antioxidant related genes

## Maintains intestinal health
by improving epithelial cell migration to the wound

# Applications of lysozyme in aquaculture

Aquaculture Nutrition

*Aquaculture Nutrition* 2011 doi: 10.1111/j.1365-2095.2011.00902.x

## Effect of dietary inclusion of lysozyme on growth performance and plasma biochemical parameters of rainbow trout (*Oncorhynchus mykiss*)

J. DENG, B. BI, Q. AN, L. KONG, Q. WANG, L. TAO & X. ZHANG
*College of Animal Science and Technology, Yunnan Agricultural University, Kunming, China*

**Table 2** Growth performance, feed utilization and liver characteristics of rainbow trout fed diets with different lysozyme levels for 10 weeks[1]

| | Dietary lysozyme level (mg kg$^{-1}$) | | | | | Pooled | ANOVA | | Regression analysis | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 150 | 300 | 450 | 600 | SD | *F*-value | *P*-value | Equation | $R^2$ | *P*-value |
| Initial weight (g) | 7.56 | 7.78 | 7.78 | 7.78 | 7.89 | 0.44 | 0.171 | 0.948 | | | |
| Final weight (g) | 41.9[a] | 48.5[b] | 48.5[b] | 49.3[b] | 50.8[b] | 3.74 | 6.145 | 0.009 | $y = 41.97 + 7.823(1 - e^{-0.0101x})$ | 0.667 | 0.001 |
| Feed intake (g per fish) | 76.7[a] | 81.1[b] | 78.4[ab] | 79.2[ab] | 79.6[ab] | 1.75 | 6.973 | 0.006 | $y = 2E\text{-}07x^3 - 2E\text{-}04x^2 + 0.0431x + 76.923$ | 0.514 | 0.041 |
| SGR (% per day)[2] | 2.14[a] | 2.29[ab] | 2.29[ab] | 2.31[b] | 2.33[b] | 0.08 | 5.390 | 0.014 | $y = 2.14 + 0.171(1 - e^{-0.0114x})$ | 0.667 | 0.001 |
| FER[3] | 0.45[a] | 0.50[ab] | 0.52[b] | 0.52[b] | 0.54[b] | 0.04 | 5.941 | 0.010 | $y = 0.447 + 0.086(1 - e^{-0.00656x})$ | 0.696 | <0.001 |
| PER[4] | 1.04[a] | 1.17[ab] | 1.19[ab] | 1.22[b] | 1.26[b] | 0.09 | 6.048 | 0.010 | $y = 1.043 + 0.214(1 - e^{-0.00478x})$ | 0.693 | <0.001 |
| Protein retention (%)[5] | 18.6[a] | 22.4[ab] | 22.6[ab] | 22.9[b] | 22.8[b] | 0.54 | 5.735 | 0.018 | $y = -2.6E\text{-}05x^2 + 0.021x + 18.948$ | 0.673 | 0.004 |
| Lipid retention (%)[6] | 31.8[a] | 38.3[b] | 38.2[b] | 38.2[b] | 37.5[b] | 0.88 | 7.065 | 0.010 | $y = -5.2E\text{-}05x^2 + 0.039x + 32.420$ | 0.565 | 0.010 |
| Survival (%) | 91.1 | 100 | 95.6 | 97.8 | 97.8 | 7.51 | 0.460 | 0.764 | $y = 3E\text{-}07x^3 - 3E\text{-}04x^2 + 0.0819x + 91.511$ | 0.128 | 0.666 |
| Liver characteristics | | | | | | | | | | | |
| Total lipid (g kg$^{-1}$) | 38.8 | 38.8 | 38.3 | 37.3 | 38.0 | 0.25 | 1.574 | 0.255 | $y = -0.0021x + 38.887$ | 0.228 | 0.072 |
| HSI (%)[7] | 1.49 | 1.45 | 1.46 | 1.44 | 1.41 | 0.04 | 1.434 | 0.292 | $y = -0.0001x + 1.483$ | 0.314 | 0.030 |

Lysozyme supplementation significantly **improved FER, PER and** slightly increased **survival rate and protein content** of rainbow trout.

# Applications of lysozyme in aquaculture

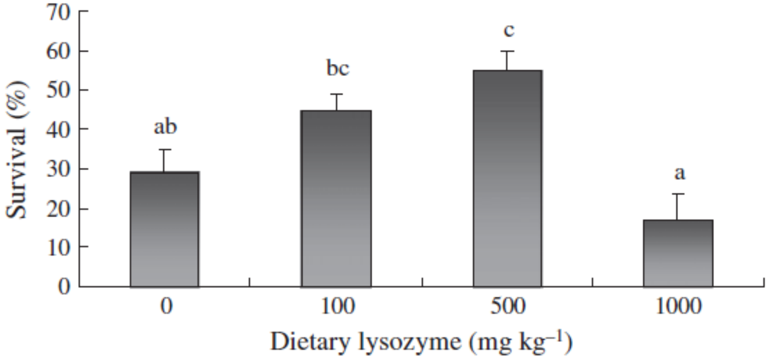
Aquaculture Nutrition

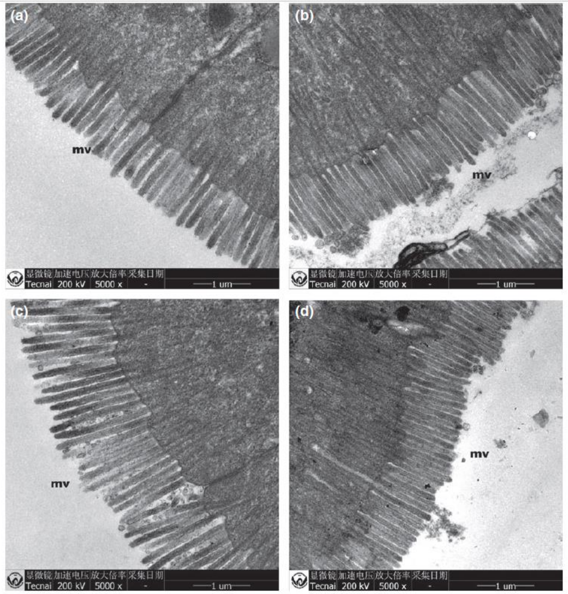
*Aquaculture Nutrition* 2014 **20**; 229–241 doi: 10.1111/anu.12069

## Effect of dietary lysozyme on growth, immune response, intestine microbiota, intestine morphology and resistance to *Aeromonas hydrophilia* in gibel carp (*Carassius auratus gibelio*)

Y. CHEN[1,2], X. ZHU[1], Y. YANG[1], D. HAN[1], J. JIN[1] & S. XIE[1]

[1] *State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, The Chinese Academy of Sciences, Wuhan, China;* [2] *University of the Chinese Academy of Sciences, Beijing, China*




Lysozyme supplementation **increased microvilli length and survival** rate of gibel carp after immersion challenge with *Aeromonas hydrophilia*

# eLysozyme for shrimp farming

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journal homepage: www.elsevier.com/locate/aquaculture

ELSEVIER

## Suppression of white feces syndrome in Pacific white shrimp, *Litopenaeus vannamei*, using hen egg white lysozyme

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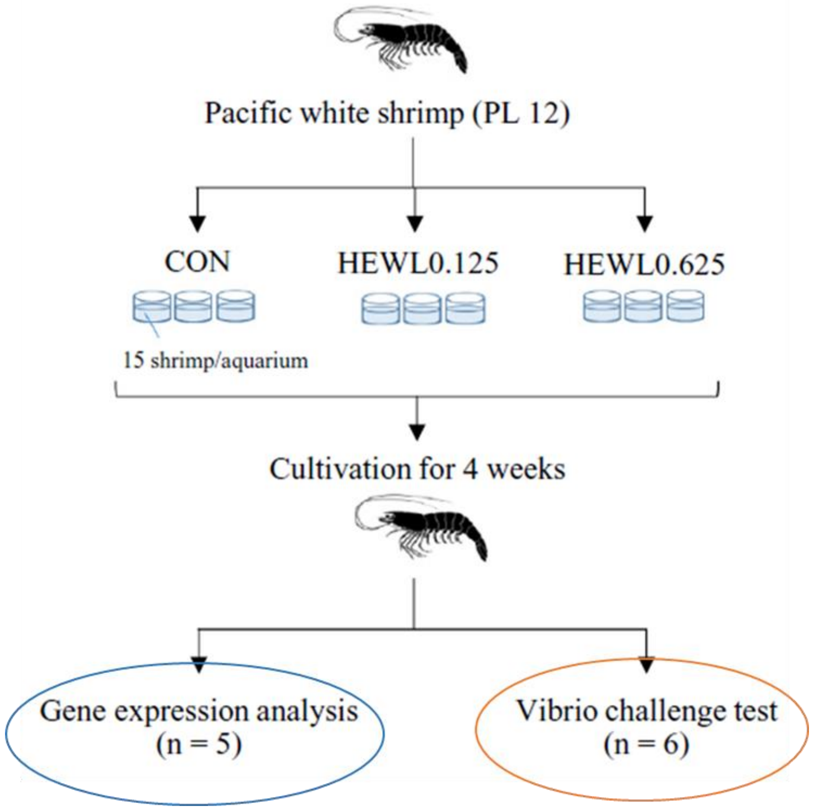
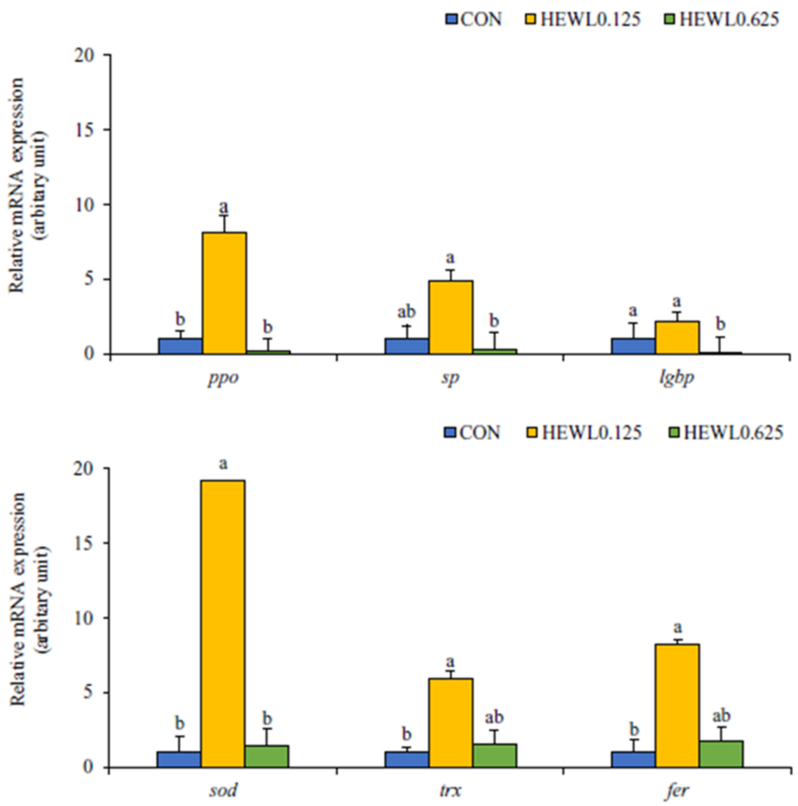
# eLysozyme for shrimp farming (pathogen challenging)

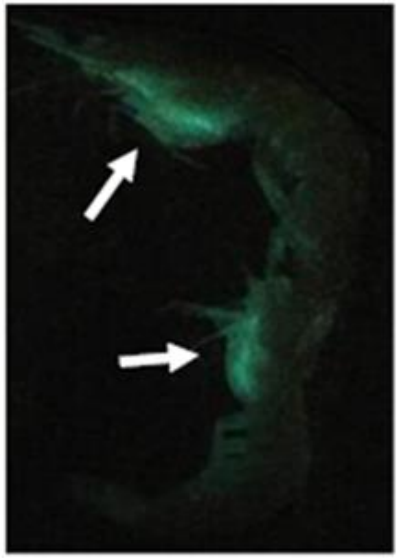


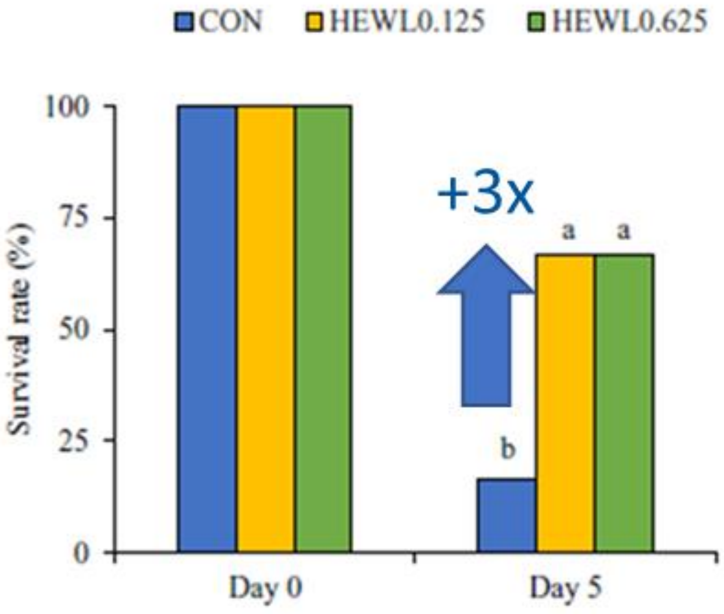

*15 ppt sea salt, pH 7.5 – 8.5, D.O. > 5 mg/L, temperature 28 – 30 degree Celsius, feeding for 4 times daily, 35% protein feed
**Immersion challenging in 7 log CFU/ml for 24 h.




**The expression level of immune-related and antioxidant-related genes** in shrimp hepatopancreas **were significantly improved by feeding** with 125 ppm **eLysozyme.**

# eLysozyme for shrimp farming (pathogen challenging)

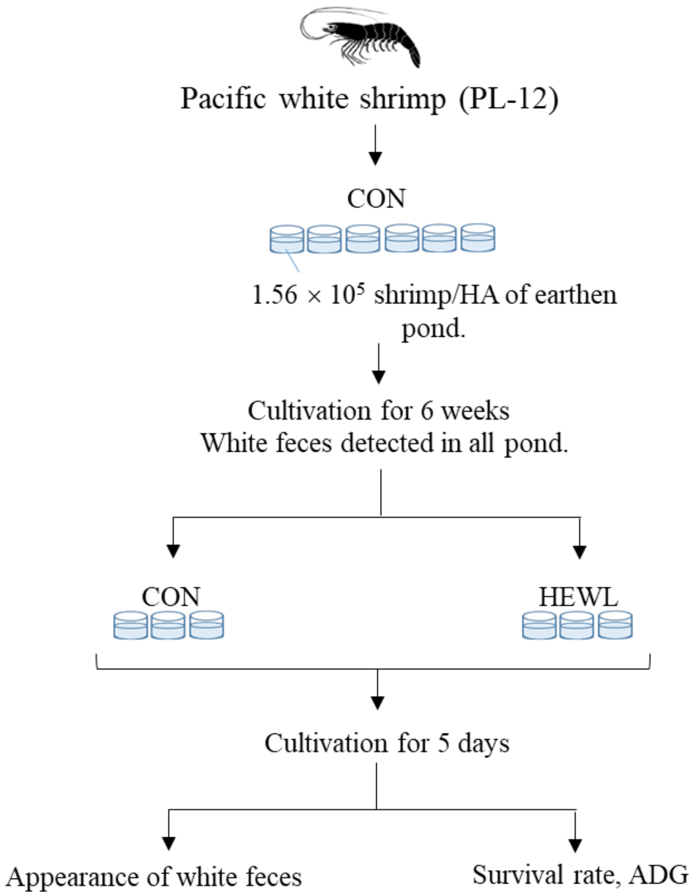


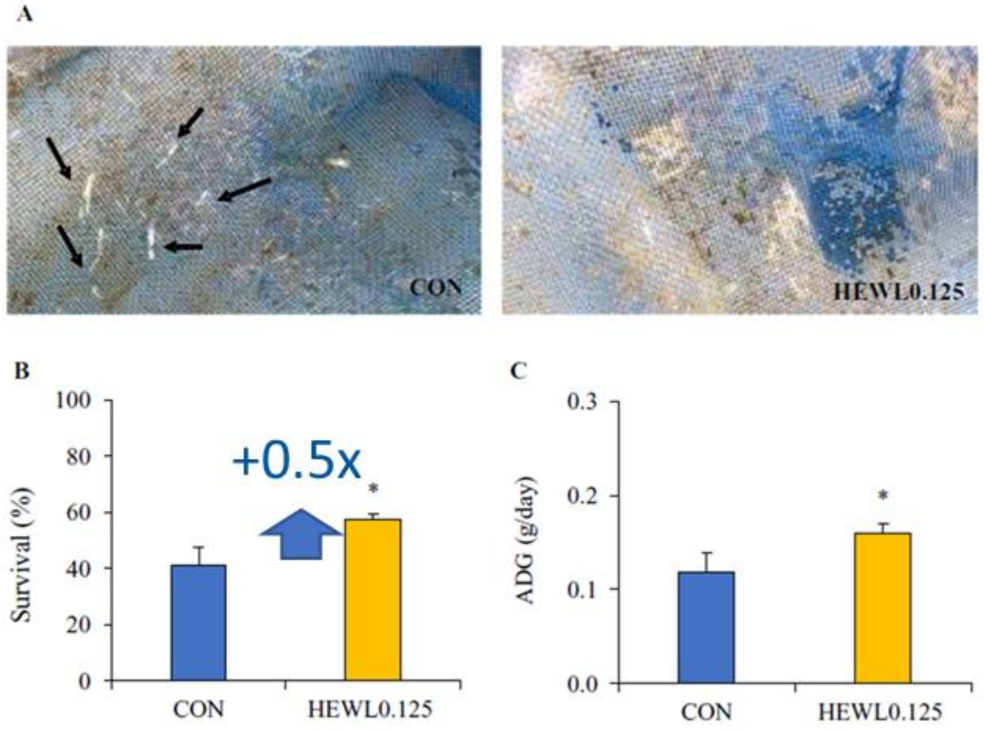



In pathogen challenging condition, eLysozyme Improved the Vibrio/disease resistance of shrimp.

After Vibrio challenging, **the survival rate of shrimp fed 125 and 625 ppm eLysozyme was significantly higher** than control (unfed eLysozyme).

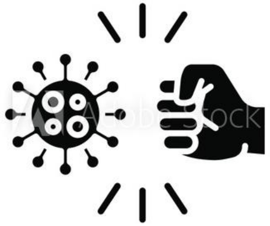
# eLysozyme for shrimp farming (control of white feces syndrome)




- Shrimp **recovered from WFS** after fed 125 ppm eLysozyme **for 5 days.**
- The **survival rate** of shrimp fed eLysozyme was **significantly higher** than control.
- **Crop yield** of eLysozyme group was **200% of control group.**

# Commercial product of eLysozyme for shrimp farming



Attacks bacteria

Improves shrimp immunity and crop yield


Avanti® Feeds Limited

Thai Union Feedmill

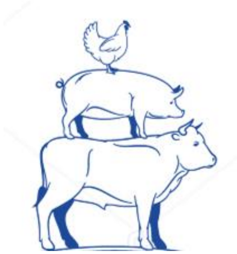
บริษัท โอโว่ ฟู้ดเทค จำกัด OVO FOODTECH CO., LTD.

BIOTEC a member of NSTDA

# Other hen egg white proteins as promising sources of functional peptides for used as antibiotic alternative and growth promoters

| Source of peptides | Functions |
|---|---|
| Ovotransferrin | Antimicrobial activity (Legros et al., 2021; Rathnapala et al., 2021)<br>Immunomodulatory (Rathnapala et al., 2021)<br>Anti-inflammatory activity (Rathnapala et al., 2021)<br>Antioxidant activity (Benedé and Molina, 2020; Ratnapala et al., 2021) |
| Ovalbumin | Antimicrobial activity (Pellegrini et al., 2004; Tan et al., 2020)<br>Antioxidant activity (Benedé and Molina, 2020) |
| Ovomucin | Antimicrobial activity (Tu et al., 2020)<br>Immunomodulatory activity (Tu et al., 2020)<br>Anti-inflammatory activity (Tu et al., 2020)<br>Antioxidant activity (Tu et al., 2020) |
| Ovomucoid | Immunomodulatory activity (Holen et al., 2001; Kovacs-Nolan et al., 2005)<br>Antioxidant activity (Abeyrathne et al., 2015; Benedé and Molina, 2020) |
| Cystatin | Antimicrobial activity (Blankenvoorde et al., 1998)<br>Antioxidant activity (Benedé and Molina, 2020) |

# Conclusions and future challenges



- Utilization of **hen egg white lysozyme, as antibiotic alternative, can effectively contribute to animal well-being** by providing antimicrobial activity against pathogenic bacteria, improving immunity and increase growth performance.

- With the rapid progress in bioinformatics, computational simulation and biotechnology, more **effective AMPs from hen egg white lysozyme can be discovered and tailor-made to meet specific user's requirements.**

- Other than lysozyme, **many hen egg proteins/peptides are promising sources of antibiotic alternative** for animal farming.

- Comprehensive research is required to **gain insight and study the feasibility of these AMPs** for used in animal farming application.

# THANK YOU

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