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Article

Evaluation of the Effects of Different Dietary Doses of an Anti-Mycotoxin Additive for Pacific White Shrimp (*Litopenaeus vannamei*)

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Abstract

Shrimp farming often suffers due to high mortalities and poor growth. Mycotoxins can be one of the causes but often underestimated. An anti-mycotoxin additive (AMA) was tested designing an experiment to assess its efficacy and determine the best dose for pacific white shrimp (*Litopenaeus vannamei*). Four treatments (0, 1, 2 and 3 g/kg of diet) were randomly allocated in 12 aquaria during larval rearing (20 days) and 12 tanks fibreglass tanks during subsequent grow-out (90 days). Results showed positive impacts on feed conversion, protein efficiency, survival and growth. Higher the dose better is the immunity as indicated by the survival of shrimp against bacterial challenge. However, the quadratic models indicated that the dose of 1.4 g/kg had the highest daily weight gain of shrimp (66.7 mg) and the dose of 2.5 g/kg of diet resulted in the highest survival (60.3%). Therefore, the doses between 1.4 and 2.5 g/kg of feed are recommended for the grow-out phase of shrimp. However, further studies should be done in outdoor pond conditions for varying feeding regimes, varying contamination levels and stocking densities.

Keywords: Pacific white shrimp; larval rearing; grow-out; mycotoxins; anti-mycotoxin additive; bacterial challenge; survival; growth

1. Introduction

Aquatic foods are the healthiest and largest sources of animal protein for humans. Globally nearly 160 million metric ton (mmt) of aquatic food is consumed annually, which is more than chicken (144 mmt) or beef (76 mmt) and lamb (17 mmt) combined. The capture or harvesting of wild fish stocks likely originated as a fundamental activity to obtain nutrient-rich food and has persisted as a long-standing tradition. Although oldest written history of fish farming dates back to 300-500 BC in China and India, modern aquaculture started only after 1970s when the wild fish catch declined [1]. Farming of fish or aquaculture grew rapidly after 1980s and has now become the fastest growing food production sector. FAO data [2] show that the production of farmed aquatic foods increased by almost 189 folds in 72 years of farming history i.e. it increased from 0.5 mmt in 1950s to 94.4 mmt in 2022 whereas wild catch was 91.0 mmt. For the first time in history, aquaculture took over the wild catch in 2022, as the wild catch remained almost constant since 1990. Approximately 51% of aquatic food production originates from farming. More than 700 finfishes, shellfishes, and aquatic plants are used in aquaculture. Major commercially farmed finfishes are carps, tilapias, salmonids, and catfishes. Mollusks such as oysters, mussels, clams, cockles, etc. are not only valuable aquatic foods but also play an important role in maintaining the ecosystem and environment as they capture CO₂ and store it in the form of shell. Crustaceans such as shrimps, prawns, crabs, etc. belong to another

category of species which contribute to considerable food and nutrition security. Among them penaeid shrimps are popular and highly traded seafood items across the globe. There are two major commercially important farmed species of crustaceans, namely Giant tiger shrimp (*Penaeus monodon*) and Pacific white shrimp (*Litopenaeus vannamei*). Total penaeid shrimp production has reached nearly 8 mmt in 2022 [2]. The recent shrimp market research report shows that the value of shrimp was over US\$75 billion in 2023 and is expected to grow at about 5% per year to more than US\$124 billion by 2032 [3] with the rise in seafood consumption owing to improved health awareness and advances in farming, storage and transportation technologies. Major penaeid shrimp exporting countries are Ecuador, India, Vietnam, Indonesia, China, Argentina, Thailand etc. and importing countries include China, US, Japan and European countries [4].

Commercial aquaculture depends on feed as the main input which accounts for 50-80% of the production cost. Assuming average feed conversion ratio (FCR) of 1.5 [5], a total of 12 mmt of feed is required globally to support 8 mmt production of penaeid shrimp. Various raw materials are utilized in the production of aquaculture feed. Fishmeal is regarded as the optimal ingredient; however, due to sustainability concerns, efforts have been made to substitute it with plant-based alternatives. Plant-based products have high chances of developing fungus which produce mycotoxins. Earlier FAO reported that 25% of the global food crops are contaminated by mycotoxins; however, other scholars have pointed out that it should be 60-80% higher than that value [6]. More importantly, low-quality or animal-grade food crops are utilized as feed ingredients, which implies that nearly all components are likely to be contaminated with mycotoxins. Around 400 mycotoxins have been identified but only 30 of them are known to be harmful [7–10]. *Aspergillus*, *Fusarium* and *Penicillium* are the main genus of fungi capable of producing mycotoxins such as aflatoxins, ochratoxin A, fumonisins, trichothecenes and zearalenone [8,11]. Mycotoxins contaminated feed negatively affects aquaculture productivity by lowering feed intake, reducing growth, increasing FCR, causing abnormality and cancer, damaging gills and liver, causing toxicity and causing high mortalities [12]. It is almost impossible to have animal feed without mycotoxins; however, their effects are underestimated and hardly recognized in aquatic species. Some studies have been done highlighting the occurrence of mycotoxins in aquaculture feeds and feed ingredients in Asia and Europe by Gonçalves et. al.[13,14]. High risk of exposure to mycotoxins is normally overlooked. Consuming fish or other products contaminated with mycotoxins might be one of the sources of entering the human food chain, threatening food safety and public health, causing toxicities and even cancer [9]. Mycotoxins can remain in the kidney, liver and muscles of fish and finally be ingested by humans which may affect the health of human as they are genotoxins, nephrotoxins, carcinogens and immunosuppressors [8,12,15]; . Therefore, the European Food Safety Authority (EFSA) panel has established guidelines for the assessment of additives and reduction of contamination of feed by mycotoxins [16,17].

Shrimp farming suffers from reoccurring slow growth, various diseases and high mortalities, that have been difficult to relate to any specific causes. Mycotoxins, especially aflatoxins, might be one of the reasons[18,19]. As mycotoxins are hard to avoid in feed, the strategy should be to minimize their effects in the animal itself. Various attempts have been made to improve digestibility and absorption of nutrients which affects nutritional value of feeds making tremendous impacts on the growth and survival [20]. A number of substances have been tested to reduce the growth of fungus or bind the mycotoxins to make them unavailable for absorption along the gastrointestinal tract. There are some mycotoxins detoxifying products and enzymes which can selectively transform mycotoxins into less toxic forms. However, due to a large number of mycotoxins are often present in the feed, many types of enzymes are needed which is not feasible. A more practical approach is the use of mycotoxin binders especially natural adsorbents, such as clays and yeast derivatives, combined with phytogenics, which can bind a wide range of mycotoxins compared to synthetic adsorbents, are less toxic, leave no residues, and provide nutritional benefits while preserving the bioavailability of other nutrients of the diet.

A commercial anti-mycotoxin additive (AMA) from BIÖNTE NUTRITION S.L. has been developed specially for aquaculture to use as a feed supplement. It is claimed to be an innovative broad-spectrum anti-mycotoxin solution as it presents three modes of action: adsorption, bio-protection and post-biotic effect, specific for aquatic species. AMA contains a unique combination of selected binding materials for adsorption, plant extracts and lysolecithin for bio-protection, and yeast cell wall and hydrolyzed yeast for post-biotic effect. Specifically, includes natural clays like bentonite and sepiolitic clay that are able to bind mycotoxins via electrostatic and van der Waals forces along the digestive tract of the animals [21–24]. By adsorbing the mycotoxins, the clays prevent them from being absorbed by the animal's digestive system, avoiding or reducing the health damage. The product includes plant extracts rich in bioactive compounds; namely, curcumin from turmeric (*Curcuma longa*) and Silymarin from milk thistle combined with lysolecithin from sunflower (*Helianthus annuus*) that mitigate the oxidative stress caused by mycotoxins [25–29]. In addition, it also contains post-biotic compounds from inactivated yeast products such as a mixture of yeast cell wall and hydrolyzed yeast from *Saccharomyces cerevisiae* that is able to agglutinate endotoxins and mycotoxins, to improve overall gut health by contributing to an enhanced resilience of the animals, increased innate immunity and ammonia nitrogen stress resistance ability, thereby improving growth performance [21,30,31]. The present study was carried out by designing two consecutive trials to evaluate the efficacy of anti-mycotoxin additive (AMA) specific for aquatic species on the survival, growth, feed utilization, immune response and health parameters in White leg shrimp (*Litopenaeus vannamei*).

2. Materials and Methods

2.1. Experimental System and Animal

The experiment was conducted at the Asian Institute of Technology (AIT), Pathum Thani, Thailand (Coordinates: 14.08N°, 100.61E°). A total of 12 glass aquaria tanks (100 L/tank) under the roof of zinc sheet (semi-outdoor condition with half sunlight allowed through a row of transparent sheets and protected by wire fence) with an independent recirculatory aquaculture system (RAS) were used considering a complete randomized design (CRD) in which, every treatment was randomly assigned to the different aquaria.

Post-larvae (0.2 mg) of Pacific white shrimp (*Litopenaeus vannamei*) were obtained from a private hatchery located in Chachoengsao province of Thailand. They were acclimatized during a period 14 days before using for the experiment providing the control diet, three times daily to apparent satiation. The experiment was divided into two parts: first 20 days as post-larval rearing in aquarium of 100 L capacity and 90 days of grow-out period in larger plastic tanks (300 L). During post-larval rearing 300 post-larvae (PLs) were stocked in each replicate aquarium and during grow-out phase (90 days), 110 post-larvae were chosen from each replicate aquaria used in the first phase trial.

2.2. Test Diets and Mycotoxins

Locally available commercial shrimp powder containing 43% crude protein (CP) (Ocean Aquaculture Platinum Plus #4 PL10-15, 400-600 micron) was used for the post-larval trial. For the grow-out trial, a commercial pellet containing 40% CP (Feed #1, Charoen Pokphand Co. Ltd., Thailand). A locally sourced corn with observed fungal growth was used as source of mycotoxins. The corn was processed into a fine powder using a grinder machine (Powder Grinder, ECO), resulting in a particle size range of 44–250 µm. The average concentration of fungus cells which produce mycotoxins in the corn was found to be 2.83×10^5 CFU. Therefore, 10% corn was mixed with the 90% standard shrimp feeds (40-43% CP) to have 2.83×10^4 CFU (toxic level is 1.0×10^4 CFU) which resulted in at least 36.8% CP after dilution due to the added corn which had approx. 8% crude protein; reasonably a good protein level. The two components were thoroughly blended using a feed mixing electric machine (Imarflex, IF-169) to ensure homogenous distribution of the contaminated material throughout the feed. Mycotoxin-contaminated feed served as the control treatment in both the nursing and grow-out trials to evaluate the protective effects of dietary supplementation of AMA.

The test diets were well dried and stored at normal refrigerator at 4-7°C, protected from light and humidity.

The concentration of specific mycotoxins was determined through standard analytical methods. In order to confirm the feed had mycotoxins, they were identified and quantified using the in-house HPLC method CH-002-TM, which is based on AOAC Official Method 991.31 [32]. For the detection of fumonisins, the in-house method CH-127-TM was applied, following the protocol outlined in EN 15662:2018. The report showed that the feed had a total of 46.6 µg fumonisins with B1 (35.1 µg/kg) and B2 (11.5 µg/kg).

During both the trial periods, shrimps were fed to near satiation; three times daily during larval nursing and two times during grow-out trial. Feeding behavior was observed and recorded. Feeding rates were used 4%, 6% and 8% of the biomass per aquarium each day for the 1st, 2nd and 3rd week respectively as suggested by Hung and Quy [33]; Antunes et. al. [34], Effendi et al. [35] for the post-larvae. During the grow-out phase, they were fed daily at the rate of 4%, 5%, 6%, 8% and 8% of the biomass per tank changing the rate weekly from week 1 to 5. For the remaining grow-out period, feeding rate was reduced to 5 - 6% daily. Uneaten feed and faeces were removed from tanks weekly and separated by a hand net. They were collected into a deep freezer (-18°C) for proximate analysis.

2.3. Feed Supplement and Treatments

A commercial anti-mycotoxin additive (AMA, BIÖNTE NUTRITION S.L., Reus, Spain) specific for aquatic species was used as a dietary supplement to evaluate its effects on survival, growth performance and health status of white shrimp (*Litopenaeus vannamei*) during both the nursing and grow-out trials. Four experimental diets were prepared by supplementing a commercial shrimp feed with AMA at varying concentrations. The control group received only the naturally contaminated commercial feed (0 g/kg), while the treatment groups were supplemented with low, medium and high levels (i.e. 1, 2, and 3 g/kg of diet) of AMA powder respectively as shown in the Table 1. The AMA powder was homogenized into each diet using an electric blender. Each treatment had three replications. Management of the trial tanks was done in accordance with the standard procedures of the aquaculture research facilities of AIT, Thailand.

Table 1. Experimental treatments.

Treatment no.	Treatments	Dose of AMA (g/kg feed)
T1	Control (no AMA)	0.0
T2	Level 1 (low)	1.0
T3	Level 2 (medium)	2.0
T4	Level 3 (high)	3.0

Note: Treatments in triplicate (no. of tanks used was 12 (i.e. 4 x 3)).

2.4. Water Quality Management

The experiment was conducted using freshwater. Water quality parameters such as temperature, dissolved oxygen (DO), pH, ammonia and nitrites were carefully monitored and maintained throughout experimental periods of both nursing and grow-out trials. The pH levels ranged between 7.5 and 8.5, while water temperature was maintained within the optimal range of 27–28°C. A multiparameter probe (YSI Professional Plus) was employed to measure pH and temperature, and DO concentrations were monitored weekly in the early morning at 07:30 hrs. using a DO meter (LAQUA, Horiba, Model 220). Photoperiod was controlled at a 12-hour light and 12-hour dark cycle to simulate natural day-night conditions. In both the trials, water exchange was performed weekly. Before exchanging water each time, 50% of the water volume in each tank was drained by siphoning to eliminate accumulated wastes i.e. uneaten feed, faeces and excess organic matters. Freshwater was then added to restore the original volume in each culture tank. Additionally, before each water exchange, water samples (500 mL per replicate) were collected from each tank for analysis

of ammonia-nitrogen (NH₃-N) and nitrite-nitrogen (NO₂-N) concentrations. These parameters were determined using the standard titration method [36].

2.5. Sampling of Feed

About 50 g of each of the test diets was randomly sampled for proximate analysis, performed at the laboratory facility of AIT. Commercial feed without mixing of the supplement as a control treatment, and different mixing ratios of the supplement were collected in three replicates each. The samples were analyzed using the standard protocols [32]. Crude protein was determined by Micro-Kjeldahl Method using FOSS Kjeltac 8100 apparatus (FOSS Analytical AB, Hoganas, Sweden). Crude lipid was tested by Soxhlet Method using FOSS Soxtec 2043 apparatus (FOSS Scino Co. Ltd, Suzhou, China). The crude fiber was estimated by following Weende Method using FOSS Fibertec 1020 apparatus (FOSS Scino Co. Ltd, Suzhou, China). Moisture was estimated by air oven Lab Tech (Model LDO-100E, Daihan Labtech Co. Ltd, Namyangju City, Korea). Ash was determined by after burning the samples at 550°C using a muffle furnace (Lab Tech Model LEF-115S-1 muffle furnace).

2.6. Sampling of Shrimp

At the end of post-larval trial of 20 days, 110 juveniles per replicate aquarium were weighed and transferred to the plastic tank for the grow-out trial. The remaining were kept in deep freezer (-80°C) for proximate analysis. At the end of grow-out trial, about 50 g (7-8 shrimps) per tank were randomly selected to sacrifice in the deep freezer for proximate analyses of whole body. The samples were analysed in triplicates. Batch weights (total bulk weight of shrimp in each tank) was taken at the end of the nursing period and every 30 days during the grow-out phase. At the final harvest, shrimps were counted to calculate the survival rate. During the experimental period, shrimp health and mortality were observed during each feeding.

2.7. Data Records

Other performance parameters such as feed intake, weight, length and survival were recorded and compiled in MS Excel file. Feed utilization indicators such as feed conversion ratio (FCR) and protein efficiency rate (PER) were also calculated and compared. For post-larvae trial as well as for grow-out trial, 20 shrimps were randomly sampled for one initial pooled sample and 10 were taken to measure length from each replicate aquarium or tank.

At the final harvest of grow-out phase, five shrimps were sampled for morphological analysis. Their internal organs such as stomach, intestine and hepatopancreas were observed with naked eye (macroscopic) and also under compound microscope. Photos were taken by treatment for demonstration.

Survival, specific growth rate, daily weight gain, daily length gain, feed conversion ratio and hepatosomatic index were compared among the treatments which were calculated based using the following equations [37] as:

$$\text{Survival rate (\%)} = (N_t / N_0) \times 100$$

$$\text{Specific growth rate (SGR, \%/day)} = [\ln(W_t) - \ln(W_0)] / t \times 100$$

$$\text{Daily weight gain (DWG, mg/day)} = (W_t - W_0) / t$$

$$\text{Daily length gain (DLG, mm/day)} = (L_t - L_0) / t$$

$$\text{Feed conversion ratio (FCR)} = \text{Fresh feed offered} / \text{dry weight of shrimp}$$

$$\text{Protein efficiency Ratio (PER)} = \text{Biomass gain (g)} / \text{protein consumed (g)} \times 100$$

$$\text{Hepatosomatic index (HSI)} = \text{weight of liver} / \text{total weight of shrimp}$$

Where,

N_t - number of larvae and post-larvae taken at the final harvest.

N₀ - number of larvae stocked

Ln - Natural log

W_t - final weight (g)

W₀ - initial weight (mg)

L_t - final length (cm)
L₀ - initial length (cm)
t - time (days)

2.8. Bacterial Challenge Trial

In order to evaluate the capacity of the AMA to ameliorate immune suppression, a bacterial challenge test against *Aeromonas hydrophila* was performed after 90 days of growth performance trial. A disease resistance on the experimental animals using a challenge test with *Aeromonas hydrophila* by immersion method, was performed. The shrimp of all the 12 tanks assigned for the four treatments (T1 to T4) replicated three times, were divided into two groups; one group for the exposure to bacteria (infected group) and the other was for control or mock-infected. Therefore, the challenge trial was conducted using 24 plastic tanks of 30 L capacity each stocking with 10 shrimp per tank. Pre-challenge test was performed in order to determine the amount or the CFU of *Aeromonas hydrophila* used for immersion method (LD50, i.e., lethal dose that caused 50% mortality). Mortality and external body conditions were closely observed for 14 days and the accumulated mortality was calculated and to compare among the treatments.

2.9. Data Analysis

The data is presented as an average of the three replicates for each of the four treatments along with their standard errors (Average ± SE). One-way analysis of variance (ANOVA) was used for each growth, survival and other parameters to test the effects of the factor (feed supplement AMA). Tukey’s HSD-test was used to compare among treatments groups. Quadratic regression was used to determine the relationship between the factor (AMA dose) and variables (survival, weight, length etc.) Significance level was considered as significant at 5% and highly significant at 1%. Data were compiled in Microsoft® Excel for MAC Microsoft 365 @2025 and statistical analyses were performed using IBM SPSS Statistics (Version 23).

3. Results

The common feed ingredient, corn contaminated with mycotoxins, was added to make the basal or control feed while AMA powder was supplemented at 1, 2 and 3 g/kg diet as a dietary additive as treatments for conducting two-stage trial; nursing of post-larvae and grow-out trials. During the nursery trial, the survival rate was not affected whereas during the grow-out phase survival rate had significant quadratic relationship (P<0.05) with the dose. Growth of shrimp was not affected but length was did vary by the dose of AMA. Therefore, quadratic relationships of the dose of AMA with the shrimp survival and length have been used to determine the optimum doses (1.4 - 2.5 g/kg diet) which are described in the following sub-sections.

3.1. Post-Larvae Stage

During the 20 days post-larvae stage, the AMA significantly (P<0.05) increased the final length and the length gain regardless of the doses (Table 2). However, there were no effects on the weight and survival of the post-larvae.

Table 2. Survival and growth performance (Mean±SE) of shrimp in post-larvae phase.

Parameters	Treatments (g/kg diet)			
	0.0	1.0	2.0	3.0
Initial number	300±0	300±0	300±0	300±0
Final number	159±21	188±15	139±41	192±12
Survival (%)	52.9±7.0	62.6±4.9	46.4±13.7	64.0±3.8
Initial length (cm)	1.1±0.02	1.1±0.02	1.1±0.02	1.1±0.02
Initial weight (mg)	9.2±0.0	9.2±0.03	9.2±0.03	9.2±0.0
Final length (cm)	2.5±0.1 ^b	2.8±0.0 ^a	2.8±0.1 ^a	2.9±0.0 ^a

Final weight (mg)	140±10	126±6	151±26	126±2
Daily weight gain (g)	6.5±0.5	5.9±0.3	7.1±1.3	5.8±0.1
Length gain (cm/day)	0.07±0.0 ^b	0.09±0.0 ^a	0.08±0.01 ^a	0.09±0.0 ^a
Specific growth rate (%)	13.6±0.4	13.1±0.2	14.0±0.9	13.1±0.1

Note: values with different superscripts in the same rows are significantly different at 0.05.

The effects were also seen in proximate compositions of the whole body of post-larvae (Table 3). Protein content of the larvae was 60% higher ($P<0.05$) in the low-dose group (56.99±3.80%) compared to the control group, whereas medium and high dose groups did not differ significantly with the control. Lipid content of the post-larvae was highest when they were fed at medium dose followed by the low dose. However, high dose did not differ with the control. Medium and higher doses did not differ with the control. Whole-body moisture content was significantly ($P<0.05$) lower in the medium and high dose group as compared to the control group. Interestingly, ash content was 25% lower all the groups with AMA, regardless of the dose as compared to the control group (23.51±0.15%).

Table 3. Proximate analysis of final whole body of shrimp in post-larvae phase.

Parameters		Treatments (g/kg diet)			
		0.0	1.0	2.0	3.0
Whole body	Moisture (%)	88.93±0.34 ^a	88.14±0.17 ^{ab}	87.86±0.66 ^b	87.92±0.22 ^{ab}
	Ash (%)	23.51±0.15 ^a	17.09±0.97 ^b	17.42±1.56 ^b	17.61±3.07 ^b
	Lipid (%)	14.25±1.06 ^c	17.26±1.11 ^b	22.22±0.19 ^a	13.22±0.37 ^c
	Protein (%)	35.75±4.32 ^a	56.99±3.80 ^b	35.15±1.73 ^a	37.88±0.56 ^a

Note: values with different superscripts in the same rows are significantly different at 0.05.

Among the water quality parameters of the post-larvae trial (Table 4), DO and pH were reasonably good as there was continuous aeration 24 hours. Temperature was also fairly constant which ranged between 27.1 and 28.4 °C. Nitrite-nitrogen ranged from 1.01 to 5.89 mg/L which are quite high levels for shrimp post-larvae or any aquatic species. However, ammonia-nitrogen remained within the acceptable range of 0.7-1.51 mg/L.

Table 4. Water quality parameters of post-larvae phase during culture (20 days).

Treatment with AMA	Water Quality				
	DO (mg/L)	pH	Temp (°C)	NO ₂ - N (mg/L)	NH ₃ -N (mg/L)
Control (0.0 g/kg)					
Average	6.85±0.17	8.24±0.09	27.92±0.38	3.27±1.04	0.81±0.45
Min-Max	6.44-7.01	8.02-8.32	27.1-28.3	1.76-4.84	0.07-1.51
Low (1.0 g/kg)					
Average	7.02±0.11	8.21±0.07	27.98±0.26	2.63±1.51	0.72±0.27
Min-Max	6.9-7.17	8.11-8.3	27.7-28.3	1.01-5.89	0.34-1.12
Medium (2.0 g/kg)					
Average	6.91±0.07	8.22±0.05	28.08±0.26	3.15±1.29	0.6±0.33
Min-Max	6.83-7.05	8.11-8.28	27.7-28.4	1.98-5.74	0.3-1.37
High (3.0 g/kg)					
Average	6.98±0.09	8.26±0.07	27.97±0.29	2.23±0.56	0.58±0.29
Min-Max	6.83-7.16	8.13-8.34	27.5-28.3	1.38-3.19	0.17-1.09

3.2. Grow-Out Phase

The survival rate of white shrimp was monitored monthly throughout the grow-out phase (Table 5; Figure 1). In the first month, all treatments began with the same number of individuals (110 shrimp). By the end of the first month, survival rates remained high across all treatments ranging

from 83% in the control group to 91% in the high-dose group. By the end of the second month, the survival continued to remain high which ranged from 75% in the control group to 89% in the high dose group. By the end of the third month survival declined significantly in all the groups. The control group had the lowest survival rate at 27%, which was significantly lower ($P<0.05$) than all other groups. All the AMA supplemented groups i.e. low, medium, and high doses maintained higher survival rates of 50%, 58%, and 59% respectively, with no significant difference among them. The medium and the high doses had more than twice the survival rate of the control group, which means the AMA was highly effective in terms of survival of shrimp during the grow-out phase. However, the survival did not increase further by the highest dose. Therefore, regression analysis was done to see the relationship (Figure 2) between survival and AMA dosage. The data analysis showed a significant quadratic model ($y = -5.3788x^2 + 26.530x + 27.591$, $R^2 = 0.9969$, $P<0.01$, $n=12$, $X_{max} = 2.5$, $Y_{max} = 60.3$) that clearly indicated that the highest survival rate of shrimp (60.3%) could be achieved by applying 2.5 g of AMA product per kg of feed.

Table 5. Average no. of shrimp and survival rate (%) by month during the grow-out phase.

Treatment dose	1-July	1-Aug	1-Sep	1-Oct
Control (0.0 g/kg)	110±0	91±11 (83%)	83±4 (75%)	30±10 (27%) ^b
Low (1.0 g/kg)	110±0	98±8 (89%)	89±15 (81%)	55±15 (50%) ^{ab}
Medium (2.0 g/kg)	110±0	98±1 (89%)	87±10 (79%)	64±4 (58%) ^{ab}
High (3.0 g/kg)	110±0	100±6 (91%)	99±2(89%)	65±9 (59%) ^a

Note: values with different superscripts in the same column are significantly different at 0.05.

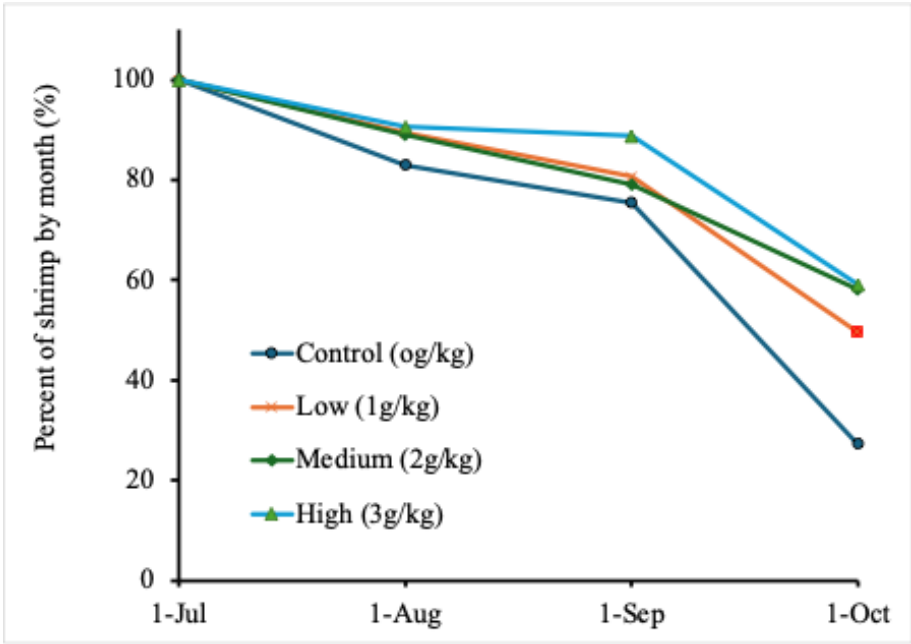


Figure 1. Survival rate (%) of shrimp during the grow-out phase by month.

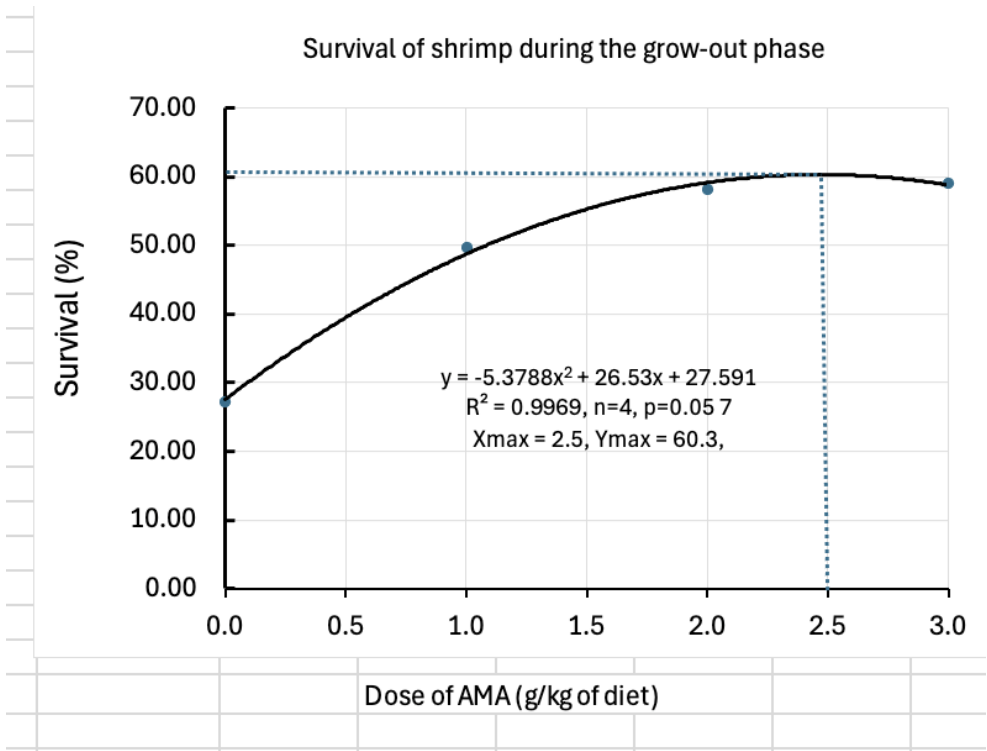


Figure 2. Average survival rate (%) of shrimp at the end of grow-out phase.

Similarly, the dose of the AMA exhibited a good quadratic relationship (Figure 3) with the daily weight gain of the shrimp ($y = -2.0706x^2 + 5.6323x + 62.8410$, $R^2 = 0.9968$) which showed the maximum daily weight gain of 66.7 mg could be obtained at 1.4 g of AMA per kg of feed. However, **specific growth rate** (SGR) did not show any clear trend. Growth performance are presented in Table 6. The length of the shrimp showed a clear relationship described by a quadratic model ($y = -0.055x^2 + 0.2163x + 9.7063$, $R^2 = 0.9692$, $X_{\max}=2.0$, $Y_{\max} = 9.9$). It showed that the highest length of the shrimp would be reached at the dose 2.0 g of AMA per kg of feed (Figure 4). Hepato somatic index (HSI) was significantly higher in the control. The inclusion of AMA significantly decreased the HSI showing a negative quadratic relationship as represented by the equation, $y = 0.3132x^2 - 1.7176x + 6.6719$, $R^2 = 0.5918$, $X_{\min} = 2.7$ and $Y_{\min} 4.3$. The lowest HSI was achieved at the dose of 2.7 g/kg of AMA (Figure 5).

Table 6. Survival and growth performance of shrimp during the grow-out phase.

Parameters	Treatments (g/kg diet)			
	0.0	1.0	2.0	3.0
Initial length (cm)	3.57±0.02	3.57±0.1	3.41±0.11	3.54±0.17
Initial weight (g)	0.28±0.0	0.27±0.0	0.28±0.0	0.27±0.0
Final length (cm)	9.70±0.33	9.89±0.74	9.90±0.94	9.87±0.55
Final weight (g)	6.1±1.3	6.4±1.3	6.3±1.1	5.9±0.6
Length gain (cm)	6.13±0.34 ^a	6.31±0.72 ^a	6.49±0.83 ^a	6.33±0.67 ^a
Weight gain (mg/day)	62.8±13.9	66.6±14.5	65.7±11.4	61.2±6.6
Length gain (mm/day)	66.6±3.7	68.6±7.9	70.5±0.01	68.8±7.3
Initial number	110±0.0	110±0.0	110±0.0	110±0.0
Final number	30±9.90 ^b	54.67±15.04 ^a	64±4.24 ^a	65±8.54 ^a
Survival (%)	27.3±9.0 ^c	49.7±13.68 ^{ab}	58.3±3.86 ^{ab}	59.1±7.8 ^a
Specific growth rate (%)	3.3±0.2	3.4±0.3	3.4±0.2	3.4±0.2
Feed conversion ratio (FCR)	2.3±0.8	1.3±0.1	1.5±0.3	1.2±0.1
Protein efficiency ratio (PER)	92±36	153±10	135±33	168±8
HSI (%)	6.71±0.87 ^a	5.15±0.64 ^b	4.60±0.70 ^b	4.30±1.07 ^b

Note: values with different superscripts in the same rows are significantly different at 0.05.

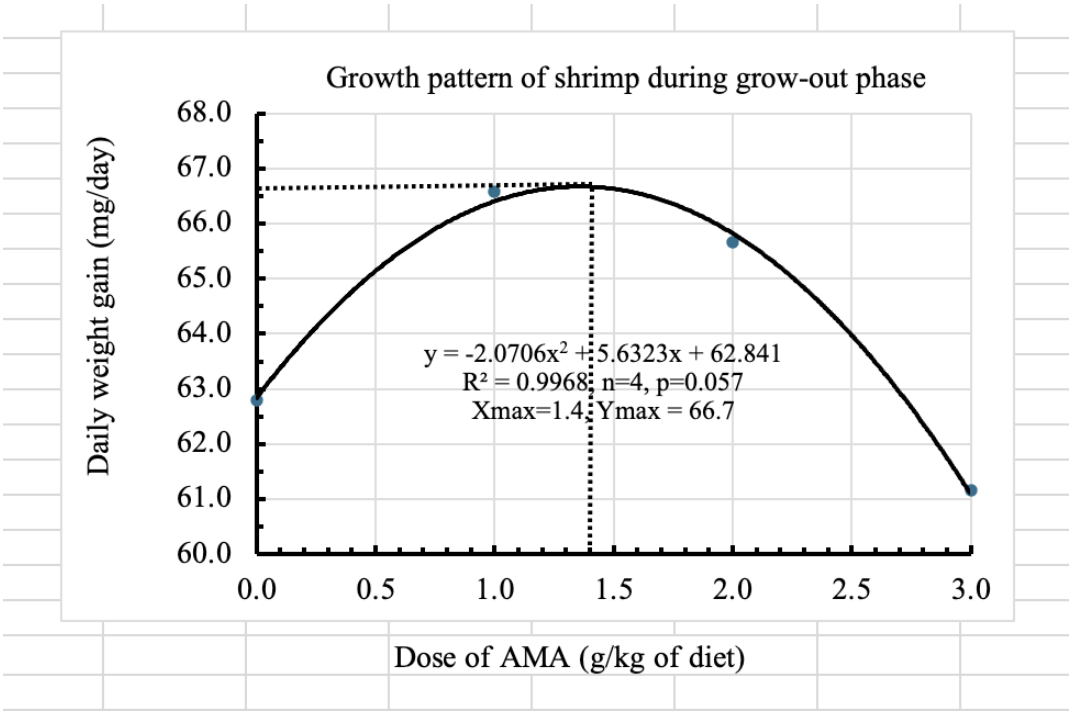


Figure 3. Average daily weight gain (DWG, mg/day) of shrimp in grow-out phase.

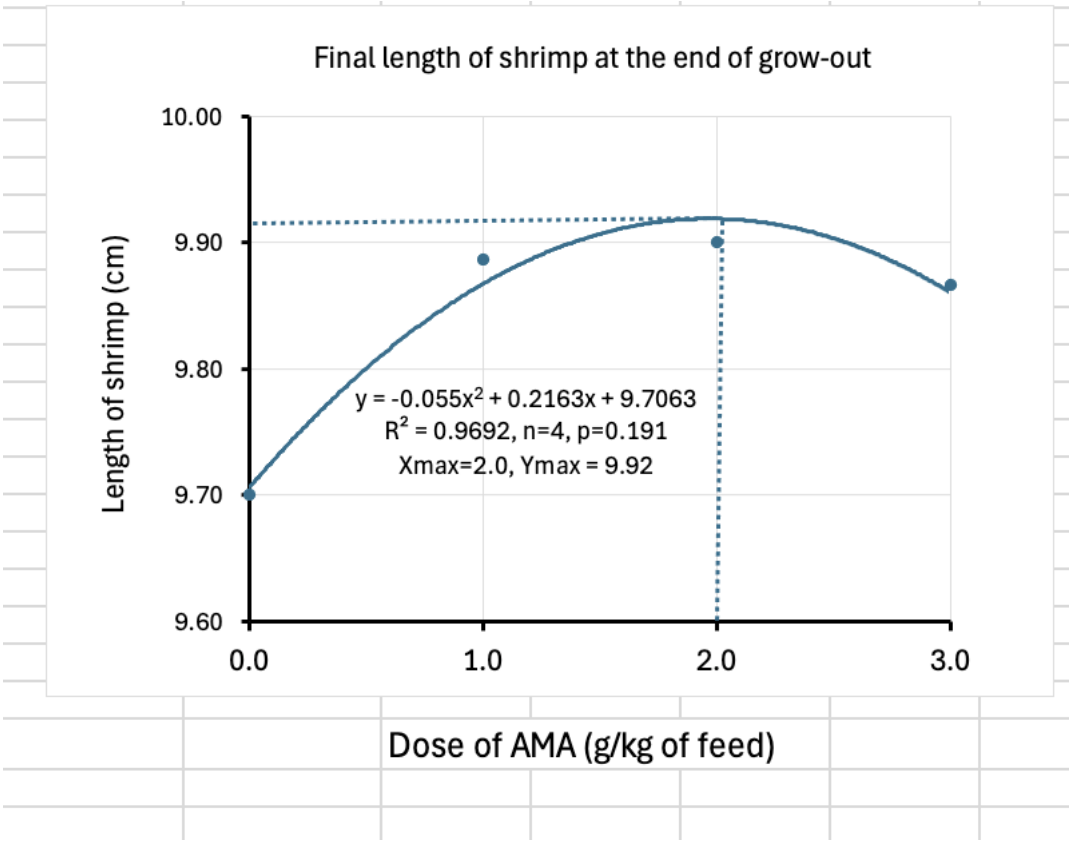


Figure 4. Final length (cm) of shrimp during grow-out phase.

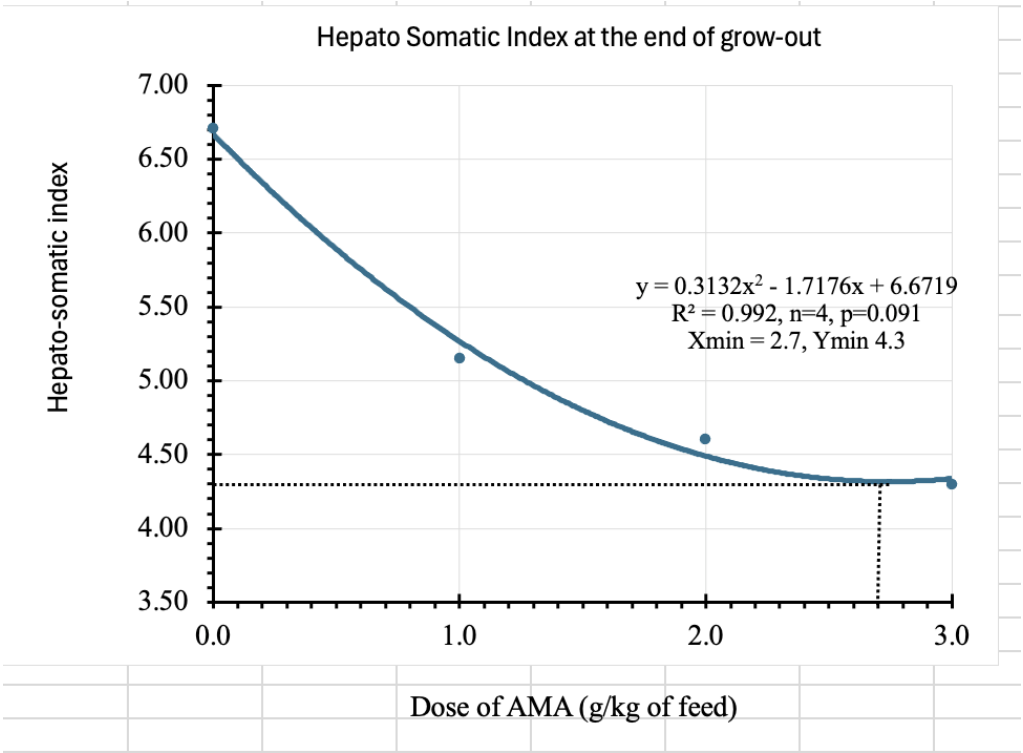


Figure 5. Hepato-somatic index (HSI) of shrimp at the end of grow-out phase.

During the **grow-out phase**, feed conversion ratio (FCR) was 2.3 ± 0.8 for control treatment, whereas it remained below 1.5 in all the treatments with AMA supplementation. Similarly, effects could be seen in terms of protein efficiency ratios which was 92 ± 36 in case of control, but AMA supplemented feeds had over 135%. The experimental feeds were analysed to determine the proximate composition after adding the AMA supplement (Table 7). The levels of the respective nutrients were similar across treatments, showing no significant variation in moisture, ash, lipid, or protein content, confirming that the experimental diets were nutritionally consistent aside from the AMA levels.

Table 7. Proximate composition (%) of feed, final whole body and faeces of shrimp at the end of grow-out phase.

Factors		Treatments (g/kg diet)			
		0.0	1.0	2.0	3.0
Feed	Moisture	13.68±0.93	14.77±0.09	14.33±0.48	14.62±0.12
	Ash	14.41±0.09 ^b	14.50±0.09 ^b	14.44±0.07 ^b	14.76±0.00 ^a
	Lipid	4.68±0.76	5.24±1.49	4.99±0.43	5.48±0.41
	Protein	38.44±0.47	38.77±0.16	38.75±0.41	38.51±0.44
Whole body	Moisture	75.41±1.43 ^a	65.21±1.91 ^b	72.14±0.19 ^a	75.57±1.45 ^a
	Ash	11.69±0.59	9.94±1.42	10.55 ^b ±1.48	13.19±2.08
	Lipid	5.15±0.61 ^a	3.32±0.11 ^b	3.45±0.26 ^b	3.26±0.1 ^b
	Protein	80.66±0.24 ^b	77.72±1.36 ^c	80.73±0.68 ^b	82.97±0.59 ^a
Faeces	Moisture	89.30±2.34	89.10±2.38	88.95±2.68	88.13±2.54
	Ash	41.36±3.18 ^b	47.15±3.96 ^{ab}	50.56±2.46 ^a	51.85±3.16 ^a
	Lipid	0.85±0.15 ^a	0.38±0.09 ^b	0.63±0.11 ^{ab}	0.37±0.05 ^b
	Protein	11.82±2.45 ^a	5.79±1.49 ^b	13.24±0.18 ^a	12.80±1.51 ^a

Note: values with different superscripts in the same rows are significantly different at 0.05.

The composition of the **final whole-body** of shrimp fed with the high dose of AMA supplemented diet showed significantly higher protein content ($82.97\pm0.59\%$) compared to the other

groups ($P<0.05$). The low-dose group exhibited the lowest moisture content ($65.21\pm1.91\%$), while the other groups remained statistically similar. Lipid content was significantly lower in all the AMA-supplemented groups in comparison to the control ($P<0.05$), suggesting improved lipid metabolism. Ash contents were not statistically significant.

Proximate composition of faeces revealed that there were no significant differences in the moisture content among treatments. Ash content was highest in the high-dose group ($51.85\pm3.16\%$) and significantly different from the control group ($41.36\pm3.18\%$). Lipid content in faeces was significantly lower in the low- and high-dose groups ($0.38\pm0.09\%$ and $0.37\pm0.05\%$, respectively) compared to the control group indicating better lipid utilization. Protein in faeces was significantly lower in the low-dose group ($5.79\pm1.49\%$), while the other treatments showed higher values similar to the control. These findings (Table 7) suggest that dietary supplementation of AMA had effects on nutrient utilization which improved body composition, particularly increasing protein deposition and reducing body lipid accumulation in white shrimp.

Water quality parameters of the grow-out trial (Table 8), DO and pH were reasonably good as there was continuous aeration 24 hours. DO ranged from 6.39-7.15 mg/L and pH between 7.65 and 8.37. Temperature ranged between 27.0 and 29.2 °C. Nitrite-nitrogen ranged from 1.01 to 5.44 mg/L which are quite high levels for shrimp post-larvae or any aquatic species. However, ammonia-nitrogen remained within the acceptable range of 0.39-2.89 mg/L.

Table 8. Water quality parameters during culture in grow-out phase.

Treatment with AMA	Water Quality				
	DO	pH	Temp	NO ₂ -N	NH ₃ -N
Control (0.0 g/kg)					
Average	6.87±0.14	8.08±0.14	27.90±0.62	2.97±1.08	1.11±0.68
Min-Max	6.59-7.14	7.88-8.37	27.00-29.00	1.08-5.14	0.39-2.47
Low (1.0 g/kg)					
Average	6.93±0.18	8.03±0.13	27.98±0.63	2.75±1.10	1.23±0.65
Min-Max	6.39-7.15	7.80-8.26	27.10-29.20	1.01-5.22	0.57-2.89
Medium (2.0 g/kg)					
Average	6.91±0.09	8.01±0.16	26.57±6.02	3.57±1.41	1.13±0.45
Min-Max	6.74-7.03	7.65-8.21	27.00-29.00	1.23-5.44	0.51-2.34
High (3.0 g/kg)					
Average	6.87±0.13	8.00±0.24	28.04±0.64	2.99±1.32	1.10±0.69
Min-Max	6.39-7.15	7.80-8.26	27.01-29.20	1.01-5.22	0.57-2.89

3.3. Bacterial Challenge Phase

The survival duration and onset of mortality of shrimp during the bacterial challenge test are summarized in Table 9. All the shrimp died before 9th day of bacterial challenge in the control group exhibiting the shortest survival time, with an average of 8.67 ± 1.53 days and the first mortality occurred as early as 3rd day post-infection. In contrast, shrimp fed with AMA-supplemented diets showed significantly longer survival durations ($P<0.05$), ranging from 11.67 ± 0.58 to 12.0 ± 0.0 days, even the lowest dose had the same effect with medium and high doses. Moreover, the onset of mortality in these groups was significantly delayed, with the first deaths recorded between days 7.33 ± 0.58 and 8.33 ± 0.58 . The high-dose AMA demonstrated the best resistance, with the more than 100% delayed occurrence of the first mortality and about 50% longer survival. These results clearly indicated that AMA has significant benefits in the immunity of shrimp.

Table 9. Mortality of shrimp during the bacterial challenge test.

Treatment (g/kg diet)	Infected group (average days)	
	Mortality starting day	Final survival days
Control (0.0)	3.00±0.0 ^a	8.67±1.53 ^a

Low (1.0)	7.33±0.58 ^b	12.00±2.00 ^b
Medium (2.0)	7.67±1.53 ^b	11.67±0.58 ^b
High (3.0)	8.33±0.58 ^b	12.00±0.00 ^b

Note: values with different superscripts within the same rows are significantly different at 0.05.

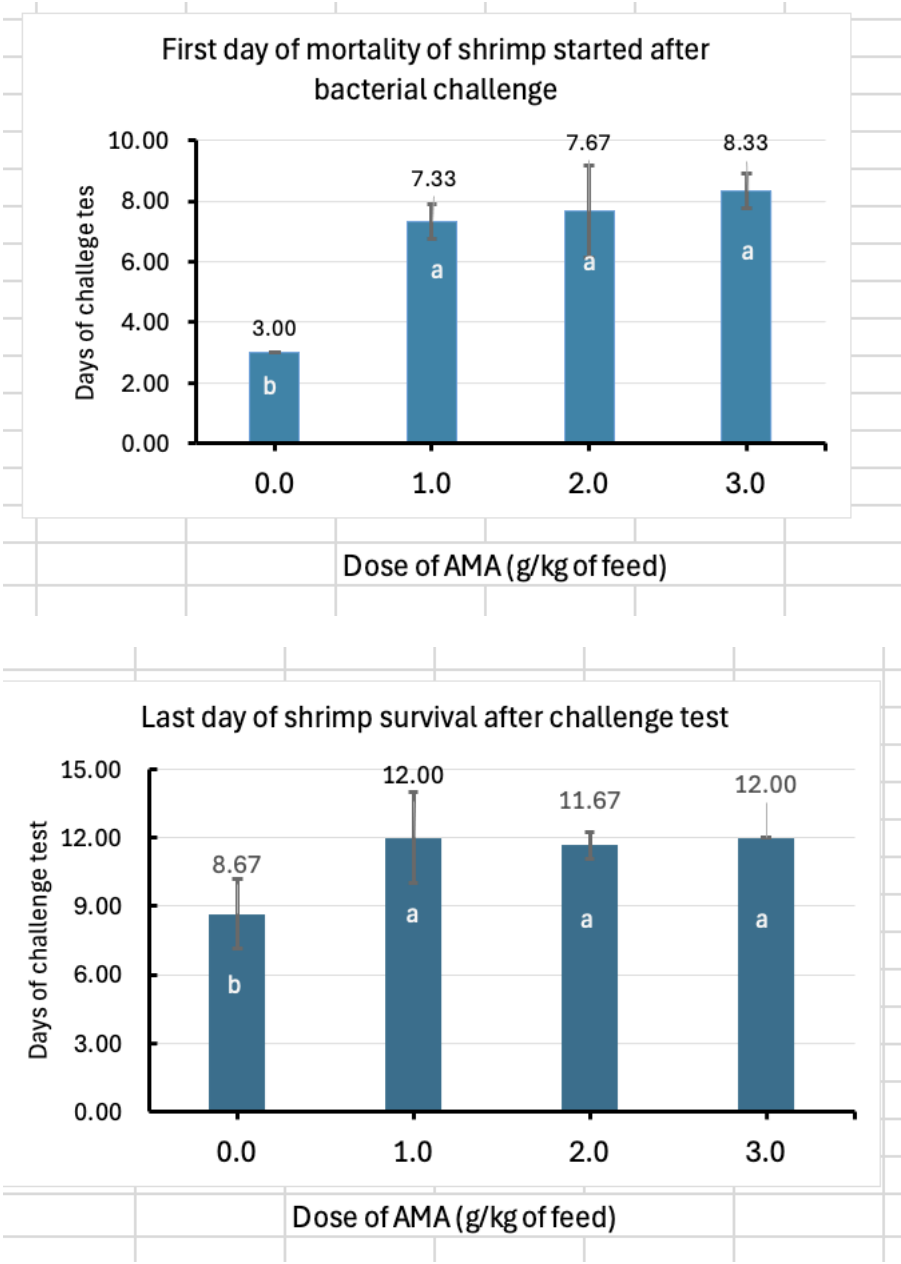


Figure 6. a Extension of survival (starting day of the first mortality after bacterial challenge test) by the different doses of supplementation of AMA. **b** Extension of survival (i.e. average day of the last shrimp death after bacterial challenge test) by the different doses of supplementation of AMA.

3.4. Morphometry Analysis

Results of the morphometry analysis of internal organs after the bacterial challenge test was carried out under microscope. Results are summarized in Table 10 and shown in Figures 7 and 8a,b. Major analytical observations were conducted in the AIT laboratories i.e body color, stomach, intestine, and hepatopancreas. The body color analysis revealed that the shrimp which received AMA had hard shell and remained normal in color whereas the color of the shrimp without AMA had the shell of pale color (Figure 7). Stomach of the shrimp was half full in the treatment with 1 to 2g/kg feed AMA, whereas the shrimp of the control and high dose treatment had empty stomach. Intestinal parts

of the shrimps from the control and low-dose of AMA were red in color indicating the inflammation whereas the shrimp from medium and high-dose had blue normal colored intestines. More importantly, the hepatopancreas was brown and dark brown in color with more darker in case of control and the high dose of AMA. But the shrimps of medium and high doses had smooth and normal hepatopancreas whereas that from control and low-dose had abnormal.

Table 10. Summary of results of the morphometry analysis of shrimp internal organs.

Organs	Treatments (g AMA/kg diet)			
	0.0	1.0	2.0	3.0
1. body color	Pale, soft shell	Normal, hard shell	Normal, hard shell	Normal, hard shell
2. stomach	Empty	Half full	Half full	Empty
3. intestine	Red & inflammation	Red & inflammation	Blue & normal	Blue & normal
4. hepatopancreas	Dark brown	Brown	Brown	Dark brown
	Less lipid	Less lipid	Less lipid	Less lipid
	Thin cell wall	Thin cell wall	Thin cell wall	Thin cell wall
	Not normal	Not normal	Smooth/normal	Smooth/normal



Figure 7. Shrimp showing their appearance after grow-out trial.

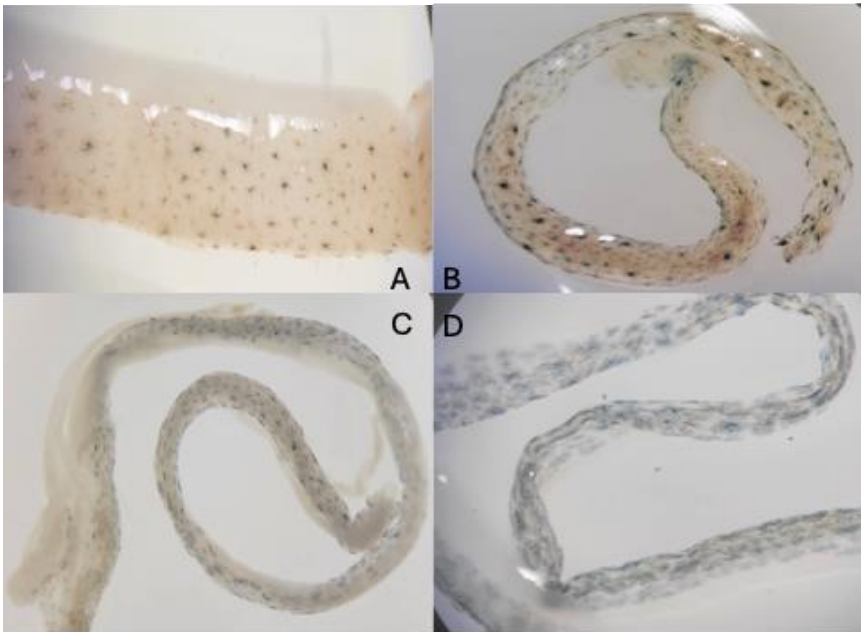
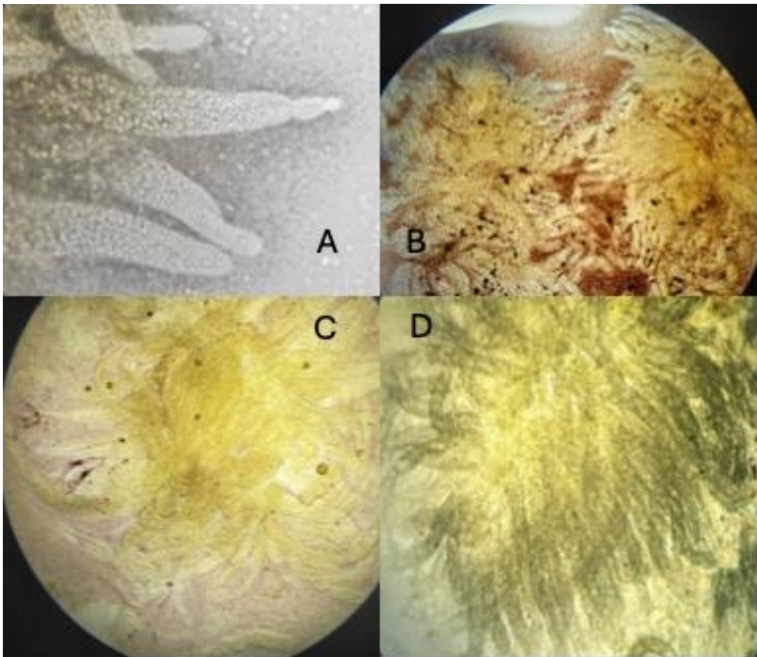


Figure 8. a Lipid tissue observations in hepatopancreas: A - Control feed, B -1.0, C – 2.0 & D – 3.0 g AMA/kg diet. **b** Pigmentation and inflammation of the shrimp intestine: A - Control feed, B -1.0, C – 2.0 & D – 3.0 g AMA/kg diet.

4. Discussion

Shrimp farming is considered a high-risk business due to the occurrence of high mortalities as a result of various causes including bacterial and viral diseases, bad physical and chemical properties of water, and poor quality of aquafeed with missing or deficient nutrients and containing mycotoxins and other pollutants. Although, high mortalities in shrimp are often untraceable, more recently, the problems related to the feed and the harms caused by the presence of various mycotoxins in feed and feed ingredients are becoming more apparent[8]. About 400 mycotoxins identified so far but only 20-

30 of them are well-known and are produced by fungi of three genera: *Aspergillus*, *Fusarium* and *Penicillium* [8–10]. In order to reduce aquatic foods production costs, the use of plant-based ingredients increased to replace fishmeal from diets. However, the risk of exposure of farmed aquatic species to feed pollutants such as mycotoxins augmented due to the higher inclusion of potentially contaminated raw materials such as cereals and grains. Then, it is almost impossible to avoid mycotoxins from aquafeed. Therefore, various nutritional strategies such as the supplementation of mycotoxin detoxifiers have been proposed as solution for aquaculture challenge [13,14,19].

The anti-mycotoxin additive (AMA) investigated is a commercial mycotoxin detoxifier specifically developed for aquatic species, including shrimp. AMA is a broad-spectrum mycotoxin detoxifier that presents three modes of action: adsorption, from a combination of selected binding materials; purified plant extracts from turmeric (*Curcuma Longa*) and milk thistle (*Silybum Marianum*), and sunflower (*Helianthus annuus*) lysolecithin for systemic health enhancement (bioprotection); and inactivated yeast derivatives from *Saccharomyces cerevisiae* that provide a post-biotic effect in the gastrointestinal tract and support the immune system function [29]. Specifically, AMA includes a combination of selected natural clays, including bentonite and sepiolitic clay, that are able to bind mycotoxins via different physicochemical mechanisms like electrostatic and van der Waals forces. These adsorbents perform their detoxification activity along the digestive tract of the animals, preventing the toxins from being absorbed in the intestine, thus being eliminated through faeces [21–24]. The purified plant extracts included in AMA present important levels of bioactive phytochemical compounds, remarking the curcumin from turmeric, the silymarin from milk thistle, and the lysophospholipids from sunflower lysolecithin; which are compounds that demonstrated to provide antioxidant, anti-inflammatory and hepatoprotective benefits [29,38–40]. The inactivated yeast products provide a source of post-biotic biopolymers such as β -glucans and mannan-oligosaccharides (MOS) from yeast cell wall, along with highly digestible nutrients from hydrolyzed yeast [31,41]. β -glucans, which constitute the insoluble fraction of the inner layer of the yeast cell wall, have demonstrated effective binding properties against some mycotoxins. This process occurs through chemical interactions between the toxins and the hydroxyl, ketone, and lactone groups of the polymers [42,43]. Furthermore, MOS may contribute to the reduction pathogenic bacteria populations and to the mitigation of the detrimental effects of endotoxins (LPS) in the gut. This is accomplished through a competitive mechanism based on the structural similarity of MOS to mannosylated receptors of the epithelial cells [30,44]. Hydrolyzed yeast is produced by enzymatic processes to break down the cell wall and other macromolecules in small peptides and amino acids. Moreover, it contains valuable trace minerals and vitamins such as from B-group. These components are highly digestible and bioavailable thus enhancing growth and immune function of aquatic animals [45,46]. In that context, the present study was designed to assess the efficacy and determine the best dosage of AMA in white shrimp fed with a naturally contaminated diet in terms of growth performance and survival, bacterial disease resistance and organs integrity.

The administration of AMA resulted in a significant increase in length gain during the post-larval phase. However, due to the limited duration of this phase, its potential effects on additional physiological or growth parameters could not be fully assessed. Therefore, a subsequent grow-out trial was conducted to evaluate longer-term impacts. The effect of the supplementation during larval stage might have accumulated in the grow-out phase. During the grow-out phase, positive effects of AMA were seen in the survival rate, daily weight gain and length gain. The survival increased with the increase in the dose of the AMA; however, it peaked at 2.5g/kg diet then declined. Daily weight gain improved at the lowest AMA dose only, but medium and high doses did not enhance the growth further. The regression analysis indicated that 1.4 g of AMA/kg of diet results in the highest weight gain. Therefore, the most suitable dose of AMA would be in between 1.4 and 2.5 g/kg diet. Within this range, lower dose enhances the growth and higher dose increases survival as a result, growth or the size of individual shrimp may be compromised due to limitation in space and food availability for a greater number of shrimps. Therefore, low dose of AMA can be used to produce larger shrimp where demand and prices are high for larger shrimp. High dose i.e. 2.5 g/kg diet would be suitable

for those who want to produce more but smaller shrimps. Same dose of another AMA was applied by Kracizy et. al. [19] who found improved growth in White shrimp. Applying lower stocking densities of shrimp might result in higher survival at low doses and enhanced growth at higher doses of AMA. However, more research is needed to prove these.

The inclusion of AMA in the diet had a clear effect on the feed conversion ratio (FCR). The FCR was reduced from 2.3 ± 0.8 in the control treatment to 1.3 ± 0.1 for the lowest dose of 1 g/kg and up to 1.2 ± 0.1 for the highest dose of 3 g/kg. Similar findings on growth factors have been reported in isolated studies of AMA components in farmed crustaceans. In Pacific white shrimp juveniles challenged by a contaminated diet with 150 μg of aflatoxin B1 (AFB1) per kg of feed, including treatments with the supplementation of bentonite ranging from 0, 2.5, and 5 g/kg; a significant ($P < 0.05$) increase in weight gain (WG) and survival rate was observed when the clay was added to the diets, the effect being greater at higher dosages [23]. Likewise, the effects on performance by the supplementation of a bentonite-based mycotoxin detoxifier at a 2.5 g/kg dose in *Litopenaeus vannamei* juveniles fed with diets including total aflatoxin levels of 1067.8 ng/kg and 1715.3 ng/kg of fumonisins (FB1+FB2) were evaluated. A reduction in total biomass and specific growth rate (SGR) was reported in both contaminated treatments in comparison to the control diet without mycotoxins, and the addition of the clay supplement resulted in a significant ($P < 0.05$) improvement in WG, SFR, and FCR [19].

In regards of turmeric extract, García-Pérez et al. [26] observed a significant increase ($P < 0.05$) in the feed intake and SGR in white shrimp juveniles fed a contaminated diet with 200 $\mu\text{g/kg}$ of total aflatoxins when turmeric extract (70% w/w curcumin) was included at a rate of 0.15 – 0.30 g/kg, achieving the maximum benefits against aflatoxin effects at a dosage of 0.20 g of curcumin per kg of finished feed. Similar response to curcumin supplementation (0.1 - 0.2 g/kg) was observed in Pacific white shrimp challenged by 500 $\mu\text{g/kg}$ of AFB1 for 56 days, with a significant ($P < 0.05$) increase in the final body weight and numerically lower mortality values [25]. Correspondingly, *L. vannamei* juveniles administered a control diet with the supplementation of 0, 0.075, 0.0150 and 0.300 g/kg of curcumin for 9 weeks, resulted in a significant ($P < 0.05$) augmentation of growth parameters, including WG, SGR, and FCR. Interestingly, the inclusion of 0.075 g/kg of curcumin achieved the greater performance improvement in comparison to the control diet without mycotoxins addition [28].

Similarly, the effects of other two components of the AMA; namely, silymarin, an extract of the seeds of the milk thistle, and lysolecithin from sunflower seeds, have also been studied in shrimp species. The inclusion of silymarin in shrimp feed in ranges from 0.5-1.0 g/kg, resulted in an enhancement in growth factors, immune response, antioxidant capacity, and gut morphology in Pacific white shrimp [38], and in 1.0-2.0 g/kg dosages it reduced inflammation and lipid peroxidation in the shrimp [39]. Likewise, *L. Vannamei* under low salinity stress were supplemented with silymarin at 0.1, 0.2, and 0.4 g/kg, which resulted in a significant ($P < 0.05$) improvement in WG, FCR, digestive enzymes activities, hepatopancreas integrity, and alleviated the oxidative damage in comparison to control diet (Huifeng et al., 2021). Regarding lysolecithin, the effects of dietary lysophospholipids in the growth performance and nutrient utilization, due to its emulsifying properties, have been reported in farmed crustaceans. In dosages of lysophospholipids ranging from 0.3-2 g/kg a significant ($P < 0.05$) increase in percentage weight gain (PWG), SGR, and FCR has been observed in Pacific white shrimp [39,40,48]. Interestingly, in tiger shrimp (*Penaeus Monodon*) the feeding of 2 g of lysolecithin per kg of diet for 42 days improved significantly SGR and daily growth coefficient [49].

Inactivated yeast products from *Saccharomyces cerevisiae* including yeast hydrolysate have been evaluated in Pacific white shrimp in terms of growth performance and feed utilization. An 8-week feeding trial in *L. Vannamei* with the supplementation of 10 g/kg of hydrolyzed yeast resulted in higher WG, SGR, and improved FCR [31]. Controversially, in a 56-day feeding trial with up to 30 g/kg of yeast hydrolysate, no significant improvement was observed in terms of growth. However, significant ($P < 0.05$) digestive enzyme activities were reported, indicating a better utilization of nutrients, including carbohydrates and lipids [41]. The reported data of the isolated materials support

the benefits provided by AMA in the growth performance of Pacific white shrimp observed in the present study.

Regarding the protein efficiency ratio, it was found to be 92% in control whereas up to 168% in the case of the highest dose of AMA. In the proximate analyses performed, the ameliorating effects of AMA were observed in the chemical composition of faeces, especially in decreased lipid content. The low lipid content in the faeces of the shrimp fed with the AMA meant that the additive might have enhanced lipid digestion and metabolism, which should have been used as a source of energy, sparing the protein, thereby improving growth and immune response [50]. This effect has been reported in other studies previously, specifically with the administration of lysophospholipids, which demonstrated enhanced lipid metabolism and, consequently, an improved performance in shrimp [39,40,48,49]. More importantly, 25% higher ash content in the faeces of the AMA supplemented groups in comparison to the control indicated that clays included in AMA to bind mycotoxins must have contributed while binding the mycotoxins in the digestive system [22–24]. AMA, especially the clay materials, does not get digested, being added to the ash content significantly as other nutrients get absorbed e.g. lipids. Moreover, the presence of indigestible clay and yeast products might have served like fiber, which supports the digestion of other nutrients [41].

Moreover, results of the bacterial challenge test showed that the AMA used in this research was able to protect the shrimp and delay the occurrence of mortality considerably. Mortality of shrimp started after 7 days in the case of animals that received AMA supplementation, whereas in the control treatment, it started from the 3rd day post-infection. In addition, the lowest dose of AMA delayed the same number of days as done by the medium and high doses. Similarly, all the shrimp died before the 9th day post-infection in the case of control group, while shrimp supplemented with AMA at all dosages tested were able to survive 3 additional days, up to 12 days post-infection. These results clearly indicated that the AMA which contains selected clay adsorbents, curcumin from turmeric extract, silymarin from milk thistle extract, lysophospholipids from sunflower lysolchitin, and inactivated yeast components, presents a significant benefit in the immunity of shrimp, improving the resistance to bacterial infections. Since mycotoxins main adverse effects include the impairment of the immune function in shrimp, the use of dietary strategies that mitigate these toxins by adsorption mechanisms complemented with active substances for the improvement of the overall health status may be able to counteract the higher induced sensitivity to infectious diseases [51–53].

The use of dietary mycotoxin detoxifier bentonite at 2.5 - 5 g/kg, demonstrated a significant ($P < 0.05$) increase in *L. Vannamei* survival from acute hepatopancreatic necrosis bacterial disease produced by pathogenic strains of *Vibrio parahaemolyticus* [54]. Similarly, the expression of whiteleg shrimp immunity-related genes improved through the supplementation of magnesium aluminosilicate clays at a range of 10 - 40 g/kg [55]. In stinging catfish under a bacterial challenge with *Aeromonas hydrophila*, sodium bentonite was administered at 5 and 10 g per kg of feed, which resulted in statistically ($P < 0.05$) enhanced complement, respiratory burst and lysozyme activities, consequently, lowering cumulative mortality (Jawahar et al., 2018). In addition, the curcumin mitigates the oxidative stress caused by mycotoxins and other stressors, consequently improving the innate immune function [25–29]. Other phytochemicals such as silymarin and lysolchitin combined with post-biotic inactivated yeast products present bio-protective capacities, including antioxidant and anti-inflammatory properties, thus improving the resilience of the shrimp to infectious diseases and other environmental or nutritional stressors [29–31,47,48]. For instance, in shrimp fed with isonitrogenous and isolipidic diets supplemented with 10 g /kg of yeast hydrolysate challenged by ammonia nitrogen stress, inflammation-related genes *TNF- α* and *IL-1 β* were reduced in the intestine, and the expression level of immune-related genes (*dorsal*, *relish*, and *proPO*) was augmented in comparison to the control diet (Jin et al., 2018). Likewise, Neto and Nunes (2015) observed significantly ($P < 0.05$) enhanced survival of *L. Vannamei* juveniles orally exposed to infectious myonecrosis virus (IMNV) when fed with 1 g/kg of β -glucans from yeast cell wall. However, what combinations of plant and yeast extracts with adsorbent materials would be more effective is not

known. More research may be conducted to determine the doses of AMA for varying levels of mycotoxin contamination and farmed aquatic species.

5. Conclusions

The commercial anti-mycotoxin additive intended for aquatic species tested in this study showed positive impacts on growth, survival, feed conversion and protein efficiency in the pacific white shrimp. A quadratic relationship on the shrimp survival and growth especially in length. Higher the dose better is the immunity as indicated by the survival of shrimp against bacterial challenge. However, the quadratic models indicated that at the dose of 2.5 kg/ton of feed results in the highest survival whereas the dose of 1.4 g/kg of diet had the highest daily weight gain of shrimp. Therefore, doses between 1.4 and 2.5 g/kg of diet are recommended for the grow-out phase of shrimp. Further studies should be done using longer period experiment in different shrimp culture systems at different stocking densities with different feeds or feeding regimes with varying levels of contamination, and for the diets of other commercial aquaculture species such tilapia, pangasius, sea bass, freshwater prawn, hybrid catfish, carps and others.

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Data Availability Statement: Data are available upon request.

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Conflicts of Interest: Authors declare that there is no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AIT	Asian Institute of Technology
AMA	Anti-mycotoxin agent
ANOVA	Analysis of variance
CP	Crude protein
CRD	Complete randomized design
DO	Dissolved oxygen
DWG	Daily weight gain
FCR	Feed conversion ratio
HPLC	High pressure liquid chromatography
HSI	Hepato-somatic index
LD ₅₀	Lethal dose at which 50% animal die
Ln	Natural log
mL	Mili-litre
PER	Protein efficiency ratio
PL	Post-larvae
pH	Potential of hydrogen ion concentration
RAS	Recirculatory aquaculture system
SGR	Specific growth rate

References

1. Bhujel, R.C. Global Aquatic Food Production. P. 99-126. In Aquatic Food Security. Crumlish, M. & Norman, R. (Eds). 5M-Books.2024, <https://5mbooks.com/product/aquatic-food-security>.
2. FAO. The State of World Fisheries and Aquaculture. Blue Transformation in Action. Rome, Italy, 2022,.. <https://doi.org/10.4060/cd0683en>
3. SKYQUEST. <https://www.skyquestt.com/report/shrimp-market/market-size> (retrieved on May 31, 2025).
4. FAO Globefish. Quarterly Shrimp analysis - February 2025. <https://openknowledge.fao.org/> (retrieved on May 31, 2025).

5. Rairat, T., Kitsanayanyong, L., Keetanon, A., Phansawat, P., Wimanhaemin, P., Natnicha, C., Suanploy, W., Chow, P.Y.E. & Chuchird, N). Effects of monoglycerides of short and medium chain fatty acids and cinnamaldehyde blend on the growth, survival, immune responses, and tolerance to hypoxic stress of Pacific white shrimp (*Litopenaeus vannamei*). *PLOS ONE*. **2024**, 19, 0308559.<https://doi.org/10.1371/journal.pone.0308559>.
6. Eskola, M., Kos, G., Elliott, C.T., Sultan Mayar, J.H., Krska, R.) Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate' of 25%. *Critical Reviews in Food Science and Nutrition*. **2020**. 60 (16): 2773-2789. <https://doi.org/10.1080/10408398.2019.1658570>
7. Alshannaq, A., & Yu, J. H. (2017). Occurrence, toxicity, and analysis of major mycotoxins in food. *International journal of environmental research and public health*. **2017**.14(6), 632.
8. Bhujel, R. C. Mycotoxins in feed – an underestimated challenge for the aquafeed industry. *Mycotoxinsite.com*. **2021**,<https://mycotoxinsite.com/mycotoxins-feed-underestimated-challenge-aquafeed-industry/?lang=en&fbclid=IwAR1LawrpbuSoMu8eFRYgTeer-gOWTx9WTLJgTlZXkeZV7HrnqFF9k7jeIos>
9. El-Sayed, R.A., Jebur A.B., Kang, W. El-Demerdash, F.M. An overview on the major mycotoxins in food products: characteristics, toxicity, and analysis. *Journal of Future Foods*. **2022**, 2(2): 91-102. <https://doi.org/10.1016/j.jfutfo.2022.03.002>
10. Khan, R., Anwar, F., Farinazleen Mohamad Ghazali, F. A comprehensive review of mycotoxins: Toxicology, detection, and effective mitigation approaches. *Heliyon*. **2024**, 10(8), e28361, <https://doi.org/10.1016/j.heliyon.2024.e28361>
11. Anater, A., Manyes, L., Meca, G., Ferrer, E., Luciano, F.B., Pimpão, C.T., Font, G. (2016). Mycotoxins and their consequences in aquaculture: A review. *Aquaculture*, 451, pp. 1-10. DOI: 10.1016/j.aquaculture.2015.08.022.
12. Oliveira and Vasconcelos... Occurrence of mycotoxins in fish feed and its effects: A review. *Toxins*. **2020**, 12 (3), art. no. 160. DOI: 10.3390/toxins12030160.
13. Gonçalves, R.A., Schatzmayr, D., Hofstetter, U., Santos, G.A. Occurrence of mycotoxins in aquaculture: Preliminary overview of Asian and European plant ingredients and finished feeds. *World Mycotoxin Journal*. **2017**, 10 (2), pp. 183-194. DOI: 10.3920/WMJ2016.2111.
14. Gonçalves, R.A., Naehrer, K., Santos, G.A. Occurrence of mycotoxins in commercial aquafeeds in Asia and Europe: a real risk to aquaculture? *Reviews in Aquaculture*. **2018**, 10 (2), pp. 263-280. DOI: 10.1111/raq.12159.
15. Rokvic, N., Aksentijevic, K., Kureljuic, J., Vasiljevic, M., Todorovic, N., Zdravkovic, N., Stojanac, N. (2020). Occurrence and transfer of mycotoxins from ingredients to fish feed and fish meat of common carp (*Cyprinus carpio*) in Serbia. *World Mycotoxin Journal*. **2020**, 13 (4), 545-552. DOI: 10.3920/WMJ2020.2580.
16. European Food Safety Authority (EFSA), (2010). Statement on the establishment of guidelines for the assessment of additives from the functional group 'substances for reduction of the contamination of feed by mycotoxins. *EFSA Journal*. **2010**,8(7): 1-8.
17. European Food Safety Authority (EFSA).Guidance on the assessment of the efficacy of feed additives. *EFSA Journal*. **2018**,16(5):52-74. doi: 10.2903/j.efsa.2018.5274
18. Bintvihok, A., Ponpornpisit, A., Tangtrongpiros, J., Panichkriangkrai, W., Rattanapanee, R., Doi, K. . Aflatoxin contamination in shrimp feed and effects of aflatoxin addition to feed on shrimp production. *Journal Food Protein*. **2003**, 66, 882–885.
19. Kracizy, R.O, Brazao, C.C., Viott, A.D.M., Ribeiro, K., Koppenol, A., dos Santos, A.M.V., Ballester, E.L.C.M. Evaluation of aflatoxin and fumonisin in the diet of Pacific White Shrimp (*Litopenaeus vannamei*) on their performance and health. *Aquaculture*. **2021**, 544: 737051.
20. Bhujel, R.C., Perera, A.D., Todorović, N., Raj, J., Gonçalves, R.A. and Vasiljević, M. Evaluation of an Organically Modified Clinoptilolite (OMC) and a Multi-Component Mycotoxin Detoxifying Agent (MMDA) on Survival, Growth, Feed Utilization and Disease Resistance of Nile Tilapia (*Oreochromis niloticus*) Fingerlings Fed with Low Aflatoxin. *Aquac. J*. **2023**, 3, 56–69. <https://doi.org/10.3390/aquacj3010007>.
21. Imani, A., Salimi Bani, M., Noori, F., Farzaneh, M., Moghanlou, K.S. The effect of bentonite and yeast cell wall along with cinnamon oil on aflatoxicosis in rainbow trout (*Oncorhynchus mykiss*): Digestive enzymes,

- growth indices, nutritional performance and proximate body composition. *Aquaculture*. **2017**, 476,160-167. DOI: 10.1016/j.aquaculture.2017.04.023.
22. Hussain, D. Effect of aflatoxins in aquaculture: Use of bentonite clays as promising remedy. *Turkish Journal of Fisheries and Aquatic Sciences*. **2018**, 18 (8),1009-1016. DOI: 10.4194/1303-2712-v18_8_10.
 23. Neeratanaphan, L., and Tengjaroenkul, B., (2018). Potential of Thai bentonite to ameliorate aflatoxin B1 contaminated in the diet of the Pacific white shrimp (*Litopenaeus vannamei*). *Livestock Research for Rural Development*, 30 (10).
 24. Philips, T.D., Wang, M., Elmore, S.E., Hearon, S., Wang, J.-S. NovaSil Clay for the Protection of Humans and Animals from Aflatoxins and Other Contaminants. *Clays and Clay Minerals*. **2019**, 67 (1), 99-110. DOI: 10.1007/s42860-019-0008-x.
 25. Yu, Y.-Y.; Niu, J.; Yin, P.; Mei, X.-T.; Liu, Y.-J.; Tian, L.-X.; Xu, D.-H. Detoxification and Immuno-protection of Zn (II)- Curcumin in Juvenile Pacific White Shrimp (*Litopenaeus vannamei*) Feed with Aflatoxin B1. *Fish Shellfish Immunol*. **2018**, 80, 480–486.
 26. García-Pérez, O.D., Tapia-Salazar, M., Nieto-López, M.G., Cruz-Valdez, J.C., Maribel Maldonado-Muñiz, M., Guerrero-Guerrero, L.M., Marroquín-Cardona, A.G.. Effects of conjugated linoleic acid and curcumin on growth performance and oxidative stress enzymes in juvenile Pacific white shrimp (*Litopenaeus vannamei*) feed with aflatoxins. *Aquaculture Research*. **2020**, 51,1051-1060. DOI: 10.1111/are.14451.
 27. Bhoopathy, S., Inbakandan, D (Inba), Rajendran, T., Chandrasekaran, K., Kasilingam, R., Gopal, D. Curcumin loaded chitosan nanoparticles fortify shrimp feed pellets with enhanced antioxidant activity. *Materials Science & Engineering C*.2021, 120, 111737.
 28. Moghadam, H., Sourinejad, I., and Johari, S.A. Growth performance, haemato-immunological responses and antioxidant status of Pacific white shrimp *Penaeus vannamei* fed with turmeric powder, curcumin and curcumin nanomicelles. *Aquaculture Nutrition*. **2021**, 27: 2294–2306. DOI: 10.1111/anu.13363.
 29. Quesada-Vázquez, S.; Codina Moreno, R.; Della Badia, A.; Castro, O.; Riahi, I. Promising Phyto-genic Feed Additives Used as Anti-Mycotoxin Solutions in Animal Nutrition. *Toxins*. **2024**, 16, 434. <https://doi.org/10.3390/toxins16100434>.
 30. Neto, H.S. and Nunes, A.J.P.. Performance and immunological resistance of *Litopenaeus vannamei* fed a β -1,3/1,6-glucan-supplemented diet after per os challenge with the Infectious myonecrosis virus (IMNV). *R. Bras. Zootec*. **2015**, 44(5),165-173.
 31. Jin, M., Xiong, J., Qi-Cun, Z, Yuan Y., Xue-Xi, W., Sun, P. (2018). Dietary yeast hydrolysate and brewer's yeast supplementation could T enhance growth performance, innate immunity capacity and ammonia nitrogen stress resistance ability of Pacific white shrimp (*Litopenaeus vannamei*). *Fish and Shellfish Immunology*. **2018**, 82 () 121–129. <https://doi.org/10.1016/j.fsi.2018.08.020>.
 32. AOAC. Official Methods of Analysis of Association of Official Analytical Chemists, 15th Edition, AOAC, Arlington, VA, 1298. (1990).
 33. Hung, L.T. and Quy, O.M. On farm feeding and feed management in White leg shrimp (*Litopenaeus vannamei*) farming in Viet Nam. In M.R. Hasan and M.B. New, eds. On-farm feeding and feed management in aquaculture. FAO Fisheries and Aquaculture Technical Paper No. 583. Rome, FAO. pp. 337–357. (2013).
 34. Antunes, C.R.N., da Silva Ledo, C.A., Pereira, C.M, Dos Santos, J. (2018). Evaluation of feeding rates in the production of *Litopenaeus vannamei* shrimp using artificial substrates. *Ciência Animal Brasileira*. **2018**, 19. 10.1590/1809-6891v19e-50805.
 35. Effendi, H., Kawaroe, M., Lestari, D. F., Mursalin, & Permadi, T. Distribution of Phytoplankton Diversity and Abundance in Mahakam Delta, East Kalimantan. *Procedia Environmental Sciences*. **2016**, 33, 496–504. <https://doi.org/10.1016/j.proenv.2016.03.102>
 36. Boyd, C.E., and Tucker, C.S. *Water Quality and Pond Soil Analysis for Aquaculture*. Alabama Agricultural Experiment Station, Auburn University publishers, USA. 1992. ISBN: 9780817307219.
 37. Maciel C.R., New M. & Valenti W.C. The Predation of *Artemia nauplii* by the larvae of the Amazon River prawn *Macrobrachium amazonicum* (Heller, 1862) is affected by prey density, time of day, and ontogenetic development. *Journal of the World Aquaculture Society*. **2012**, 43 (5), 659–669.
 38. Hasanthi, M., Jo, S., Han-se K., Kwan-Sik, Y., Lee, Y., Lee, K-J. Dietary supplementation of micelle silymarin enhances the antioxidant status, innate immunity, growth performance, resistance against *Vibrio*

- parahaemolyticus infection, and gut morphology in Pacific white shrimp (*Litopenaeus vannamei*). *Animal Feed Science and Technology*. **2024**, 311,115953. <https://doi.org/10.1016/j.anifeedsci.2024.115953>.
39. Song Z., Liu, H., Liu, Y., Ye, Z., Ma, Q., Wei, Y., Xiao, L., Liang, M., and Xu, H. Effects of the Supplementation of Lysophospholipid in Low-Lipid Diets on Juvenile Pacific White Shrimp. *Aquaculture Research*. **2024**, 11. DOI: 10.1155/2024/9594116
 40. Limwachirakhom R., Triwutanon S., Zhang Y., and Jintasataporn O. Effects of dietary lysophospholipids on the performance of Pacific white shrimp (*Litopenaeus vannamei*) fed fish oil- and energy-reduced diets. *Front. Mar. Sci*. **2025**, 12:1624057. DOI: 10.3389/fmars.2025.1624057.
 41. Yang, X., Chi, S., Tan, B., Nie, Q., Dong, X., Yang, Q., Liu, H. and Zhang, S. Yeast hydrolysate helping the complex plant proteins to improve the growth performance and feed utilization of *Litopenaeus vannamei*. *Aquaculture Reports*. **2020**, 17, 100375. DOI: <https://doi.org/10.1016/j.aqrep.2020.100375>
 42. Yiannikouris, A., Andre, G., Poughon, L., François, J., Dussap, C.G., Jeminet, G., Bertin, G., and Jouany, J.P. Chemical and Conformational Study of the Interactions Involved in Mycotoxin Complexation with β -D-Glucans. *Biomacromolecules*. **2006**, 7, 1147-1155. DOI: 10.1021/bm050968t
 43. Xu, R., Yiannikouris, A., Shandilya, U.K., and Karrow, N.A. Comparative Assessment of Different Yeast Cell Wall-Based Mycotoxin Adsorbents Using a Model- and Bioassay-Based In Vitro Approach. *Toxins*. **2023**, 15, 104. DOI: 10.3390/toxins15020104
 44. Sharma, N. Exploring the potential of mannan oligosaccharides in enhancing animal growth, immunity, and overall health: A review. *Carbohydrate Polymer Technologies and Applications*. **2025**, 9, 100603. DOI: 10.1016/j.carpta.2024.100603
 45. Jach, M.E., Serefko, A., Ziaja, M., and Kieliszek, M. Yeast Protein as an Easily Accessible Food Source. *Metabolites*. **2022**, 12, 63. DOI: 10.3390/metabo12010063
 46. Rahimnejad, S., Leclercq, E., Malinovskiy, O., Penka, A., Kolárová, J., and Policar, A. (2023). Effects of yeast hydrolysate supplementation in low-fish meal diets for pikeperch. *Animal The international journal of animal biosciences*. **2023**, 17, 100870. DOI: 10.1016/j.animal.2023.100870
 47. Huifeng L., Erchao, L., Chang, X., Li, Z. and Liqiao, C. Effects of silymarin on growth, activities of immune-related enzymes, hepatopancreas histology and intestinal microbiota of the Pacific white shrimp (*Litopenaeus vannamei*) at low salinity. *Journal of Fisheries of China*. **2021**, 45 (1), 98-114. DOI: 10.11964/jfc.20200612291
 48. Wang, J., Peng, H., Jin, M., Li, M., He, Y., Li, S., Zhu, T., Zhang, Y., Tang, F., and Zhou, Q. Dietary lysophospholipids supplementation promotes growth performance, enhanced antioxidant capacity, and improved lipid metabolism of *Litopenaeus vannamei*. *Aquaculture Reports*. **2024**, 39, 102476. DOI: 10.1016/j.aqrep.2024.102476
 49. Khan, H. I., Madhubabu, E. P., Jannathulla, R., and Ambasankar, K. Effect of partial replacement of marine protein and oil sources in presence of lyso-lecithin in the diet of tiger shrimp *Penaeus monodon* Fabricius, 1978. *Indian Journal of Fisheries*. **2018**, 65(2), 100-107. DOI: 10.21077/ijf.2018.65.2.74154-12.
 50. Chyng-Hwa, L., Vu-An, T., Zhong-Fu, Z., Yu-Hung L. The effect of dietary lecithin and lipid levels on the growth performance, body composition, hemolymph parameters, immune responses, body texture, and gene expression of juvenile white shrimp (*Litopenaeus vannamei*). *Aquaculture*. **2023**, 567 739260. <https://doi.org/10.1016/j.aquaculture.2023.739260>.
 51. Santacroce, M. P., Conversano, M. C., Casalino, E., Lai, O., Zizzadoro, C., Centoducati, G., and Crescenzo, G. Aflatoxins in aquatic species: metabolism, toxicity and perspectives. *Reviews in Fish Biology and Fisheries*. **2007**, 17, 107–120. DOI: 10.1007/s11160-007-9064-8
 52. Mexía-Salazar, A. L., Hernández-López, J., Burgos-Hernández, A., Cortez-Rocha, M. O., Castro-Longoria, R., and Ezquerro-Brauer, J. M. Role of fumonisin B1 on the immune system, histopathology, and muscle proteins of white shrimp (*Litopenaeus vannamei*). *Food Chemistry*. **2008**, 110(2), 471–479.
 53. Sun, Y.; Song, Y.; Long, M.; and Yang, S. Immunotoxicity of Three Environmental Mycotoxins and Their Risks of Increasing Pathogen Infections. *Toxins*. **2023**, 15, 187. DOI: 10.3390/toxins15030187
 54. Ng, W.-K., Mong, M.-L., and Abdul-Hamid, A.-A. . Dietary montmorillonite clay improved *Penaeus vannamei* survival from acute hepatopancratic necrosis disease and modulated stomach microbiota. *Animal Feed Science and Technology*. **2023**, 297, 115581. DOI: 10.1016/j.anifeedsci.2023.115581

55. Pourjam, F., Sotoudeh, E., Bagheri, D., Ghasemi, A., and Esmaeili, N. Dietary montmorillonite improves growth, chemical composition, antioxidant system and immune-related genes of whiteleg shrimp (*Litopenaeus vannamei*). *Aquaculture International*. **2024**, 32, 5699–5717. DOI: 10.1007/s10499-024-01442-2

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