Arm & Hammer Animal Nutrition
469 North Harrison Street | Princeton, NJ 08540
Phone: 800-562-3563 | Fax: 609-403-7403 AHanimalnutrition.com

Study location: Can Tho University, Vietnam

July 22, 2024 Report on file

Title: CELMANAX Aqua reduced mortality and improved immune function of white leg shrimp (*Litopenaeus vannamei* sp.) challenged with *Vibrio parahaemolyticus*.

CELMANAX Aqua is a multicomponent, all-natural postbiotic and prebiotic feed supplement that has Generally Recognized as Safe (GRAS) status as a feed ingredient.

Study Overview

- Acute hepatopancreatic necrosis disease (AHPND), caused by *V. parahaemolyticus* is a serious problem in shrimp farming and can cause up to 100% mortality.
- The objective of this study was to determine the effect of dietary CELMANAX Aqua supplementation on the performance of *Litopenaeus vannamei* challenged with *V. parahaemolyticus*.
- White leg shrimp with starting BW 1.45 g were stocked in tanks with sixty shrimp/tank and six replicate tanks per treatment group.
- Experimental shrimp were fed 5% body weight, five times a day, a diet formulated according to NRC (NRC 2011) and supplemented with 0, 50 ppm, 100 ppm, 200 ppm, and 400 ppm CELMANAX Aqua liquid.
- All shrimps were fed the experimental diets for 21 days. This was followed by an immersion challenge with $2X10^5$ cfu/ml of *V. parahaemolyticus*.
- Cumulative mortality was recorded for 21 days post challenge.
- Typical gross signs of AHPND and hepatopancreas (HP) were documented.
- Shrimp hemolymphs samples were analyzed for immune markers and antimicrobial peptide expression.
- Data were analyzed using the software SPSS 22.0 with statistical significance noted at $P<0.05$ and a tendency at $P<0.1$.

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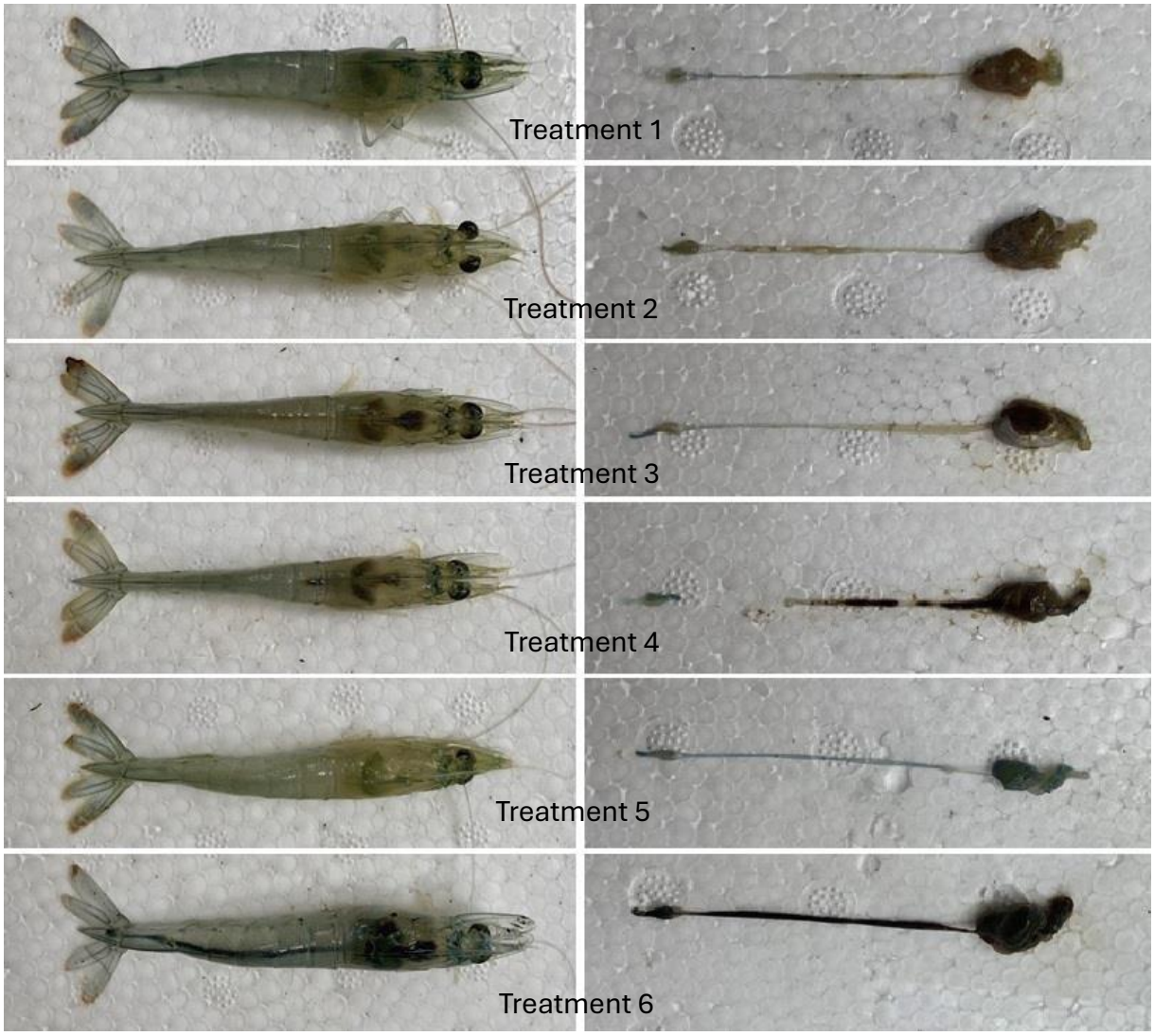
Results

Clinical signs and mortality

- Shrimp challenged with *V. parahaemolyticus* displayed clinical signs starting about 12 hours post-challenge and typical gross signs of AHPND 48 h post-challenge. All the challenged groups of shrimp showed signs of inflammation/necrosis, empty gut and pale HP to various degree. However, shrimp supplement with 200 ppm and 400 ppm of CELMANAX Aqua showed minimal effect. Shrimp supplemented with 400 ppm CELMANAX Aqua maintained normal feed intake and digestion.

Figure 1. Gross signs of APHND and HP of experimental shrimps at 48 h after challenge.



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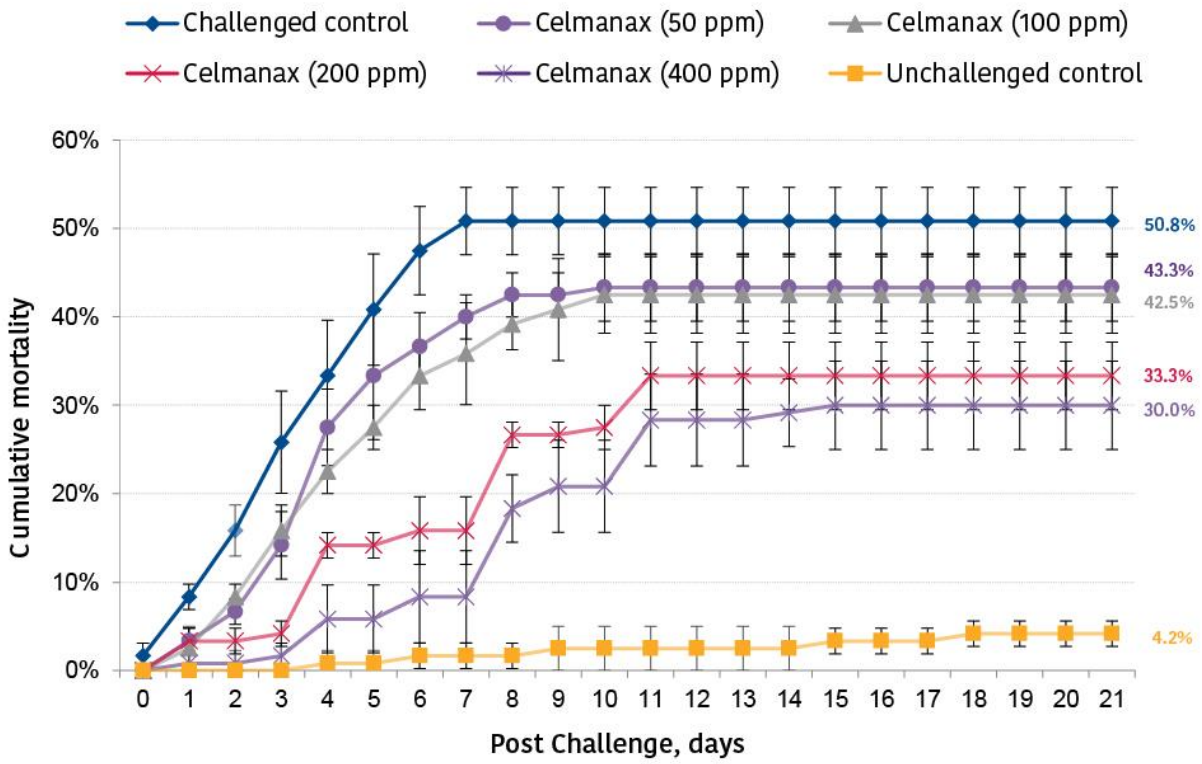

Immunity

- Phenoloxidase activity was significantly stimulated by a higher dose of CELMANAX Aqua (200 & 400 ppm, $P<0.05$) indicating higher encapsulation activity to the pathogens (Table 1).
- Respiratory burst activity was significantly activated by a higher dose of CELMANAX Aqua (400 ppm, $P<0.05$) indicating higher activity to eliminate the pathogens (Table 2).

Antimicrobial peptide expression

- Expression of Penaedin-3a increased 12h post-challenge in CELMANAX Aqua 400 and showed a tendency for increase at other CELMANAX Aqua concentrations compared to control (Figure 2).
- Expression of Crustin increased in all treatments following challenge, but the challenge control had the lowest levels. 12-hour post challenge, CELMANAX Aqua 200 PPM treatment had significantly higher levels of Crustin. 48-hour post

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challenge, Crustin expression was significantly higher in all CELMANAX Aqua treatments compared to compared to challenge control (Figure 3).

Table 1. Phenoloxidase activity of shrimp with or without supplementation of CELMANAX Aqua and challenged by *V. parahaemolyticus*. **Treatment Group**

Treatment Group	Before challenge	48h after challenge	At 50% mortality
Phenoloxidase activity			
CELMANAX Aqua 50 ppm	0.086±0.008^{Ac}	0.046 ± 0.013^{Aa}	0.059 ± 0.004 ^{Ab}
CELMANAX Aqua 100 ppm	0.088±0.011^{Ac}	0.050 ± 0.011^{Aa}	0.069 ± 0.020 ^{ABb}
CELMANAX Aqua 200 ppm	0.089±0.009^{Ac}	0.055 ± 0.009^{Aa}	0.073 ± 0.018 ^{ABb}
CELMANAX Aqua 400 ppm	0.094±0.015^{Ac}	0.054 ± 0.008^{Aa}	0.074 ± 0.017 ^{Bb}
Challenged Positive control	0.087±0.019^{Ab}	0.053 ± 0.007^{Aa}	0.064 ± 0.006 ^{ABa}
Unchallenged Negative control	0.088±0.016 ^{Ac}	0.051 ± 0.009^{Aa}	0.067 ± 0.009 ^{ABb}

Superscripts within a column ^{AB} P≤ 0.05 and superscripts within a row abc P≤ 0.05 indicate significant differences between treatments.

Table 2. Respiratory burst activity of shrimp with or without supplementation of CELMANAX Aqua and challenged by *V. parahaemolyticus*.

Treatment Group	Before challenge	48h after challenge	At 50% mortality
Respiratory activity			
CELMANAX Aqua 50 ppm	0.090 ± 0.009^{Ab}	0.071 ± 0.009 ^{Aa}	0.076 ± 0.012 ^{Aa}
CELMANAX Aqua 100 ppm	0.088 ± 0.008 ^{Ab}	0.073 ± 0.007 ^{Aa}	0.076 ± 0.009 ^{Aa}
CELMANAX Aqua 200 ppm	0.089 ± 0.005 ^{Ab}	0.076 ± 0.009 ^{Aa}	0.080 ± 0.014 ^{ABab}
CELMANAX Aqua 400 ppm	0.089 ± 0.009 ^{Aa}	0.088 ± 0.008 ^{Ba}	0.088 ± 0.009 ^{BCa}
Challenged Positive control	0.089 ± 0.005 ^{Ab}	0.074 ± 0.012 ^{Aa}	0.078 ± 0.006 ^{ABa}
Unchallenged Negative control	0.089 ± 0.006 ^{Aa}	0.095 ± 0.005 ^{Ba}	0.095 ± 0.010 ^{Ca}

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Superscripts within a column ^{ABC} P≤ 0.05 and superscripts within a row ab P≤ 0.05 indicate significant differences between treatments.

Conclusions

- *Inclusion of CELMANAX Aqua in shrimp diets can reduce mortality and tissue damages in the gut caused by V. parahaemolyticus.*
- *Improvement in immune function and expression of antimicrobial peptides in challenged shrimp may play a role in helping manage V. parahaemolyticus infection.*

Figure 2. *Relative expression level of Penaedin-3a mRNA on shrimp from different treatment groups at different sampling times were calculated using **β**-actin as reference gene.*

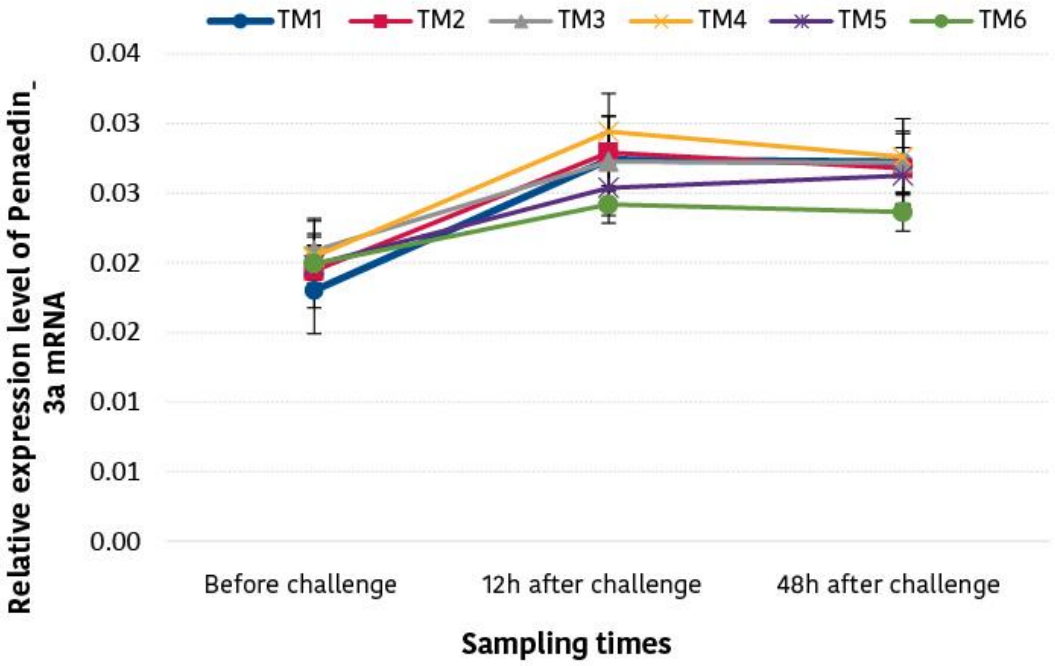

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Figure 3. Relative expression level of Crustin mRNA on shrimp from different treatment groups at different sampling times were calculated using β-actin as reference gene.