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COVID-19: Shrimp Demand & Supply

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Partial substitution of fish meal with novel fermented soybean meal enhances tolerance against AHPND

Inclusion levels 10% to 30% enhanced tolerance of whiteleg shrimp against AHPND in challenge trials conducted in Vietnam

By Sergio F. Nates

A challenge trial was conducted to determine the effectiveness of diets containing a novel fermented soybean meal in mitigating the severity and impact of the early mortality syndrome/acute hepatopancreatic necrosis disease (EMS/AHPND) in the whiteleg shrimp ***Litopenaeus vannamei***. The 33-day trial which began on January 16, 2020 was conducted at the ShrimpVet Laboratory in Ho Chi Minh City, Vietnam. It included a 1-day adaptation period, 21 days of feeding, a day of challenge and 10 days of post-challenge.

Tanks and water

Trials were carried out in 120L plastic tanks. All tanks were outfitted with an activated coral biological filter and aerator. They were covered with a plastic cap to reduce the risk of cross contamination. Brackish water of 20 ppt salinity was used in each trial. Water quality parameters such as dissolved oxygen (DO), pH, and temperature were measured daily. Total ammonia nitrogen (TAN), nitrite, and alkalinity were measured twice a week.

Shrimp

The specific pathogen free (SPF) shrimp *L. vannamei* used in this trial were progenies from broodstock originally from Hawaii. They were checked for disease pathogens. PCR analyses included pathogens for *Enterocytozoon hepatopenaei* (EHP), white spot syndrome virus (WSSV), Taura syndrome virus (TSV), infectious myonecrosis virus (IMNV) and EMS/AHPND. Nauplii and post larvae were reared in a strict biosecurity facility. Post larvae were also checked again with PCR for important pathogens including EHP, WSSV, TSV, IMNV, and EMS/AHPND. One day prior to the start of the study, shrimp were group weighed to determine initial weight. The initial average shrimp weight was 0.56 ± 0.04g.

Test diets

The trial had five groups; test diets in groups D1-D3 contained the novel fermented soybean meal, ME-PRO® (Prairie Aquatech, USA) at three inclusion levels, 10% (D1), 20% (D2) and 30% (D3). There was a positive control (D4) as well as a negative control (D5). There were four replicates per group.

All feeds were formulated using a commercial feed formulation software and manufactured using commercial extrusion methods. Chemical analysis (proximate analysis and mineral composition) of feeds were analysed using third party laboratories (Midwest Laboratories, Omaha, NE). Shrimp were fed *ad libitum* with their respective diets with four meals per day during the trial. Feed consumption was recorded during the trial. Feeding amount was adjusted depending on the biomass and actual feed consumption.

Challenge method

An immersion challenge method was used in this trial. There was a total of 28 tanks used as treatment and positive controls tanks. Tryptic soy broth+2% sodium chloride (TSB+) inoculated with a consistently virulent strain of *Vibrio parahaemolyticus*, was incubated for 24 hours. The bacterial suspension was added into tanks to achieve the bacterial density measured by optical density absorbance (OD_{600} nm); this density was expected to kill 90% in the positive control (LD_{90}) within 10 days. Negative control (four tanks) was treated with sterile TSB+ added directly to the tanks. The challenge dosage was $3.25x10^5$ CFU/mL which was 90% of the lethal dose (LD_{90}). Standard histopathology and H&F stain were applied for shrimp tissues.

Molecular and histological analyses

Five days before the start of the trial, two shrimp samples were sacrificed to check for WSSV, TSV, EMS/AHPND, EHP and IMNV and confirm the animal's health status. During the challenge trial, two-representative moribund shrimp per treatment were collected for histological analysis. Samples were collected within 120 hours of post-challenge. PCR results showed that experimental shrimp were not infected with the above pathogens before the trial started.

Gross signs of EMS/AHPND in infected shrimp included a pale-to-white hepatopancreas (HP), significant atrophy of the HP, soft shell and gut with discontinuous or no contents. In addition, the HP will not squash easily between the thumb and forefinger. This may probably be due to increased fibrous connective tissue and haemocytes (Figures 3-6).

Statistical analysis

Water quality parameters (temperature, DO, pH, TAN, nitrite, and alkalinity) and survival of shrimp were analysed by one-way analysis of variance (ANOVA), followed by Duncan test to determine differences among treatments. All tests were performed at 5% significance level ($P<0.05$). Survival data was arcsine-transformed for analysis but only the original values were presented. Results were presented as mean ± standard deviation. Data were analysed using the SPSS software version 22.

Water quality parameters

Throughout 33 days of the trial, water quality parameters were recorded and are presented in Table 1.

	Treatments with ME-PRO®			Control Treatments	
Treatment	**D1 10%**	**D2 20%**	**D3 30%**	**D4 Positive**	**D5 Negative**
Temp (°C)	27.53 ± 0.54[a]	27.38 ± 0.49[a]	27.49 ± 0.50[a]	27.39 ± 0.51[a]	27.45 ± 0.46[a]
DO (mg/L)	6.19 ± 0.06[a]	6.19 ± 0.06[a]	6.19 ± 0.06[a]	6.20 ± 0.06[a]	6.18 ± 0.05[a]
pH	7.78 ± 0.05[a]	7.79 ± 0.05[a]	7.78 ± 0.05[a]	7.79 ± 0.05[a]	7.79 ± 0.06[a]
Alkalinity (ppm)	124.44 ± 5.27[a]	125.56 ± 5.27[a]	124.44 ± 5.27[a]	124.44 ± 7.26[a]	124.44 ± 5.27[a]
TAN (ppm)	0.28 ± 0.26[a]	0.22 ± 0.26[a]	0.11 ± 0.22[a]	0.22 ± 0.26[a]	0.22 ± 0.26[a]
Nitrite (ppm)	3.50 ± 2.26[a]	3.61 ± 2.15[a]	3.61 ± 2.15[a]	3.61 ± 2.15[a]	3.61 ± 2.15[a]
Salinity (ppt)	20.00 ± 0.00[a]	20.00 ± 0.00[a]	20.00 ± 0.00[a]	20.00 ± 0.00[a]	20.00 ± 0.00[a]

Values are presented as mean ± sd. The same letters on the same row were not significantly different (P < 0.05).

Table 1. Water quality parameters during the trial.

Survival rate

During the trial, shrimp were counted daily to assess the estimated survival rate. After 21 days of pre-challenge, there was no statistically significant difference in terms of survival rate among treatments (P>0.05) (Figure 1). Survival rates of treatments D1, D2, D3, D4, and D5 were 85.00 ± 2.00%, 87.00 ± 2.00%, 86.00 ± 2.31%, 87.00 ± 2.00%, and 87.00 ± 2.00%, respectively.

During the challenge and post-challenge, shrimp in the negative control did not show any clinical signs of EMS/AHPND and final survival rate was significantly higher than other treatments (88.53 ± 2.51%). This indicated that the trial set up was acceptable and no cross-contamination happened to the negative control.

After 10 days of post-challenge, survival rate of the positive control was 8.12 ± 7.00%. Survival rates of treatments D1, D2, and D3 were 9.36 ± 5.32%, 17.32 ± 7.13%, and 35.17 ± 21.60% respectively. The results showed that the survival rate of shrimp in treatment D3 (35.17 ± 21.60%) was significantly higher than the positive control (8.12 ± 7.00%) (P<0.05) (Figure 2).

While the survival of shrimp in the D1 and D2 treatments appears to be not statistically different from the positive control, there is a definitive trend of higher survival concomitant with the inclusion levels of ME-PRO in the diet. This clearly indicates that this ingredient clearly enhances tolerance against AHPND in *L. vannamei* juveniles and the inclusion rate of 30% has positive effects compared with the positive control in improving survival rate of the challenged shrimp.

Discussion

Recent studies on the development of practical diets for shrimp production systems using a microbial enhanced protein, ME-PRO, have shown to be a promising solution to produce eco-friendly aquafeeds. Besides having over 70% crude protein content and highly available phosphorus content, it presents numerous physical characteristics including high viscosity, which can improve faecal material stability, low dust and small particle size, both of which can improve water quality.

The protein is processed at a state-of-the-art plant using non-GMO (genetically modified organism) soybean

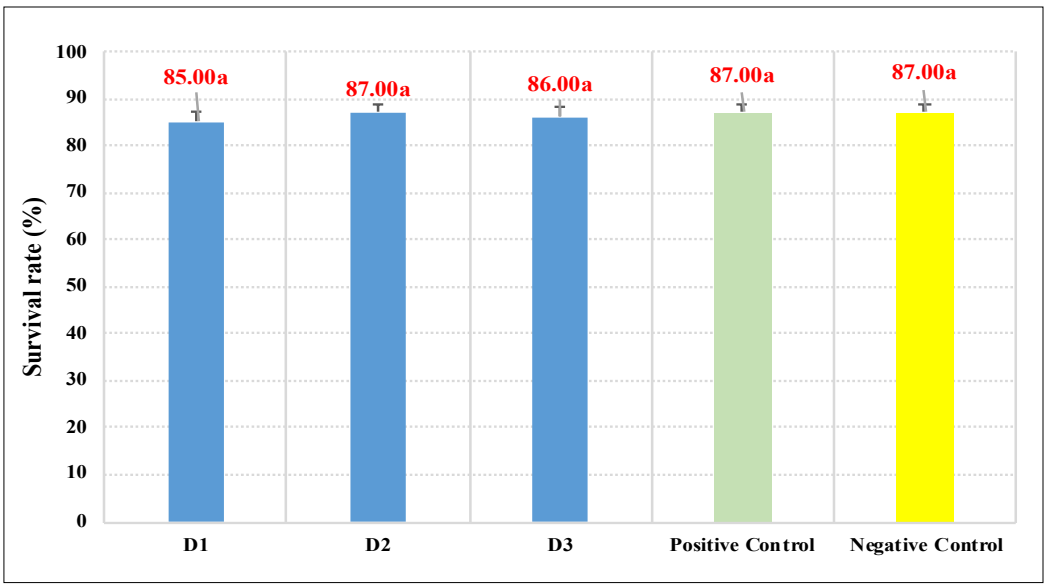

Figure 1. Survival rate at day 21 of the pre-challenge. (Values are presented as mean ± sd; n=5; P < 0.05)

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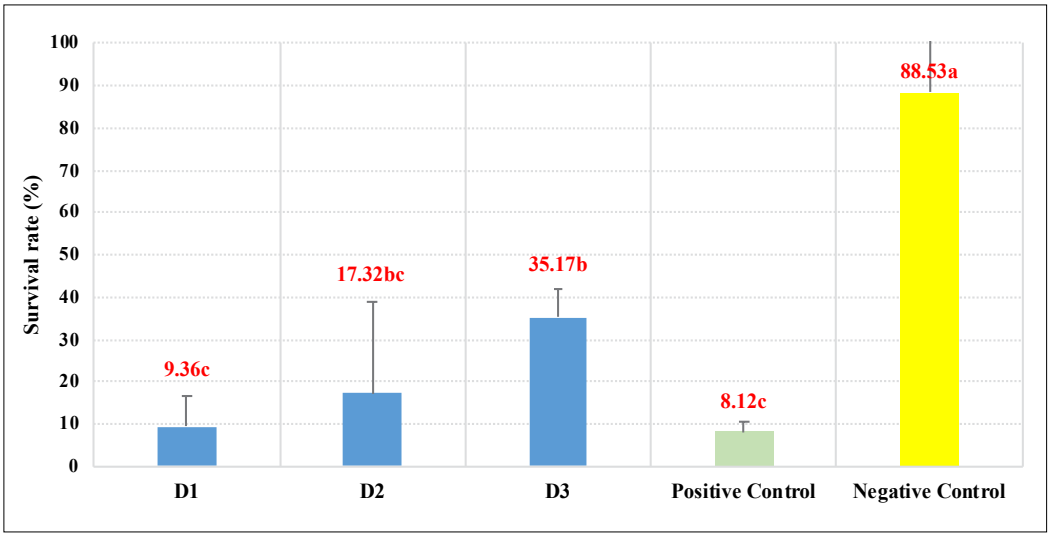
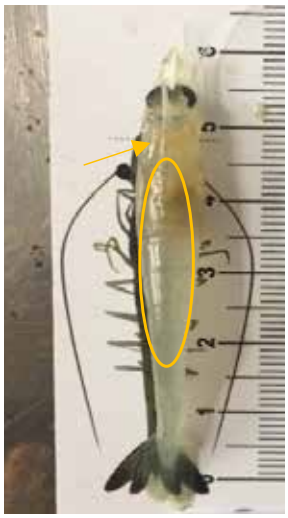
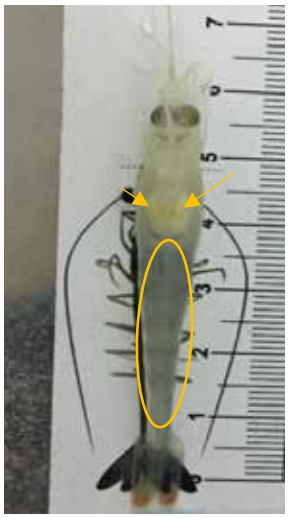


Figure 2. Survival rate at the end of the trial (Day 10 of post-challenge) (Values are presented as mean ± sd; n=5; P < 0.05)

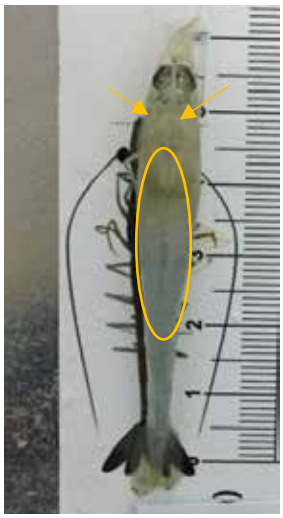
Figures 3. Healthy shrimp in negative control (brownish HP, full stomach and gut)



Figures 4. EMS-infected shrimp in positive control (pale HP and empty gut)



Figures 5. EMS-infected shrimp in a treatment (pale HP, empty stomach and gut)



Figures 6. Gross signs of EMS in shrimp hepatopancreas (pale and shrunken HP, empty stomach and gut)

meal and a natural occurring, non-toxigenic, fungi, *Aureobasidium pullulans*. The fermented co-product also offers significant amounts of short-chain peptides and free amino acids that confer excellent attractability and palatability properties. The presence of biologically active factors can increase gut microbiota, reduce intestinal inflammation, and boost metabolic processes for which lead to improved animal health which makes it a perfect match for the Asian aquaculture industry.

Results from numerous feeding trials has demonstrated that ME-PRO can sustain shrimp health, high-performance growth, and feed efficiency with inclusion levels as high as 50% of the total amount of ingredients in the diet. The results obtained in this study suggest that its inclusion levels in formulated feeds can have a positive effect on improving the survival rate of EMS/AHPND infected shrimp. This ingredient can also be considered a functional ingredient in disease prevention, used when formulating sustainable, practical and economical feeds for the shrimp aquaculture industry.

Sergio F. Nates, PhD is the Executive Vice-President at Prairie Aquatech which specialises in the development and use of plant-based products for the animal feed industry. Email: sergio@prairieaquatech.com