

Comparative evaluation of the immunostimulatory activities of autolyzed yeast and purified β -glucan in Pacific white shrimp (*Litopenaeus vannamei*)

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Research highlights

- Purified β -glucan elicited stronger immunostimulatory effects than autolyzed yeast in *Litopenaeus vannamei*.
- Dietary supplementation with BG200 significantly improved weight gain and survival during the nursery stage.
- Dietary supplementation with BG200 and AY6 enhanced resistance to *Vibrio parahaemolyticus*-induced AHPND.
- Purified β -glucan significantly induced broad upregulation of innate immune-related genes in *Litopenaeus vannamei*.
- Dietary BG400 supplementation significantly increased hemocyte phagocytic activity in shrimp.

Comparative evaluation of the immunostimulatory activities of autolyzed yeast and purified β -glucan in Pacific white shrimp (*Litopenaeus vannamei*)

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Abstract

Disease outbreaks pose a major challenge to the aquaculture industry, often resulting in antimicrobial abuse. To address this, enhancing host immunity to naturally combat pathogens offers a sustainable solution. Functional feed additives, including autolyzed yeast and purified β -glucan, have emerged as promising alternatives to antimicrobials, effectively boosting shrimp health and growth performance. This study evaluated the effects of dietary supplementation with autolyzed yeast at 3 Kg/ton (AY3) and 6 Kg/ton (AY6), alongside purified β -1,3/1,6-glucans at 200 g/ton (BG200) and 400 g/ton (BG400), on the growth performance, post-challenge survival against acute hepatopancreatic necrosis disease (AHPND), immune gene expression, and phagocytic activity of *Litopenaeus vannamei*. Dietary supplementation with BG200 resulted in significantly higher weight gain during the PL12–PL34 stage ($P = 0.047$) and increased survival during the PL34–PL49 stage ($P = 0.003$) compared to the control (Basal Feed, BF) group. Following an immersion challenge with *Vibrio parahaemolyticus* (AHPND strain), shrimp fed

AY6 and BG200 exhibited significantly reduced cumulative mortality ($P = 0.008$ and $P = 0.002$, respectively). Continuous 70-day feeding trials (2 g shrimp) demonstrated the upregulation of immune-related genes (e.g., *PO1*, *PO2*, *PPAE2*, *Crustin 1*, *ALF1*, *ALF3*, *Peroxinectin*, and *HSP70*) in the BG200 and BG400 groups. Shrimp fed with AY3 and AY6 induced moderate gene expression responses. Furthermore, phagocytic activity was significantly elevated in the BG400 group ($P = 0.004$). Overall, these results indicate that purified β -glucan exerts a more pronounced impact on growth, immune response, and disease resistance than autolyzed yeast. These findings highlight the potential of dietary β -glucan supplementation to enhance shrimp health and mitigate disease risks.

Keywords: White leg shrimp (*Litopenaeus vannamei*); Autolyzed yeast; Yeast β -glucan; Immunostimulant; *Vibrio parahaemolyticus* AHPND; Alternative to antimicrobials (ATA).

Introduction

Aquaculture is one of the fastest-growing food production sectors globally, with shrimp farming playing a pivotal role in sustaining international seafood supply and trade. However, intensive production systems expose shrimp to multiple environmental and pathogenic stressors that compromise growth performance, disrupt immune homeostasis, and increase disease susceptibility (Kumar et al. 2025; Rowley et al. 2024). Among these, *Vibrio parahaemolyticus*, the etiological agent of acute hepatopancreatic necrosis disease (AHPND), represents one of the most devastating bacterial pathogens in shrimp aquaculture. Since its emergence, AHPND has caused extensive mortality and severe economic losses across Asia and Latin America (Tran et al. 2013; Leet et al. 2015; Kumar et al. 2021; Sanches-Fernande et al. 2022). The disease is driven by the pVA1 plasmid harboring the *pirAB* toxin genes, which encode binary toxins that induce acute hepatopancreatic epithelial necrosis (Intriago et al. 2023; Joshi et al. 2014; Han et al. 2015; Sirikharin et al. 2015). Notably, horizontal gene transfer has facilitated interspecies dissemination of the pVA1 plasmid, broadening the host range and accelerating the epidemiological spread of AHPND-associated pathogens. Beyond *V. parahaemolyticus*, the plasmid has been identified in other *Vibrio* species, including *V. campbellii*, *V. harveyi*, and *V. alginolyticus*, as well as in *Shewanella khirikhana* (Dong et al. 2019; Muthukrishnan et al. 2019; Paopradit et al. 2021; Prachumwat et al. 2020).

Environmental stressors further exacerbate disease vulnerability. Under intensive farming conditions, shrimp are routinely exposed to high stocking densities, temperature and salinity fluctuations, and deteriorating water quality, all of which impair immune competence. Stress-induced immunosuppression is characterized by reduced total hemocyte counts, diminished phenoloxidase activity, and downregulation of antimicrobial peptide gene expression (Chen and He 2019; Zheng et al. 2017; Xu et al. 2019a; Wang et al. 2020; Farhadi et al. 2023). Unlike vertebrates, shrimp lack adaptive immunity and rely exclusively on innate immune defenses (Tassanakajon et al. 2018). These defenses comprise integrated cellular responses (phagocytosis, encapsulation, and nodulation) and humoral effectors, including the prophenoloxidase (proPO) activating cascade, antimicrobial peptides (Penaeidins, Crustins, and Anti-lipopolysaccharide factors (ALF)), and stress-associated proteins such as HSP70 (Söderhäll and Cerenius 1998; Wang and Wang 2013). Enhancing these innate immune mechanisms is therefore essential to sustain shrimp health under intensive culture systems.

Functional feed additives have emerged as strategic alternatives to antimicrobials, whose excessive use poses risks related to antimicrobial resistance and food safety (Defoirdt et al. 2011; Dawood et al. 2018). Among these, yeast-derived products particularly autolyzed yeast and purified β -glucan have attracted considerable attention as immunostimulants (Perricone et al. 2022). β -glucan, a principal structural polysaccharide of yeast cell walls, acts as a pathogen-associated molecular pattern (PAMP) recognized by pattern recognition receptors (PRRs) on shrimp hemocytes, thereby activating the proPO cascade, enhancing phagocytosis, and upregulating immune-related genes (Akramienė et al. 2007; Meena et al. 2013; Liu et al. 2025). Autolyzed *Saccharomyces cerevisiae*, produced through enzyme-mediated self-digestion, contains β -glucans, mannan-oligosaccharides, nucleotides, and bioactive peptides that synergistically modulate gut microbiota, strengthen antioxidant defenses, and stimulate hemocyte proliferation (Li and Gatlin 2006, 2012; Li et al. 2019).

Despite accumulating evidence supporting the immunomodulatory potential of yeast-derived products, direct comparative assessments of autolyzed yeast and purified β -glucan under commercial aquaculture condition remain limited. Autolyzed yeast and yeast β -glucans are well recognized for enhancing shrimp immune competence by stimulating the prophenoloxidase (proPO) cascade, antimicrobial peptide production, phagocytosis, hemocyte adhesion, and clotting responses (Jin et al. 2018; Ma et al. 2020; Ceseña et al. 2021; Kong et al. 2025). However,

commercial yeast-derived products are not biologically equivalent. Differences in yeast strain, raw material, production technology, extraction and purification methods, and the resulting molecular characteristics—including molecular weight, branching structure, solubility, and purity—can substantially influence their composition and immunomodulatory activity (Meena et al. 2013; Liu et al. 2021). Therefore, the present study aimed to systematically evaluate the effect of Leiber® CeFi Pro, an autolysate (AY) derived from brewer's yeast (*Saccharomyces cerevisiae*) and Leiber® Beta-S consists predominantly of purified yeast β -1,3/1,6-glucans (BG) on growth performance, immune gene expression, phagocytic activity, and resistance to *V. parahaemolyticus* (AHPND) infection in *L. vannamei*.

Materials and Methods

1. Feed Supplementation

Five experimental feed formulations were supplied by Leiber GmbH (**Table 1**). The commercial pellets were originally produced at a size of 2.2×4.0 mm. To accommodate the developmental stages of post-larval (PL) shrimp, the pellets were ground and sieved into four particle-size fractions. The appropriate feed size was administered according to the shrimp growth stage.

Table 1: The experimental feeding groups for experiment trial

Group	Feed supplementation
BF	Basal feed (Control)
AY3	Basal feed + 3 Kg ton ⁻¹ autolyzed yeast
AY6	Basal feed + 6 Kg ton ⁻¹ autolyzed yeast
BG200	Basal feed + 200 g ton ⁻¹ purified β -glucan
BG400	Basal feed + 400 g ton ⁻¹ purified β -glucan

2. Experimental Design

A feeding trial was conducted to evaluate the effects of dietary supplementation. A total of 90,000 naïve PL12 shrimp were randomly allocated to five dietary treatment groups (**Table 1**). Each treatment was conducted in triplicate, with 6,000 shrimp per replicate at an initial stocking

density of 20 shrimp L⁻¹ (**Figure 1**). Shrimp were fed to apparent satiation (approximately 5-10% of body weight per day) over a 70-day experimental period. As shrimp developed, stocking densities were progressively reduced to 1.67 shrimp L⁻¹ at PL34 and to 0.67 shrimp L⁻¹ at PL49 (**Table 2**). The trial was conducted during 01/10/2022 to 07/12/2022.

Table 2: Summary of stocking density and culture temperature used in this study

Culture period	Culture phase (Day)	Shrimp stage	Stocking density (shrimp L ⁻¹)	Shrimp per replicate	Temperature (°C)
01/10/2022	Start feeding – D21	PL12 – PL34	20	6,000	26 – 28
to	D21 – D37	PL34 – PL49	1.67	500	
07/12/2022	D37 – D70	PL49 – 2 g	0.67	200	

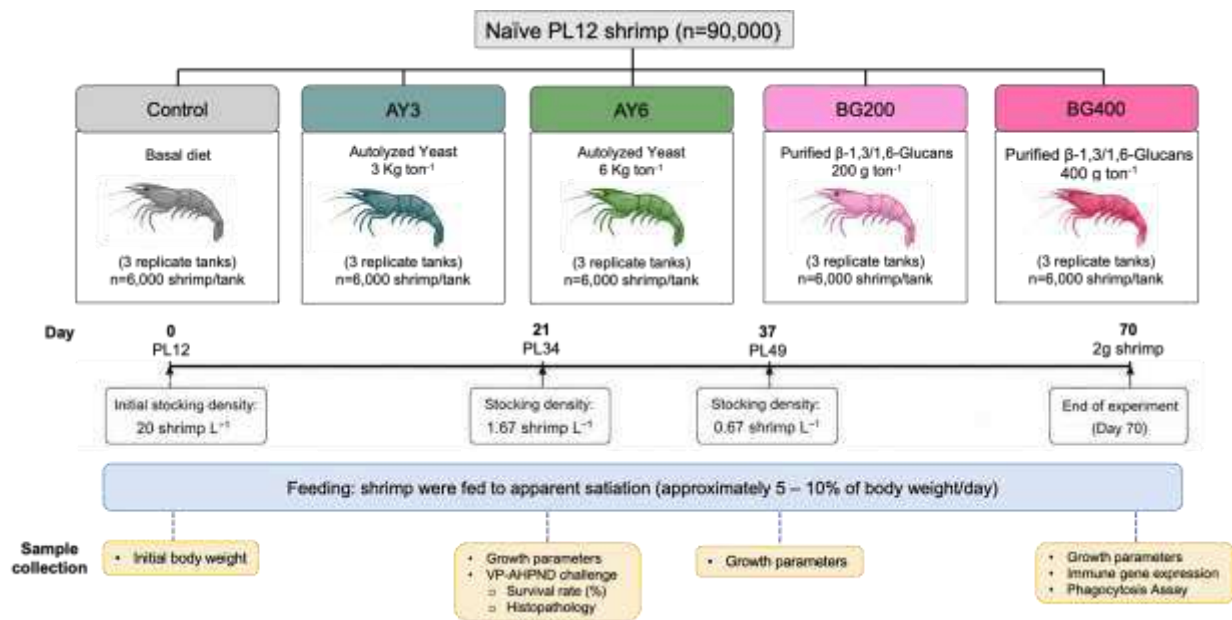


Figure 1: Overview of experimental design and sample collection.

3. Water Quality

Throughout the experimental period, water quality parameters were maintained within optimal ranges. The dissolved oxygen (DO) was maintained above 5 mg L⁻¹, total ammonia nitrogen remained below 5 mg L⁻¹, nitrite levels were below 1 mg L⁻¹, and pH ranged from 7.8 to 8.3. Water quality parameters were monitored every three days using a commercial test kit

(ParaTest, Aquacare 2000.1). A 50% water exchange was performed whenever measured values exceeded optimal thresholds.

4. Determination of Immune Gene Expression

The expression of ten immune-related genes in *L. vannamei* (PO1, PO2, PPAE2, PEN2, PEN3, crustin I, ALF1, ALF2, peroxinectin, and HSP70) was quantified in hemolymph (or hemocytes) by quantitative real-time PCR (RT-qPCR). Elongation factor 1- α (EF1 α) was used as the internal reference gene. Gene-specific primer sequences are listed in **Table 3**. Fifteen shrimp per treatment group were randomly sampled for gene expression analysis. Hemolymph (50 μ L per shrimp) was collected and immediately mixed with 500 μ L of TRIzol reagent (Invitrogen, USA), followed by snap-freezing in liquid nitrogen. Total RNA was extracted according to the manufacturer's protocol and used for downstream analysis. Each biological sample was analyzed in duplicate for each target gene. RT-qPCR was performed using a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA). Reactions were conducted in a final volume of 20 μ L containing 10 μ L of 2x iTaq Universal SYBR® Green Supermix (Bio-Rad), 0.4 μ L each of forward and reverse primers (10 μ M), 2 μ L of total RNA template (5 ng), and 7.2 μ L of nuclease-free water. The thermal cycling conditions consisted of reverse transcription at 50°C for 10 min, initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 10 s and annealing/extension at 60°C for 30 s. Cycle threshold (Ct) values were obtained using CFX Maestro software (Bio-Rad). Relative gene expression levels were calculated using the $2^{-(\Delta\Delta Ct)}$ method, normalized to EF1 α expression within the same sample (Livak and Schmittgen, 2001). Results are presented as fold changes relative to the control group.

Table 3: Primer sequences used for RT-qPCR analysis of immune-related genes in *L. vannamei*.

Genes/ Accession no.	Function	Primers	Amplicon	Reference
PO1 EU284136.1	Key enzyme in the melanization pathway	AAGTCCATTCCGTCCTCTG	121 bp	Janewanthanakul et al. 2020
		GGCTTCGCTCTGGTTAGGAT		
PO2 EU373096.1	Key enzyme in the melanization pathway	CTCAGCGTGAAGTCGCCTTA	216 bp	Hu et al. 2014
		CCTGCTCAGTGTACGGTCT		
PPAE2 JX644453.1	Prophenoloxidase activating enzyme	AAGGTATGTTGTGCCTCCTC	133 bp	Janewanthanakul et al. 2020
		AATTCGTGACGAACAGCATC		

Genes/ Accession no.	Function	Primers	Amplicon	Reference
<i>PEN2</i> DQ206401.1	Antimicrobial activity	TCGTGGTCTGCCTGGTCTT	122 bp	Hsu et al. 2022
		CAGGTCTGAACGGTGGTCTTC		
<i>PEN3</i> DQ206403.1	Antimicrobial activity	CACCCTTCGTGAGACCTTTG	140 bp	Hsu et al. 2022
		AATATCCCTTTCCCACGTGAC		
<i>Crustin I</i> MT375571.1	Antimicrobial activity	GCTGGCCTCGATAAGTGTTG	158 bp	Janewanthanakul et al. 2020
		CATCGGTCGTTCTTCAGATG		
<i>ALF1</i> MF135540.1	Anti-lipopolysaccharide factor (Antimicrobial peptide)	CCTTCTATAGGTGGGAGCTTTAC	161 bp	In this study
		CGTCCTCCGTGATGAGATTAC		
<i>ALF3</i> GGKO01008470.1	Anti-lipopolysaccharide factor (Antimicrobial peptide)	CTAAGCGGTGGCTGAACATA	115 bp	In this study
		CGTGGAAGAGAGAGATACAACAG		
<i>Peroxinectin</i> KC708021.1	Cell adhesion, degranulation, encapsulation enhancement, opsonin, peroxidase activity	AACCTGGCTTGACTGCTATT	130 bp	Xu et al. 2019
		CTGCCACCACAAACCTTCTA		
<i>HSP70</i> AY645906.1	Maintenance of cellular proteostasis	CTCCTGCGTGGGTGTGTT	119 bp	Janewanthanakul et al. 2020
		GCGGCGTCACCAATCAGA		
<i>EF1α</i> DQ021452.1	Internal control gene	TTCCGACTCGAAGAACGACC	122 bp	Leelatanawit et al. 2008
		GAGCAGTGTGGCAATCAAGC		

5. Phagocytosis Assay

Hemocyte phagocytic activity was evaluated following the method described by Htut et al. (2021). Briefly, 50 μ L of hemolymph was dispensed into each well of a 24-well culture plate containing a 13-mm round glass coverslip. Subsequently, 10 μ L of 3- μ m latex beads (Sigma-Aldrich, USA) were added to each well to achieve approximately 1×10^6 beads per well. The number of hemocyte in each well was approximately 1×10^5 cells per well. The hemolymph-bead suspension was gently mixed and incubated at 25°C for 30 min to allow phagocytosis. Following incubation, the supernatant was carefully removed, and adherent hemocytes were fixed with 0.2 mL of fixative solution (FAS) for 20 min. Fixed cells were further stained with Rose Bengal for 20 min and hematoxylin for 2 min. Samples were then rinsed under running tap water for 10 min, dehydrated in 95% ethanol and 100% ethanol (1 min each), and air-dried. Coverslips were mounted upside down onto clean glass slides using Permunt mounting solution. Phagocytosis was observed

under a light microscope at 400× magnification. Approximately 100 hemocytes per sample were counted to determine phagocytic activity.

$$\text{Phagocytic activity (\%)} = \left[\frac{\text{Number of bead-ingested hemocytes}}{\text{Number of counted hemocytes}} \right] \times 100$$

The **phagocytic index (PI)**, representing the average number of beads ingested per phagocytic hemocyte, was calculated as:

$$\text{Phagocytic index} = \frac{\text{Total number of ingested beads}}{\text{Total number of bead-ingested hemocytes}}$$

6. Sample Collection and Performance Assessment

Growth performance parameters, including final body weight, weight gain, average daily growth (ADG), feed conversion ratio (FCR), and survival rate, were evaluated at PL34, PL49, and at the 2 g stage. For immunological assays and immune gene expression analysis, five shrimp per replicate (n = 15 shrimp per treatment group) were randomly collected at the designated sampling points.

7. VP-AHPND Challenge Test

A bacterial challenge test was conducted at two developmental stages (PL34 and 2 g). At the PL34 stage, a total of 1,200 shrimp were randomly distributed into six tanks (200 shrimp per tank). Three tanks were exposed to *V. parahaemolyticus* (AHPND strain) via immersion at a final concentration of 1×10^4 CFU mL⁻¹, while the remaining three tanks served as non-challenged controls. Mortality was recorded daily throughout the challenge period. Mortality was monitored and recorded throughout the experimental period. Moribund shrimp from each group were collected and fixed in Davidson's fixative for subsequent histopathological examination.

8. Statistical analysis

All data are presented as mean ± standard deviation (SD). Statistical comparisons between the basal feed group (BF; control) and each dietary treatment group were performed using Student's *t*-test. Differences were considered statistically significant at $P < 0.05$. Graphical representations

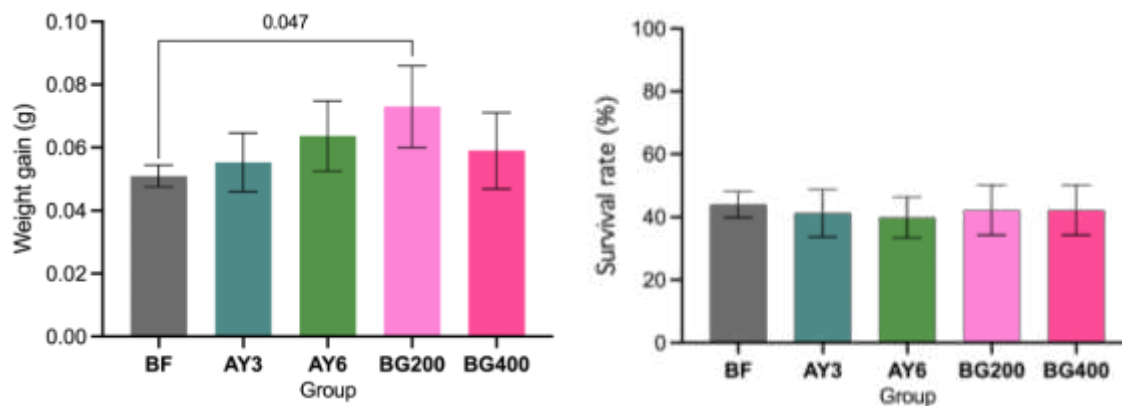
and statistical analyses were conducted using GraphPad Prism version 10.0 (GraphPad Software, USA).

Results

1. Effect of Stocking Density on Growth Performance

The trial was conducted under stocking densities of 20 PL12/L and 1.67 PL34/L, and 0.67 PL49/L, respectively. Shrimp continued cultured until 70 days (shrimp of 2 g each). During the PL12–PL34 stage, shrimp fed the BG200 diet exhibited significantly greater weight gain (0.073 ± 0.013 g) compared with the basal feed (BF) control group (0.052 ± 0.003 g) ($P = 0.047$) (**Figure 2**). No significant differences were detected among dietary treatments in average daily growth (ADG), feed conversion ratio (FCR), or survival rate during this period (**Figure 2A**). Between PL34 and PL49, shrimp receiving the BG200 diet showed a significantly higher survival rate than those fed the BF control diet ($P = 0.003$) (**Figure 2B**). From PL49 to the 2 g stage, no significant differences in all growth performance parameters were detected among the dietary groups ($P > 0.05$) (data not shown). However, no significant differences were observed among treatments in weight gain, ADG, or FCR among other dietary groups to the BF group ($P > 0.05$).

(A) PL34



(B) PL49

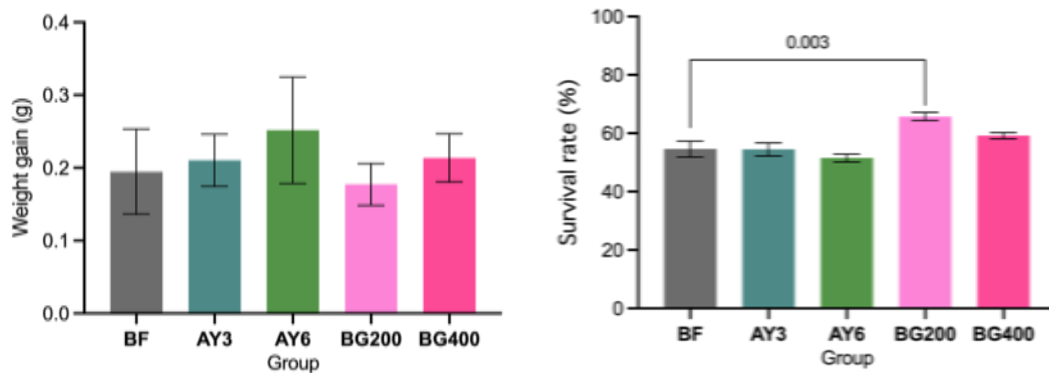


Figure 2. Growth performance of *L. vannamei* at 3 observation stages (A) PL34, (B): PL39 from PL12 stage fed five experimental diets. Data are presented as mean $\pm$ SD for weight gain, average daily growth (ADG), feed conversion ratio (FCR), and survival rate. A significant increase in weight gain was observed in shrimp fed the BG200 diet compared with the basal feed (BF) control ($P = 0.047$; Student's t -test). No significant differences were detected in ADG, FCR, or survival rate among treatments.

2. VP-AHPND Challenge at PL34

Shrimp at the PL34 stage were subjected to immersion challenge with VP-AHPND. Survival rate at 3 days post-challenge was shown in (Figure 3). Statistical analysis (Student's t -test) revealed significantly higher survival rate in shrimp fed AY6 ($P = 0.008$) and BG200 ($P = 0.002$) compared with the BF control group. These findings indicate that dietary supplementation with purified BG200 and AY6 improved survival following VP-AHPND challenge at the PL34 stage. The protective effect did not demonstrate a clear dose-dependent pattern under the conditions tested.

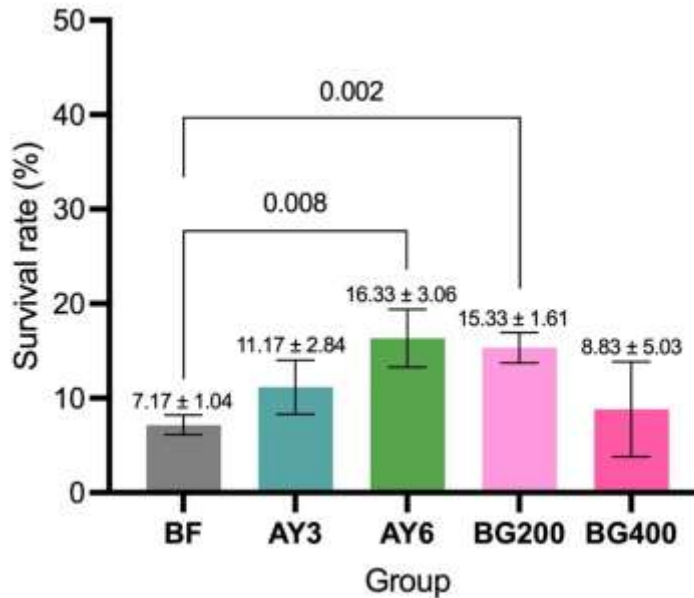


Figure 3. The bar graph showing survival rate (%) of PL34 shrimp at 3 days post-immersion challenge with VP-AHPND across five dietary groups. Survival data are presented as mean $\pm$ SD ($n = 3$ tanks per group). Asterisks indicate statistically significant differences compared with the basal feed (BF) control group ($P < 0.05$; Student's t -test).

To confirm the AHPND infection, histopathological examination of the hepatopancreas was conducted (**Figure 4**). H&E-stained photomicrographs of the non-challenged group displayed normal epithelial cells within the hepatopancreatic tubules. Conversely, shrimp in the AHPND-challenged group exhibited AHPND pathological characteristics, including sloughing of epithelial cells into the tubule lumens and hemocyte infiltration in the inter-tubular spaces.

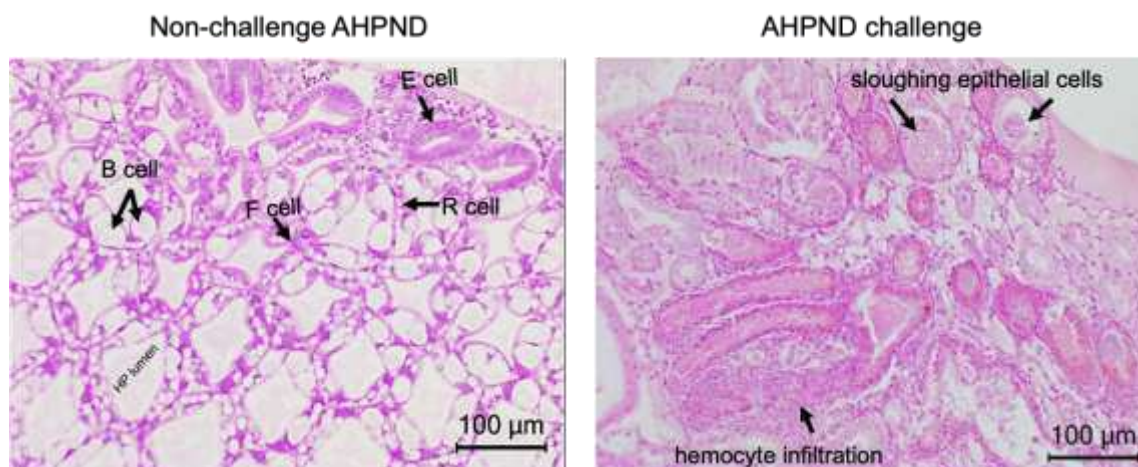


Figure 4: Representative H&E photomicrographs of the hepatopancreas from PL34 shrimp, observed under microscopic examination at 20X magnification. The non-challenged group displays normal epithelial cells within the hepatopancreatic tubules, whereas the AHPND-challenged group exhibits severe sloughing of epithelial cells and hemocyte infiltration.

3. Modulation of Shrimp Immune-related Genes Expression

Following 70 days of dietary supplementation, shrimp (approximately 2 g body weight) were randomly sampled from each dietary treatment group. Hemolymph was collected, and total RNA was extracted to quantify the transcriptional expression of immune-related genes. Interestingly, there were no down-regulation of immune-related genes detected in all 4 groups, when compared to the BF (Basal feed) control. The AY3 and AY6 groups showed significant upregulation of immune-related gene expressions of PO1 and *Crustin 1*, when compared to the BF control group. The AY3 group also showed upregulation of *Peroxinectin* gene (**Figure 5A**). The fold changes in both AY groups relative to the BF control are presented in **Table 4**.

The BG-treated group revealed higher fold changes of expression of immune-related genes, when compared to the AY groups and BF group. Both BG200 and BG400 exhibited a broader immunostimulatory profile, characterized by significant upregulation of multiple immune related genes including PO1, PO2, PPAE2, *crustin 1*, ALF1, ALF3, *peroxinectin*, and HSP70, compared with the BF control group. The expression levels of PEN2 and PEN3 were not significantly affected by any dietary treatment (**Figure 5B**).

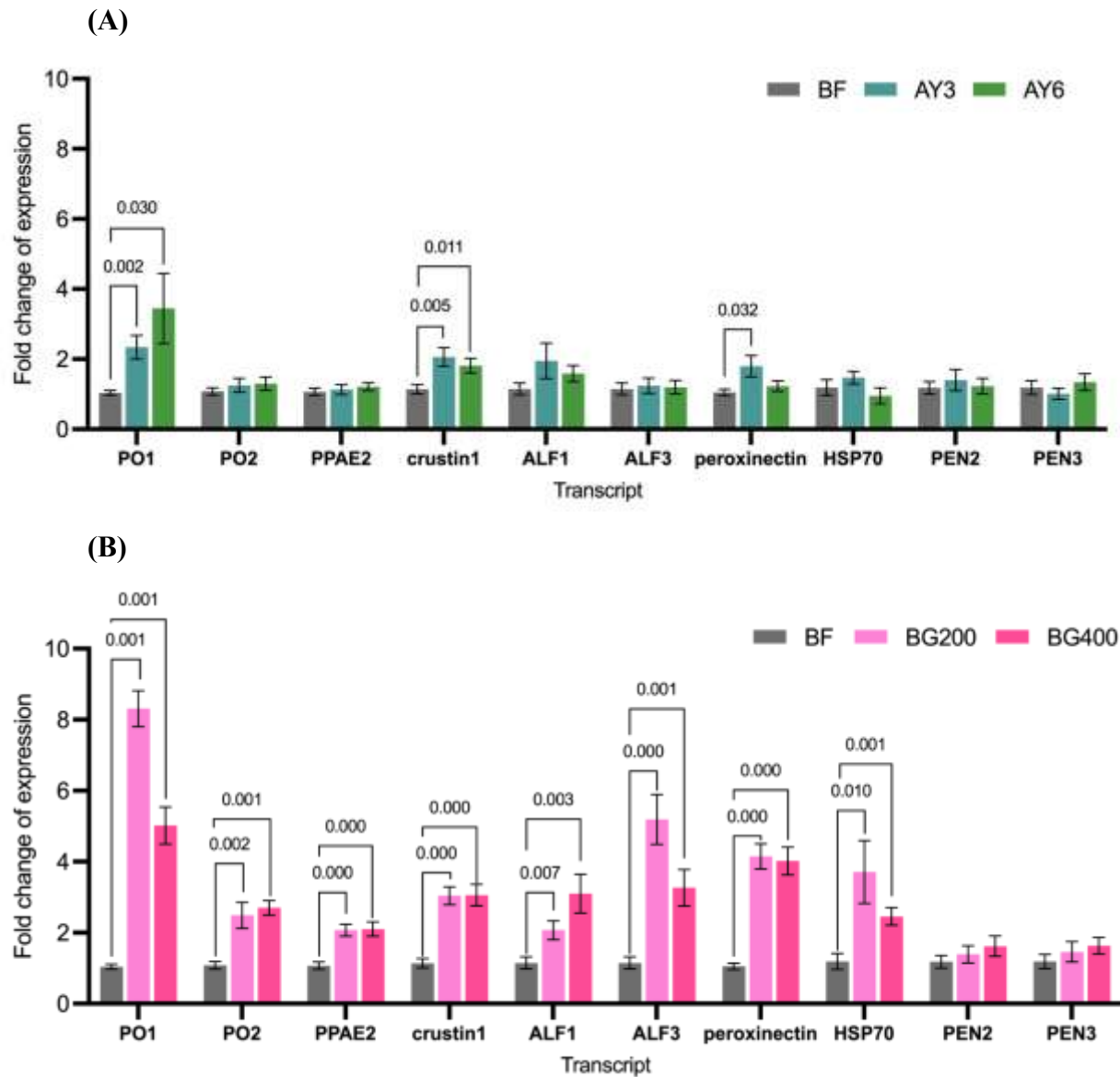


Figure 5. Bar graph showing mean $\pm$ SD fold changes in the expression of ten immune-related genes in shrimp fed five experimental diets ($n = 15$ per group).

Table 4: Summary of mean $\pm$ SD fold changes in immune-related gene expression among the five dietary groups ($n = 15$ per group). Asterisks indicate statistically significant differences compared with the basal feed (BF) control group.

Immune gene	Fold change of expression of immune gene (mean±SD)				
	BF	AY3	AY6	BG200	BG400
PO1	1.04 ± 0.26	2.34 ± 1.30*	3.45 ± 3.87*	8.31 ± 1.97*	5.02 ± 2.02*
PO2	1.08 ± 0.41	1.25 ± 0.77	1.30 ± 0.73	2.49 ± 1.41*	2.70 ± 0.80*
PPAE2	1.07 ± 0.40	1.13 ± 0.55	1.21 ± 0.45	2.07 ± 0.67*	2.10 ± 0.79*
crustin 1	1.14 ± 0.51	2.06 ± 1.03*	1.81 ± 0.81*	3.04 ± 0.96*	3.06 ± 1.16*
ALF1	1.15 ± 0.65	1.95 ± 1.95	1.59 ± 0.89	2.07 ± 1.02*	3.10 ± 2.11*
ALF3	1.15 ± 0.66	1.24 ± 0.85	1.20 ± 0.75	5.19 ± 2.72*	3.27 ± 1.99*
peroxinectin	1.05 ± 0.34	1.80 ± 1.18*	1.23 ± 0.57	4.15 ± 1.38*	4.02 ± 1.51*
HSP70	1.19 ± 0.88	1.47 ± 0.69	0.95 ± 0.89	3.71 ± 3.42*	2.46 ± 0.98*
PEN2	1.18 ± 0.69	1.40 ± 1.18	1.23 ± 0.86	1.39 ± 0.95	1.62 ± 1.11
PEN3	1.19 ± 0.77	1.01 ± 0.62	1.34 ± 0.93	1.46 ± 1.11	1.63 ± 0.90

4. Phagocytic Activity

The mean phagocytic activity across dietary groups ranged from 7.90% to 15.21% (**Figure 6A**), whereas the phagocytic index ranged from 1.22 to 1.43 (**Figure 6B**). Statistical analysis revealed a significant increase in phagocytic activity in shrimp fed the BG400 diet compared with those fed the basal feed (BF) control diet ($P = 0.004$). These results indicate that phagocytic activity was consistently elevated in shrimp fed purified BG-supplemented feed, particularly BG400, compared with both the BF control and AY-supplemented feed. No other significant differences were detected among dietary treatments.

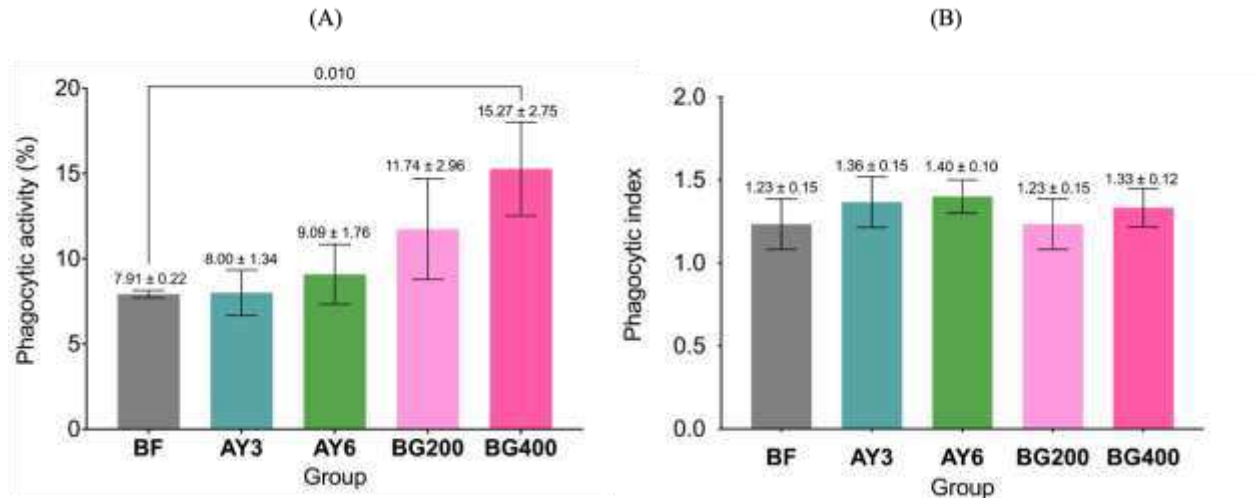


Figure 6. The bar graph showing mean±SD of hemocyte phagocytic activity (A) and phagocytic index (B) in the 2g-shrimp fed with 5 different feeds (n = 15 per group).

Discussion

Shrimp aquaculture is facing disease outbreak issues, making it impossible to increase shrimp production in farming countries worldwide. Managing these outbreaks is therefore an issue that requires urgent resolution. Current solutions focus on treating or managing pathogens in already-infected shrimp, including the inappropriate use of chemicals and antibiotics. This has exacerbated the problem due to its negative environmental impacts. An effective solution should focus on strengthening the shrimp's health from the early larval stage through to the juvenile stage reared in outdoor ponds. Enhancing shrimp health using appropriate biological immunostimulants will enable them to better fight off pathogens. When combined with proper aquaculture management, this approach will provide a pathway to sustainable production growth.

Leiber® CeFi Pro is an autolysate (AY) derived from brewer's yeast (*Saccharomyces cerevisiae*) that provides a broad spectrum of intracellular bioactive constituents, including nucleotides, peptides, amino acids, vitamins, and residual yeast cell wall polysaccharides. In contrast, Leiber® Beta-S consists predominantly of purified yeast β -1,3/1,6-glucans (BG), the principal pathogen-associated molecular patterns (PAMPs) recognized by the innate immune system. The distinct biochemical composition of these products is expected to result in different mechanisms of immune modulation. While β -glucans primarily activate innate immunity through interactions with β -glucan recognition proteins and other pattern recognition receptors, leading to

activation of the prophenoloxidase cascade, phagocytosis, and antimicrobial defence, the broader range of bioactive compounds present in yeast autolysate may additionally support cellular metabolism, tissue repair, and immune cell function. These complementary mechanisms may explain the differential transcriptional responses observed in the present study.

Although there was no significant effect found with the growth performance indicators such as ADG and FCR in all observed stages, the significant weight gain and survival rate were significantly elevated in PL34 and PL49 post rearing with BG200 for 21 days and 37 days, respectively. The effects were confirmed by the higher survival post challenge with VP-AHPND. Overall, the findings demonstrate that purified BG elicited pronounced immunomodulatory effects compared with AY under this intensive condition. The mechanism of AY6 to improve survival rate post VP-AHPND challenge might not be only from their immunostimulant effect, but from other mechanisms too. The activation of phagocytosis by BG400 also support their immunostimulant effect to fight with the VP-AHPND bacterial infection.

At the transcriptional level, purified β -glucan elicited a broad and robust immune activation profile. Significant upregulation was observed in genes associated with the prophenoloxidase (proPO) cascade (PO1, PO2, PPAE2), antimicrobial peptides (Crustin I, ALF1, ALF3), Peroxinectin, and HSP70 in β -glucan-fed shrimp (**Figure 7**). These pathways constitute the core components of crustacean innate immunity and are typically induced following recognition of β -glucan and other pathogen-associated molecular patterns (Söderhäll and Cerenius 1998; Meena et al. 2013). The coordinated upregulation of proPO-related genes and antimicrobial peptides suggests a state of immune priming, whereby dietary β -glucan elevates basal immune preparedness. Concurrently, increased HSP70 expression implies enhanced cellular stress tolerance and maintenance of cellular protection (Wang and Wang 2013). In contrast, autolyzed yeast elicited comparatively moderate transcriptional responses. Although it contains β -glucan, its bioactive composition—including mannan-oligosaccharides, nucleotides, and peptides—likely mediates broader nutritional and gut-associated benefits rather than potent receptor-driven immune activation (Li and Gatlin 2006). This pattern supports the notion that the functional impact of dietary immunostimulants becomes highly evident when shrimp are subjected to environmental or physiological stress, where the baseline immune demand is elevated.

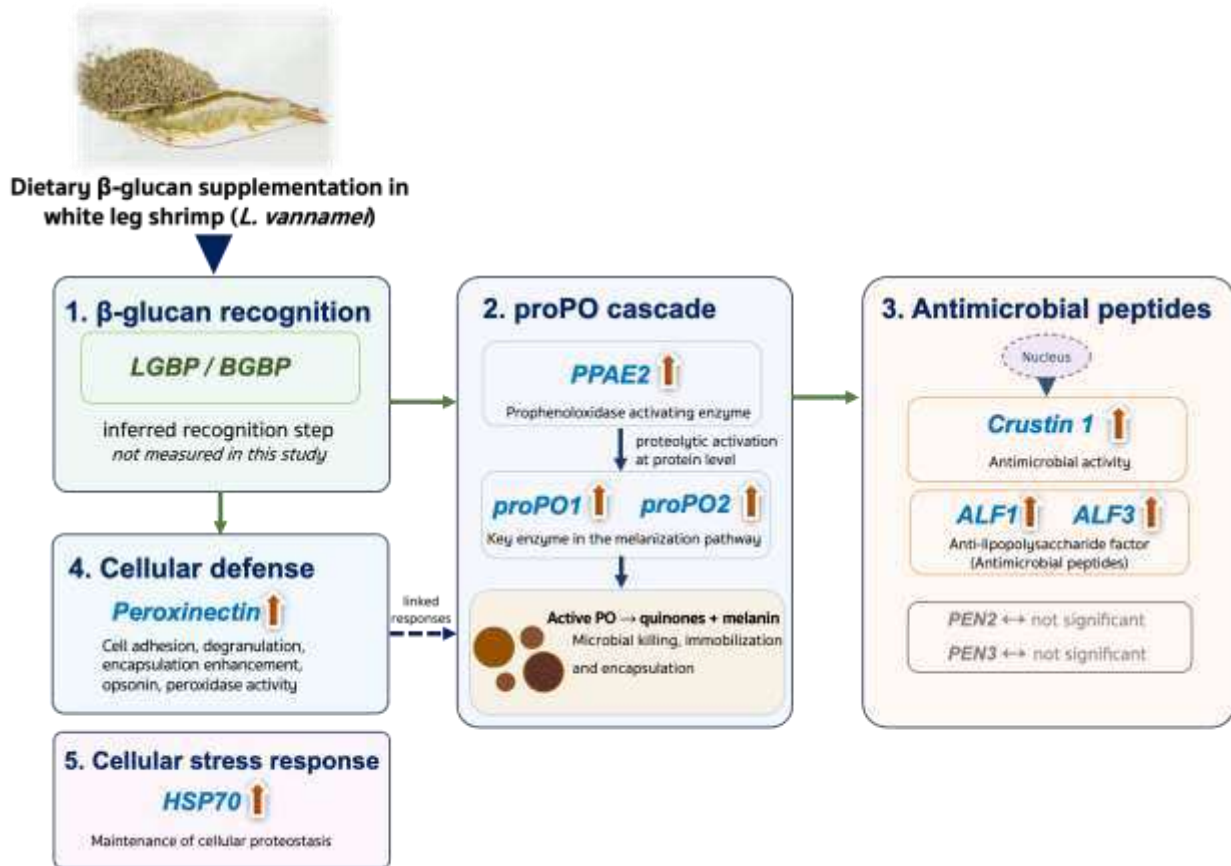


Figure 7. Summary of immune activation in Leiber® Beta-S–fed shrimp

Functional immune enhancement was further corroborated by the phagocytosis assay. Shrimp fed BG400 displayed significantly higher hemocyte phagocytic activity compared with the control group. Phagocytosis is a fundamental hemocyte-mediated defense mechanism in crustaceans and serves as a functional indicator of active cellular immune competence (Wang and Wang 2013). The increased proportion of phagocytic cells in β -glucan–fed shrimp therefore suggests an enhanced capacity for pathogen recognition and clearance. Notably, the strongest functional response was observed at the higher β -glucan inclusion level (BG400), although transcriptional responses were not strictly dose-dependent. This observation suggests that immune activation may operate within a functional threshold, beyond which further increases in gene transcription do not proportionally translate into enhanced effector responses at the cellular level. In contrast, autolyzed yeast supplementation did not significantly augment phagocytic activity relative to the control, consistent with its comparatively moderate transcriptional effects.

During immersion challenge with VP-AHPND at the PL34 stage, shrimp fed BG200 and AY6 exhibited significantly lower cumulative mortality compared with the control group. Although overall mortality remained high across treatments, the observed reduction indicates a partial protective effect of dietary supplementation. β -glucan has been previously reported to enhance resistance against *Vibrio* infections by activating the prophenoloxidase (proPO) cascade and stimulating antimicrobial peptide expression, thereby strengthening innate immune defense mechanisms (Chang et al. 2003; Pooljun et al. 2025). The reduced mortality observed in β -glucan-fed shrimp is consistent with these proposed immunological mechanisms and supports the functional relevance of proPO activation and antimicrobial peptide induction in enhancing resistance to infection. However, the high overall mortality across treatments likely reflects the inherent susceptibility of shrimp at the PL34 stage to AHPND. Autolyzed yeast at the higher inclusion level likewise reduced mortality, suggesting a moderate enhancement of immune defense; however, its protective effect was less pronounced than that achieved with purified β -glucan supplementation.

In conclusion, dietary supplementation with purified β -glucan enhanced immune-related gene expression, increased hemocyte phagocytic activity, and improved resistance to VP-AHPND challenge under stress-associated culture conditions. Autolyzed yeast conferred moderate immunological support but elicited comparatively less pronounced molecular and functional responses. These findings support the strategic application of purified β -glucan as a functional feed additive to enhance shrimp health and disease resilience in intensive aquaculture systems. Nevertheless, further validation under commercial farm conditions is warranted to determine its practical efficacy, cost-effectiveness, and long-term impact on production performance.

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Author contributions

PJ conceived the study and designed the experimental trials; RS, CK, ST and SK performed the experiments; RS, CK, ST, SK and KS analyzed the data; RS, KS and PJ drafted the manuscript; all authors critically revised the manuscript.

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Ethic statement

All experimental procedures involving animals were reviewed and approved by the Mahidol University Institutional Animal Care and Use Committee (IACUC; Protocol No. MUSC61-055-457) and were conducted in accordance with the relevant institutional guidelines and regulations. The project was initiated as part of an academic service program at Mahidol University. Following sample collection, laboratory analyses were carried out at Rajamangala University of Technology Tawan-ok (RMUTTO). All animal experiments and sample collection were conducted under the approved IACUC protocol.

Conflicts of interest

The authors declare no conflict of interest.

Data availability statement

Experimental data are available on request.

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