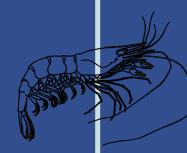


Pondguard as an Essential Oil Blend Supplement to Reduce the Disease Risk and Increase the Productivity of Aquatic Animals





PONDGUARD INTRODUCTION



FIELD OBSERVATION - Shrimp



FIELD OBSERVATION - Fish



LABORATORY REPORT



CONCLUSION

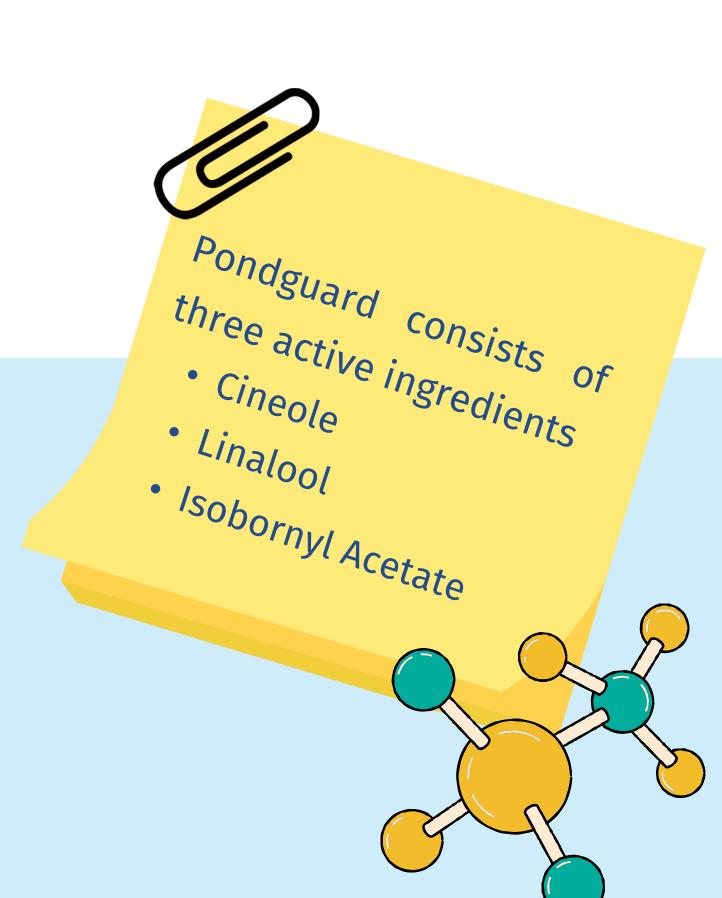




INTRODUCTION



- Pondguard helps in overall improvement in the quality and productivity.
- It maintains the general health of shrimp and fish by improving the immune system.
- It works directly against specific viral pathogens like WSSV,IMNV, TiLV, Iridovirus and KHV.
- It works directly against specific bacterial and other pathogens like Vibrio harveyi, V. campelii, V. parahaemolyticus-AHPND-EMS, Aeromonas, Streptococcus, and EHP

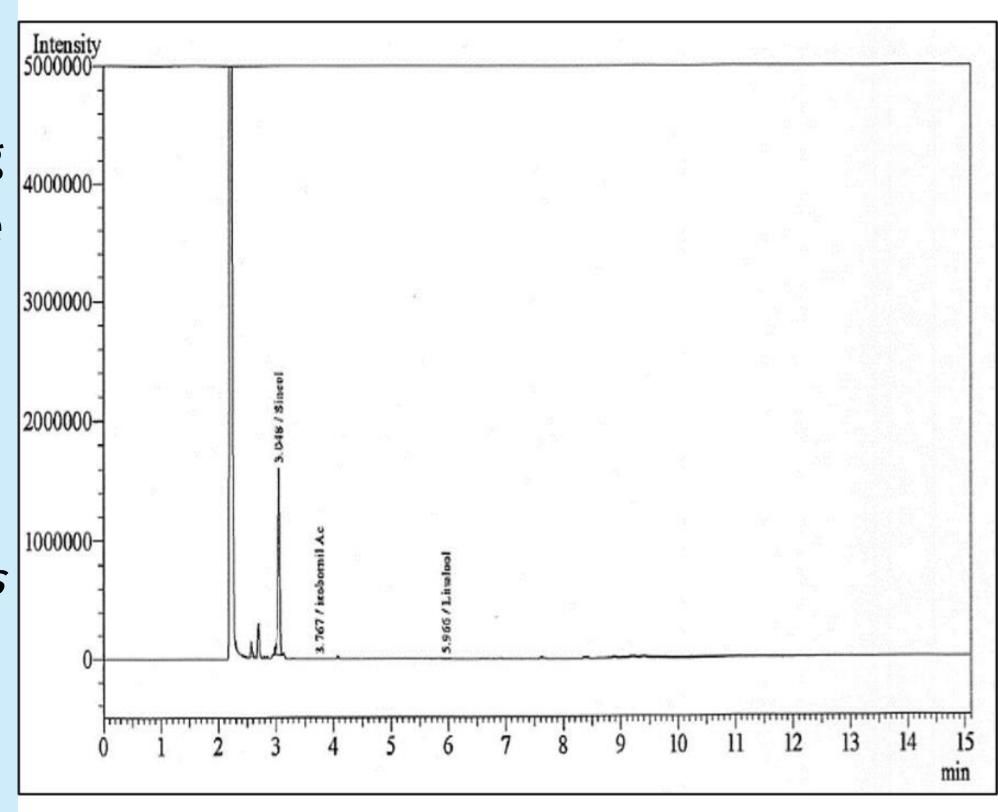


PONDGUARD FORMULATION



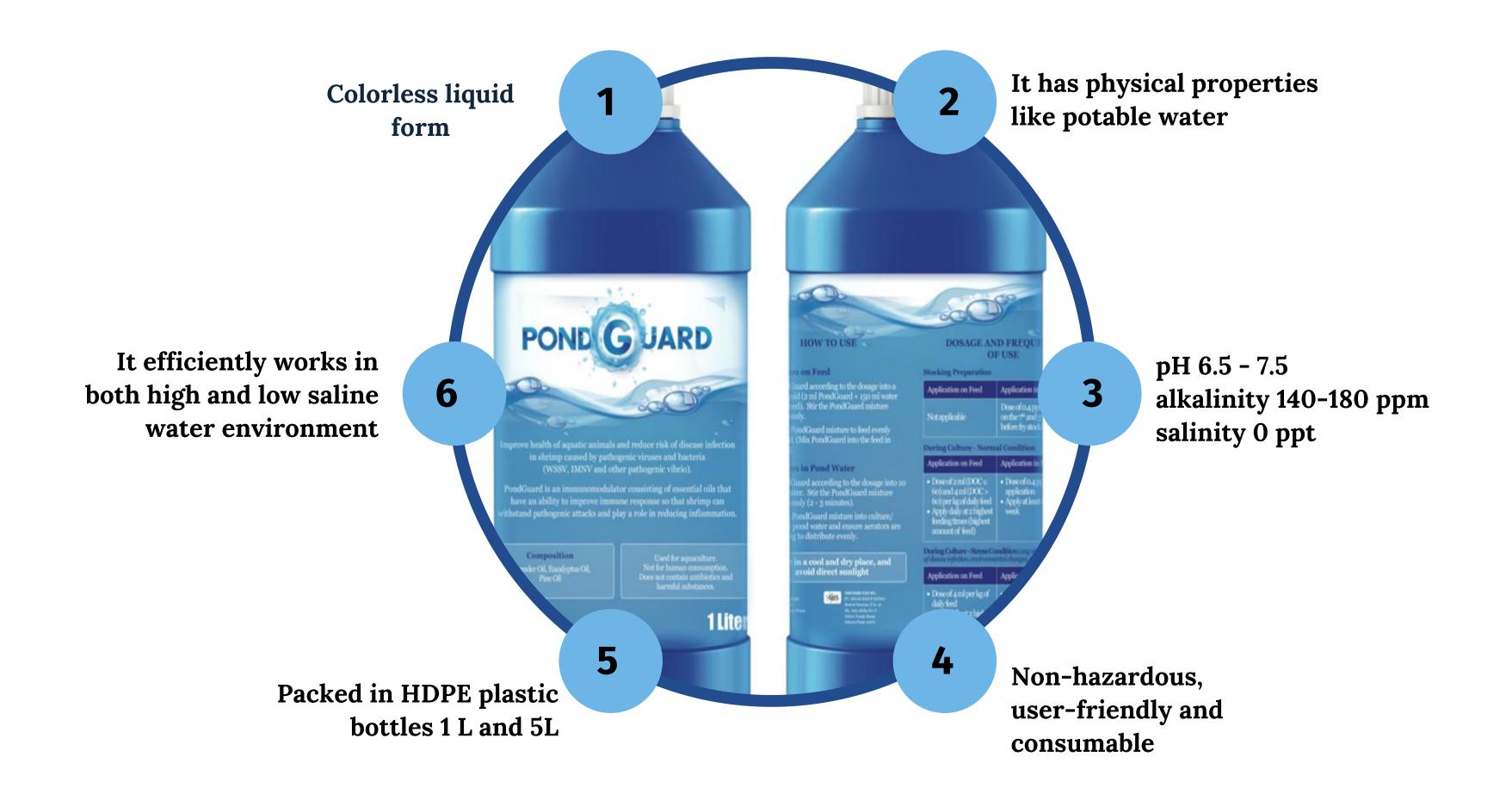
The selection of blend oils done based on anti-viral properties, immunomodulating properties and their compatibilities. The targeted active ingredients are as follows:

- Cineole extracted from *Eucalyptus* globulus,
- Isobornyl acetate extracted from *Pinus* sylvestris, and
- Linalool extracted from *Lavandula latifolia*



Chromatogram of complete NOBF compound's peak i.e. Cineole, Linalool and Isobornyl acetate





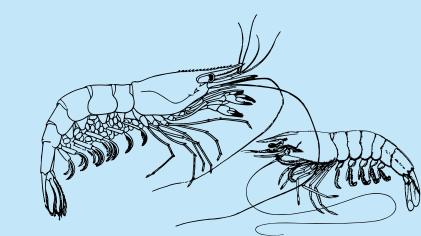
RECOMMENDED DOSE OF PONDGUARD





		PON	DGUARD A	APPLIED IN	WATER	PONDGUARD MIXED WITH THE FEED		
	Before PL Stocking		During Culture		Emergency cases	During Culture		Emergency cases
Parameters of Pondguard Application	Day -7	Day -3	DOC 1 - DOC 40	DOC 40 - harvest	High Vibrio load, sudden drop in DO, sudden increase in afternoon pH, and appearance of diseses like, WSSV, EMS, heavy moulting, etc.	DOC 1 - DOC 60	DOC 60 - harvest	High Vibrio load, sudden drop in DO, sudden increase in afternoon pH, and appearance of diseses like, WSSV, EMS, heavy moulting, etc.
Dose	0.4 ppm	0.4 ppm	0.4 ppm	0.4 ppm	0.4 ppm	2 ml/kg feed	4 ml/kg feed	8 - 10 ml/ kg feed
Frequency	Once	Once	Daily	Two times a week	Two times a day for 5 days or till mortality stops.	Two times a day at the higher	Two times a day at the higher feeding rate	Two times a day at the higher feeding rate

- Dose 0.4 ppm = 4 L for 1 Ha area and 1 m depth of water,
- The dose must be adjusted according to depth and area of pond.



PREPARATION and DILUTION METHOD

Mix recommended dose of Pondguard in 10 liter of clean water.

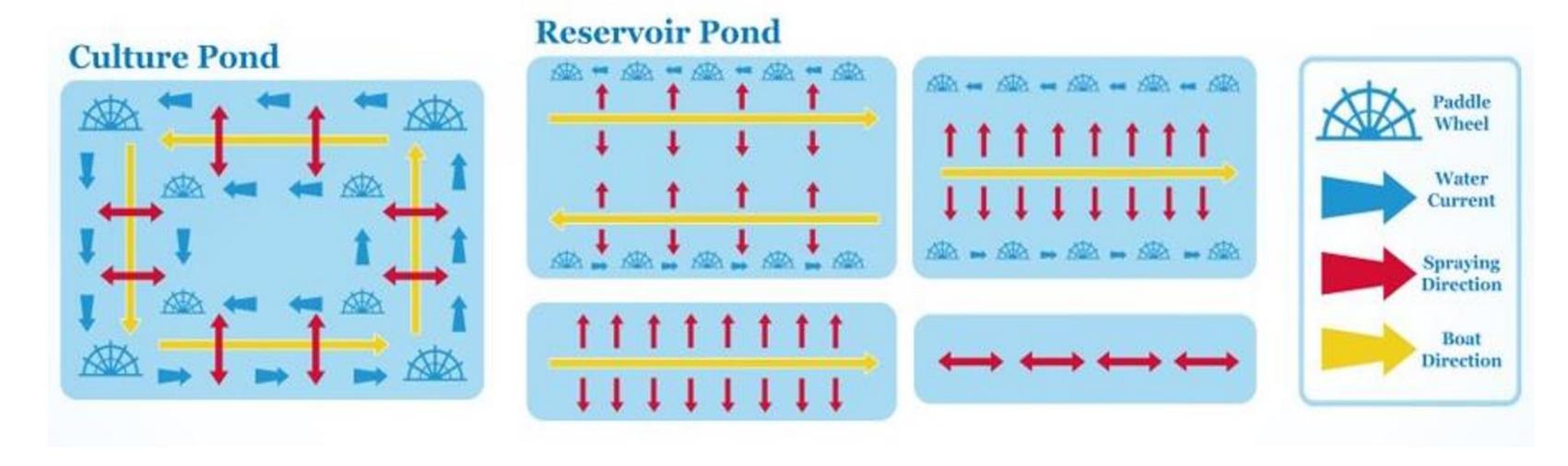
Mix well for 2 to 3 minutes.

Apply over pond in maximum current area.
Paddle wheel should be kept running for at least 1 hour, for homogenous and rapid mixing.



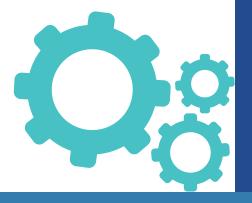
APPLICATION METHOD





Time of application: Suitable time of application 9.00 to 16.00 hrs.

Storage and handling: stored at room temperature (25° - 32° C), keep in the shade.



FIELD OBSERVATION



- Multiple pathogens and factors disrupted shrimp farming in Indonesia.
- That encouraged CPP-RNS to develop the new normal Standard Operational Procedure (SOP) to overcome them.
- The New Normal SOP helped to improve the productivity and decrease disease incidence.
- The role of Pondguard was very critical in the success of the New Standard Operational Procedure (SOP).
- The outcome is explained in the following slides



Shrimp Culture Using Pondguard

Vannamei & Monodon



SHRIMP CULTURE SOP in INDONESIA

Description	New Normal SOP	Old SOP	
	Sedimentation pond: 8%	Sedimentation pond: 8%	
Lavout	Treatment pond : 17%	Treatment pond : 22%	
Layout	Recondition pond : 8%	Culture pond : 70%	
	Culture pond : 67%		
Stocking Density	Density 120 pcs/m2	Density 134 pcs/m2	
Water Exchange/cycle	900%	800%	
Pond bottom cleaning	Started from the 2 nd week of culture	Started from 2 nd month of culture	
Disease Surveillance	Tighten	Relax	
DO level (dissolved oxygen)	> 4.5 ppm	> 4 ppm	
Culture system	Balancing on the number of plankton and bacteria (keeping the plankton number stable).	Balancing on the number of plankton and bacteria	



SHRIMP CULTURE SOP in INDONESIA

Description	New Normal SOP	Old SOP
Pondguard	Treated with Pondguard	No Pondguard treatment
Pond Preparation	Use lime CaO around 1.5 - 2 Ton/Ha (to deactivate EHP Spore)	Use lime CaO around 1 Ton/Ha (to deactivate EHP Spore).
Biosecurity - PCR Based Monitoring	RT-PCR	Nested PCR
	Frequency of monitoring: two times a week	Frequency of monitoring : once a week
TOM and Vibrio load monitoring	TOM less than 100 ppm	TOM less than 100 ppm
TOW and VIDIO load monitoring	Green vibrio less than 100 cfu/ml	Green vibrio less than 100 cfu/ml
	Yellow vibrio less than 1000 cfu/ml	Yellow vibrio less than 1000 cfu/ml





SUMMARY OF TRIALS AND OBSERVATION







Beneficial Properties	Test Methodology	Results
Immunomodulatory	In Vivo (CPP)	Enhance the hemocyte count and maintain at the optimum level.
Anti-WSSV	In Vitro & In vivo (CPP)	Effective, 100% deactivation and no infection recorded in Pondguard supplemented shrimp.
Anti-IMNV	In Vitro & In vivo (CPP)	Effective, 100% deactivation and no infection recorded in Pondguard supplemented shrimp.
Anti-AHPND/EMS	In Vitro & In vivo (University of Can Tho, Vietnam)	Effective, 100% deactivation and no infection recorded in Pondguard supplemented shrimp.
Anti-Vibrio	In vivo (CeRaf, Vietnam)	Pathogenic Vibrio load reduce to 0% in 24 hrs of application.
Growth performance in Vannamei	In Vitro (CPP) & Pond level in Indonesia, India, Saudi, China, Vietnam	Enhance the survival and productivity and lowers the FCR
Growth performance in Monodon	Pond trial - India	
Growth performance in Tilapia	In Vitro & Pond, Cage & Tank level	Enhance the survival and productivity and lowers the FCR
Biofloc technique in Tilapia	In vivo (CPP)	Enhance the survival and productivity
Grass carp growth performance	In Vitro & In vivo (SOU, China)	Enhance the growth rate, reduce the FCR, higher productivity

VANNAMEI POND PERFOMANCE in INDONESIA

Culture Performance "Technical Partner" CP Prima

Description	2019	2020	2021
Description	2013	2020	Q1
Number of Pond (pcs)	859	1,054	105
Area (ha)	277	328	31
Stocking Density (pcs/m²)	130	126	115
Avarage Day of Culture (DOC) (days)	82	85	88
Harvesting Size (pieces/kg)	61	58	52
Mean Body Weight (MBW) (gr)	17	17	19
Average Daily Weight Gain (gram/day)	0.201	0.204	0.218
Feed Conversion Ratio (FCR)	1.26	1.29	1.24
Survival Rate (SR) (%)	89	82	89
Productivity (Ton/ha)	19	18	20

- There is an increase in productivity from 18 tons per hectare to 20 tons per ha despite reducing stocking density.
- The day of culture (DOC) increased, which showed better health conditions of shrimp.
- The survival rate of shrimp increased to 89 % as compared to 82 % in 2020.
- The FCR reduced to 1.23 in 2019 as compared to 1.29 in 2020.



PONDGUARD OVERSEAS PERFOMANCE in VANNAMEI PONDS

Parameters	Saudi	India	China	Vietnam
Area (ha)	100	80	10	15
Stocking Density (pcs/m²)	35	35 - 40	600	120-180
Survival Rate (SR) (%)	10% higher using Pondguard	15% higher using Pondguard	ON GOING	ON GOING
Disease	Effective against WSSV and Low survival rate	Effective against WSSV and IMNV	EMS and WSSV target. Started trial in late 2021	Effective against WSSV and EMS. SR 5-10% higher using Pondguard
Pondguard Uses	Water and Feed	Water and Feed	Water and Feed	Water and Feed

*REMARKS

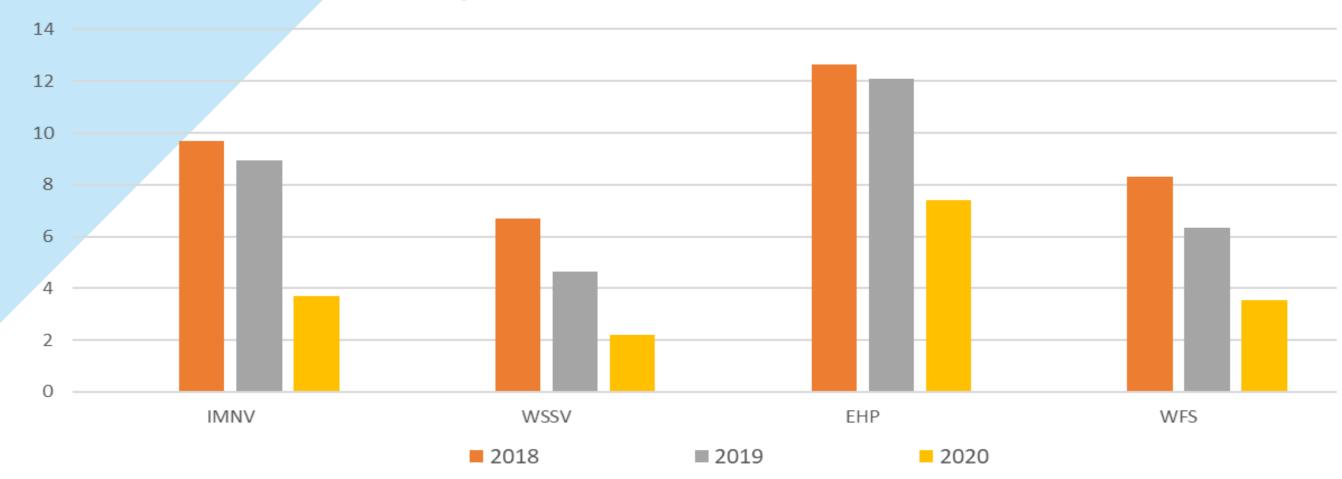
Our main target are:

- 1. To Reduce EMS incidence in Vietnam
- 2. To Reduce the number of WSSV, IMNV and EHP cases in India
- 3. To Reduce the WSSV incidence Saudi

FIELD OBSERVATION







Diseases	2018	2019	2020
IMNV	9.7	8.95	3.7
WSSV	6.7	4.65	2.2
EHP	12.635	12.1	7.4
WFS	8.3	6.35	3.55

- There is a decrease in all the pathogen monitored in 2020 and 2021 as compared to 2019 and 2018.
- There is a reduction in IMNV incidence from 8.95 % to 3.7 % and 4.8 %.
- There is a reduction in WSSV incidence from 6.7 % to 2.2 % and 3.6 %.
- There is a reduction in EHP incidence from 12. 6 % to 7.4 % and 4.5 %.
- There is a reduction in White Feces Syndrome incidence from 8.3 % to 3.5 % and 5.2 %.

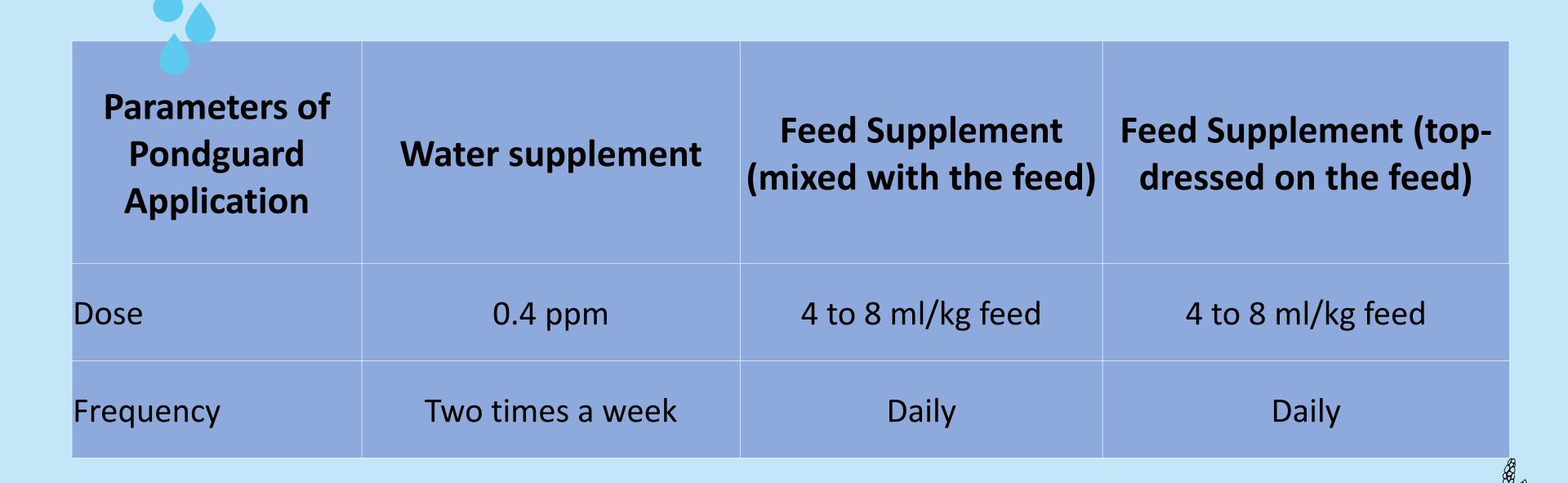


Fish Culture Using Pondguard

Tilapia, Common Carp, Seabass, Seabream, Grouper & Yellow Croaker



RECOMMENDED DOSE FOR FISH





PONDGUARD PERFOMANCE ON TILAPIA FISH (TANK TRIAL)

Group	MBW (gr)	ADG (gr/day)	Biomass (gr)	Feed Intake (gr)	FCR	SR (%)	Productivity (Ton/Ha)
Normal CP Feed	104.4a	1.621a	3,132a	2,743a	1.34a	96.7%a	62.64
PG Feed *	110.9a	1.790a	3,251a	3,183b	1.44b	98.8%a	65.02

*The PG Feed provide better performance than normal feed, but statistically not significantly different.

Group	MBW (gr)	ADG (gr/day)	Biomass (gr)	Feed Intake (gr)	FCR	SR (%)	Productivity (Ton/Ha)
Normal CP Feed	104.4a	1.621a	3,132a	2,743a	1.34a	96.7%a	62.64
PG-top dressed Feed*	114.2a	1.857a	3,427a	3,335b	1.43b	100%a	68.54

*The PG-top dressed Feed provide better performance than normal feed, but statistically not significantly different.

^{*}PG Feed was produced from normal CP feed mixed with Pondguard (4L/ton feed) in the feedmill

^{*}PG-top dressed feed was made from normal CP feed top-dressed with Pondguard (4 ml/kg feed) in the field.

PONDGUARD PERFOMANCE ON TILAPIA FISH (CAGE TRIAL)

Danfannanaa	Treat	Dugluo	
Performance	Normal CP Feed	PG Feed	P-value
MBW (g)	260.4 ^a	274.3 ^a	0.157
ADG (g/d)	2.35°	2.48 ^a	0.114
Biomass (g)	15,718°	18,163 ^b	0.005
Feed accumulation (g)	26,333ª	27,373 ^a	0.157
FCR	1.81 ^b	1.60 ^c	0.003
SR (%)	75.4°	82.9 ^b	0.013
Productivity (Kg/Ha)	39.30	45.40	

Dorformonco	Trea	P-value	
Performance	Normal CP Feed	PG-top dressed Feed	P-value
MBW (g)	260.4 ^a	286.2 ^a	0.157
ADG (g/d)	2.35 ^a	2.60 ^a	0.114
Biomass (g)	15,718 ^a	19,170 ^b	0.005
Feed accumulation (g)	26,333 ^a	26,082 ^a	0.157
FCR	1.81 ^b	1.44 ^a	0.003
SR (%)	75.4 ^a	83.8 ^b	0.013
Productivity (Kg/Ha)	39.30	47.50	

	Treat		
Performance	Normal CP Feed	PG Feed & PG in the water	P-value
MBW (g)	260.4 ^a	273.9 ^a	0.157
ADG (g/d)	2.35 ^a	2.50 ^a	0.114
Biomass (g)	15,718 ^a	18,720 ^b	0.005
Feed accumulation (g)	26,333 ^a	26,666 ^a	0.157
FCR	1.81 ^b	1.50 ^{ac}	0.003
SR (%)	75.4 ^a	85.4 ^b	0.013
Productivity (Kg/Ha)	39.30	46.80	

- PG Feed was produced from normal CP feed mixed with Pondguard (4L/ton feed) in the feedmill.
- PG Feed is able to provide better performance than the normal feed, especially in MBW, ADG, Biomass, FCR and SR

- PG-top dressed feed was made from normal CP feed topdressed with Pondguard (4 ml/kg feed) in the field.
- PG-top dressed Feed is able to provide better performance than the normal feed, especially in MBW, ADG, Biomass, FCR and SR

- *PG feed from feedmill were used and water were treated with Pondguard 0.4 ppm per week.
- This treatment is able to provide better performance than the normal feed, especially in MBW, ADG, Biomass, FCR and SR



PONDGUARD PERFOMANCE ON TILAPIA FISH (POND TRIAL)

Group	MBW (gr)	ADG (gr/day)	Biomass (kg)	FCR	SR (%)	Productivity (kg/Ha)
Normal CP Feed	180.1	1.683	2,501	2.088	49.6	17.9
PG Feed	192.3	1.798	2,161	2.406	46.8	18.0

Group	MBW (gr)	ADG (gr/day)	Biomass (kg)	FCR	SR (%)	Productivity (kg/Ha)
Normal CP Feed	180.1	1.683	2,501	2.088	49.6	17.9
PG-top dressed feed	198.3	1.853	2,744	2.236	53.2	21.1

- Pondguard dose was optimized on Tilapia
- The selected doses were:
 - ➤ In feed during production: Pondguard 8 liter/ton of feed. Daily
 - > On feed Spray: Pondguard 8 mL/ Kg of feed. Daily
- The outcome of 106 days trial suggested that Pondguard has positive impact on the Biomass increment, lowering the FCR and increasing the survival rate of tilapia.



Biofloc System advance technique on Tilapia Fish Farming

		Bioflo	c pond		
Parameter	Unit	Standard feed			Control 2
Volume	m3	3	3	192	14
No of tank	unit	3	3	1	1
Initial stocking	pcs	254	253	19,200	1,400
Stocking Density	animal/m2	85	84	10	21
Initial MBW	gram	28	28	10	21
Initial Biomass	Kg	7	7	19	29
Harvest DOC	days	90	90	95	75
MBW (harvest)	gram	285	295	213	160
ADG (harvest)	gram/day	2.86	2.96	2.13	1.85
Harvest Biomass	Kg	67	71	3,264	174
Population	Pcs	237	240	15,360	1,092
SR	%	93	95	80	78
ACC. Feed	Kg	67	70	3,150	150
FCR		1.12	1.1	1.03	1.04
Water exchange		287%	287%		
Productivity	Kg/m3	22.5	23.6	17	12.4





^{*}Premium Feed has a higher crude protein content

^{*}Control group is a group of conventional tilapia farming systems.



Cage trial on Grass Carp Fish Farming- SOU China



Effects of pondguard on growth performance of grass carp

GROUP	the average rate of	Specific growth rate	Survival rate	Feed coefficient
	gain (%)	$(SGR, \% \cdot d^{-1})$	(SR, %)	(FCR)
0	13.00 ± 0.71^{d}	0.36 ± 0.01^{d}	80.00 ± 0.00^{b}	2.26±0.11 ^a
1% (W4)	20.36±2.66 ^c	0.54 ± 0.07^{c}	92.50±2.50a	1.27 ± 0.19^{c}
2% (W5)	26.35 ± 2.37^{a}	0.69 ± 0.06^{a}	91.67 ± 3.82^{ab}	0.97 ± 0.11^{c}
3% (W6)	21.99±0.04bc	0.59 ± 0.01 bc	93.65±5.16 ^a	1.16 ± 0.09^{c}

Notes: Value with different letter superscripts in the same row mean significantly different (P < 0.05).

Effects of pondguard on growth performance of grass carp

GROUP	the average rate of gain	Specific growth rate	Survival rate	Feed coefficient
	(%)	(SGR,%·d ⁻¹)	(SR, %)	(FCR)
0	13.00±0.71 ^d	0.36 ± 0.01^{d}	80.00 ± 0.00^{b}	2.26±0.11 ^a
1% (W4)	20.36±2.66°	$0.54 \pm 0.07^{\circ}$	92.50±2.50a	1.27 ± 0.19^{c}
2% (W5)	26.35±2.37a	$0.69 \pm 0.06^{\mathrm{a}}$	91.67 ± 3.82^{ab}	0.97 ± 0.11^{c}
3% (W6)	21.99±0.04 ^{bc}	0.59±0.01 ^{bc}	93.65±5.16 ^a	1.16±0.09°

Notes: Value with different letter superscripts in the same row mean significantly different (P < 0.05).

^{* 2 %} pondguard was the best feed for grass carp, so it was more appropriate to choose 2 % concentration when adding pondguard as feed additive.



CONCLUSIONS

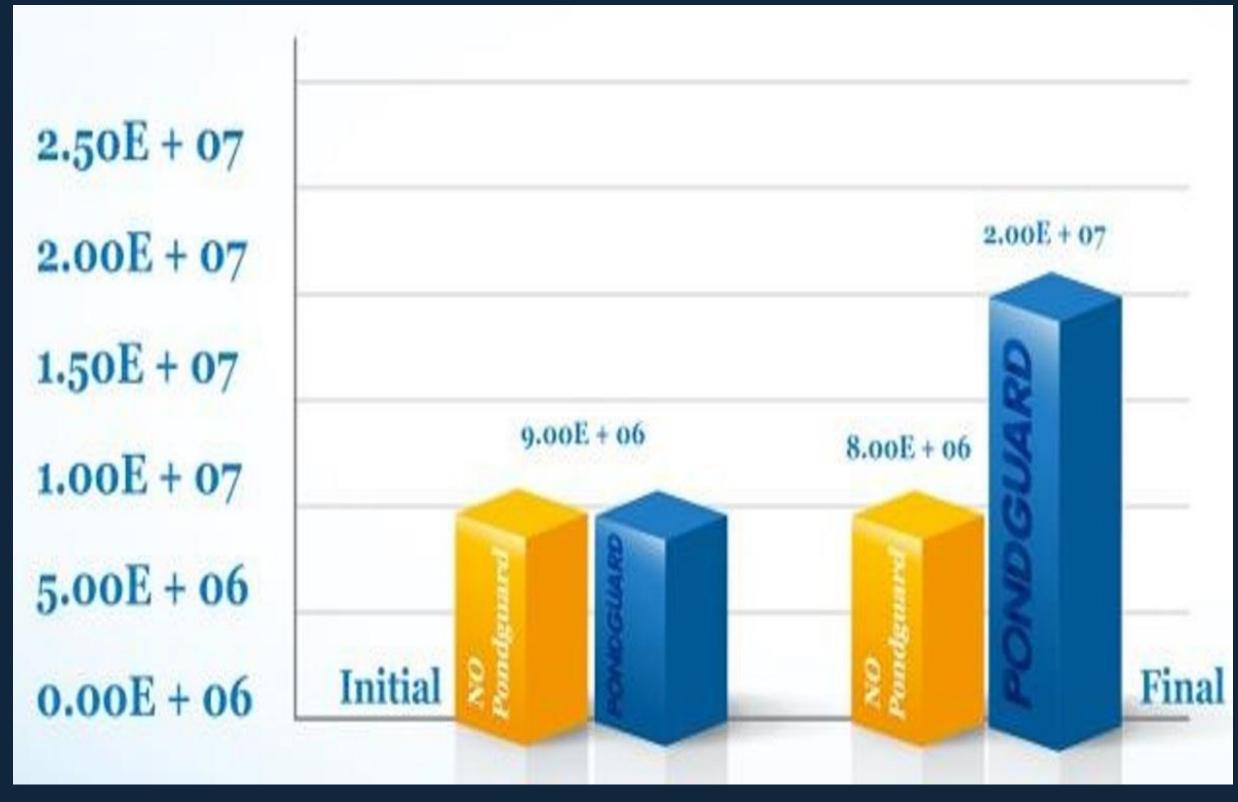
The developed blend formulation Pondguard is an effective immunomodulator and anti-pathogenic agent which supports shrimp and fish to grow in its optimum condition, which resulted in better productivity.





PONDGUARD AS IMMUNOMODULATOR

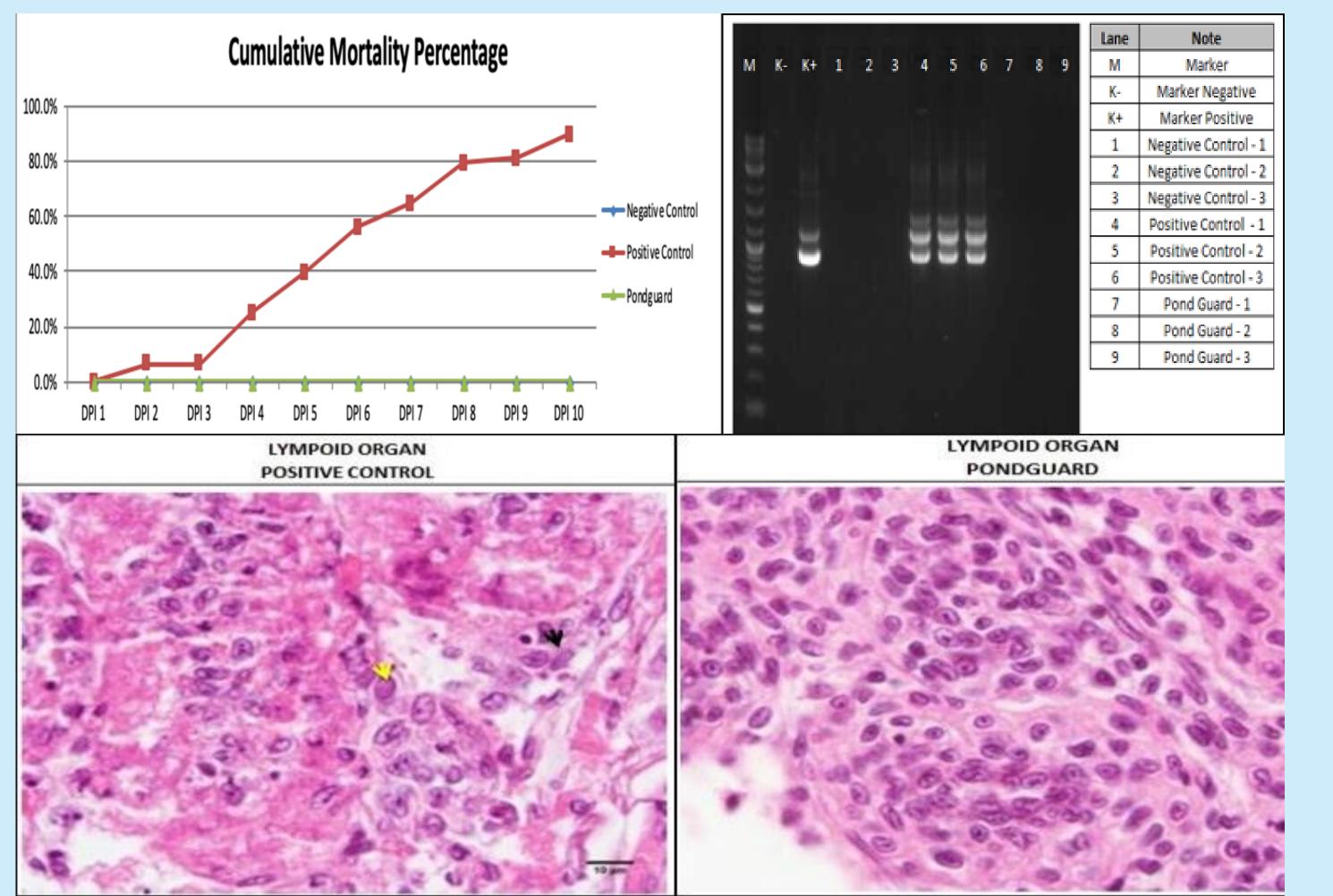
Total Hemocyte Count: Shrimp Total Hemocyte Count increased after 3-4 weeks of PG application.



- This trial was designed to observe the efficacy of Pondguard as immunomodulator.
- A hemolymph count of a normal healthy shrimp should be about log 7.
- A 3 weeks controlled small scale trial was conducted.
- The initial and final hemolytic count of shrimps were analyzed from Pondguard applied tanks and not applied tanks.
- The hemolytic count of shrimp in Pondguard applied tanks were found in optimum level i.e. log 7 after 3 weeks of application.
- The trial shows that Pondguard is acting as immunomodulator.



Pondguard against White Spot Syndrome Virus (WSSV)

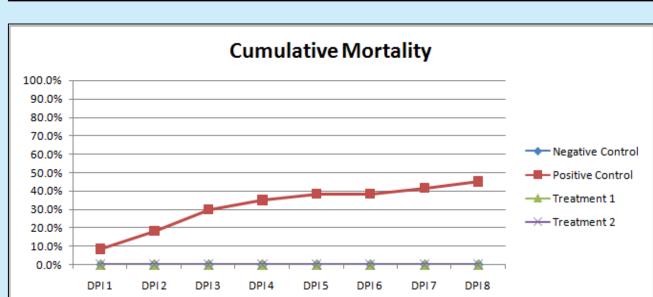


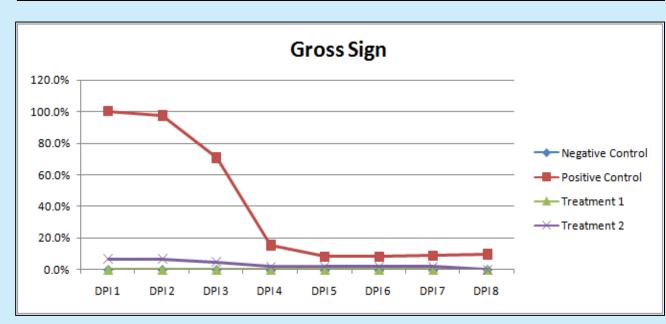
- There was 100%
 protection achieved by applying PG against WSSV.
- The PCR test of the remaining shrimp on the day of termination i.e., DPI 10 stated that all the shrimp in PG group were negative to WSSV. The positive control shrimp were positive to WSSV.
- The sub-lethal dose of virus was submerged in the PG and incubated for 2-3 hours before feeding to the shrimp.
- The trial was repeated two times leading to similar results.



Pondguard against EMS/AHPND in Shrimp

		Shrimps		Challenge M	Product	Water E	xchange	
Group	R	MBW	Number of shrimps	Volume of Bacteria/TSB	Immersion Time	Application	Rate	Frequency
Negative Control			oili lilipo	30 mL of TSB	Time			
Positive Control	,	06.00	20	30 mL of bacteria	45		200/	Every day
Treatment 1	3	0.6 - 0.8	20	30 mL of bacteria	15 minutes	0.2%	20%	started at dpi 3
Treatment 2				30 mL of bacteria		0.3%		upi 3











The state of the s		
	•	Vibrio paraho
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 Vibrio parahaemolyticus-EMS strains performed the mortality in shrimps with typical gross signs of EMS/AHPND.

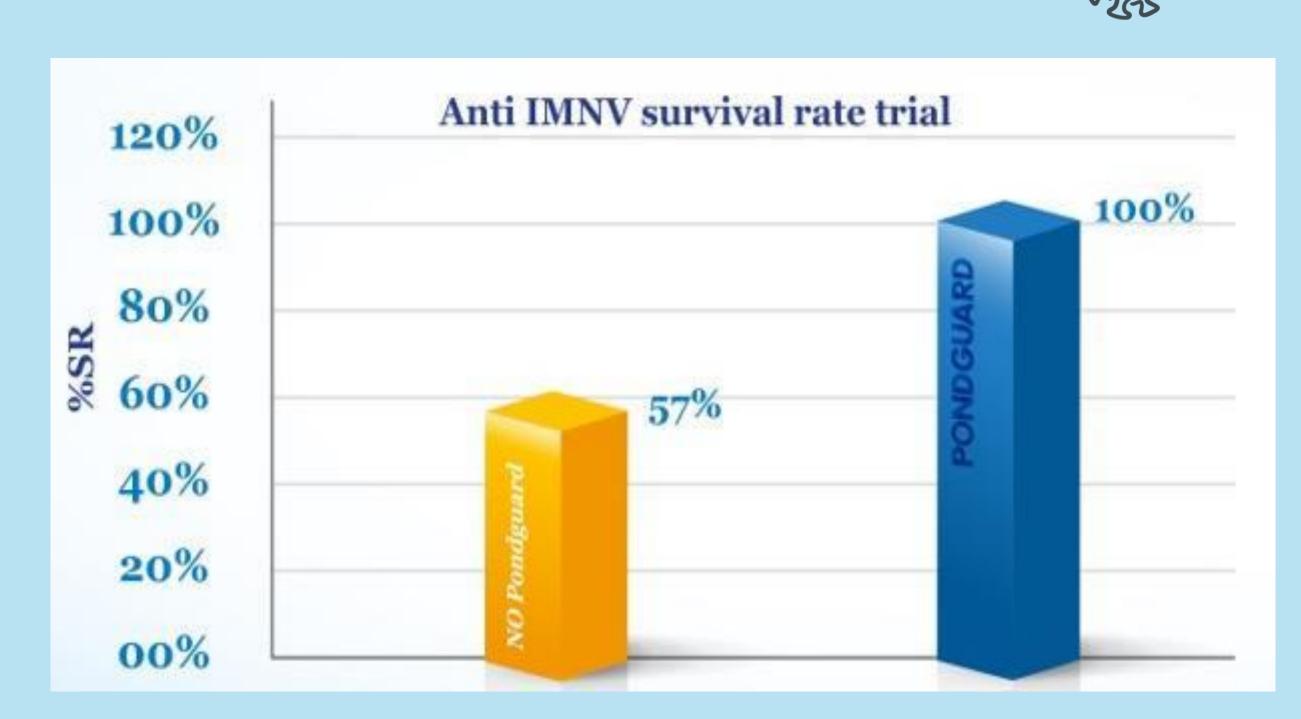
Conclusions:

 Pondguard has ability to inhibit the Vibrio parahaemolyticus-EMS growth, therefore, NO mortality and gross sign of EMS were appeared in the treated shrimps.

			RT PCR Results (CT Value)									
Group	R	Bacteria	acteria Shrimps Samples									
Стоир		after Incubation	After Challenge	DPI 1	DPI 2	DPI 3	DPI 4	DPI 5	DPI 6	DPI 7	DPI 8	
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Negative Control	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	1	24.3	33.4									
Positive Control	2	23.7	33.6	28.4	28.4	27.1	23.2	21.8	33.6	0.0	32.7	0.0
	3	25.4	33.6									
	1	13.9	0.0									
Treatment 1	2	14.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	3	13.8	0.0									
	1	13.8	36.5									
Treatment 2	2	15.8	34.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	3	16.2	35.4									



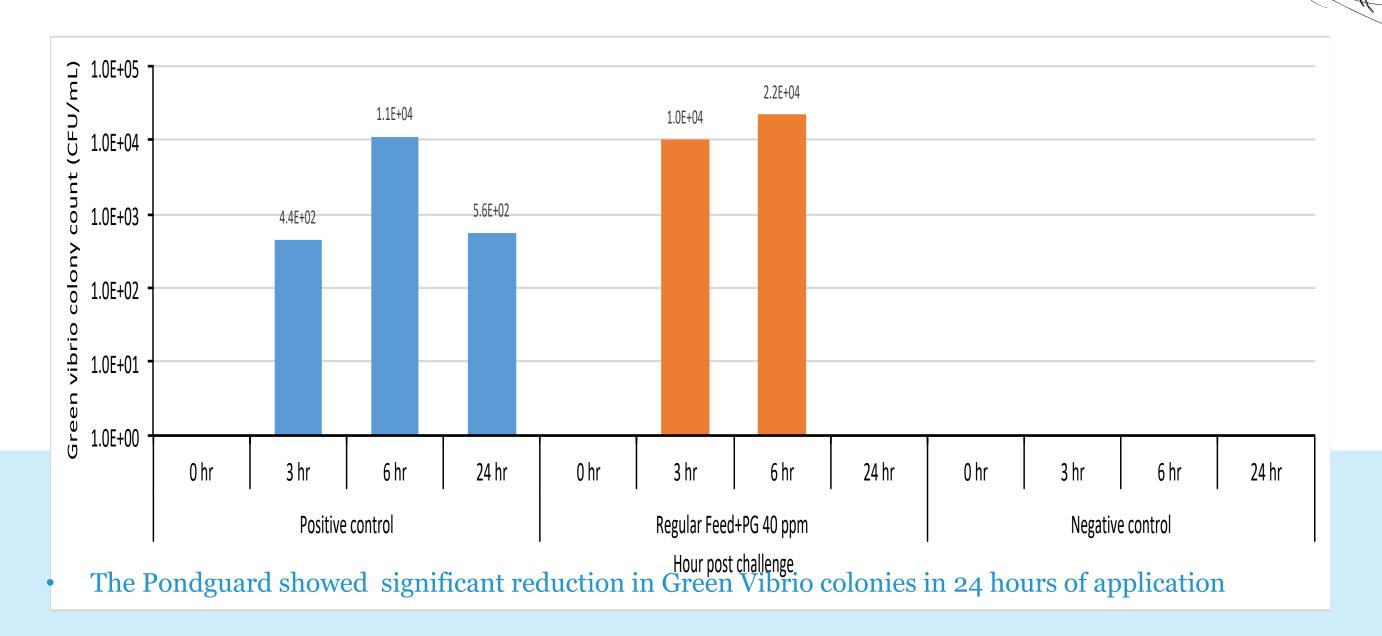
Pondguard against IMNV in Shrimp



Pondguard application was able to maintain SR 60-100% against Infectious Mionecrosis Virus (IMNV) in several trials conducted.

- A small scale bioassay trial was conducted to determine the efficacy of Pondguard against IMN Virus of shrimp.
- Pondguard was applied for
 weeks in the tanks prior
 to challenge.
- The shrimp were challenged by sub-lethal dose of IMN Virus using the per os method.
- The higher survival rate i.e.
 100% was recorded in Pondguard applied tanks after 10 days of observation.
- The trial results prove that Pondguard improved the immune system of shrimp to fight against IMN Virus.

Pondguard against PATHOGENIC GREEN VIBRIO in Shrimp



- A small scale bioassay trial was conducted to determine the efficacy of Pondguard against Green Vibrio.
- Pondguard was applied for 2 weeks in the tanks prior to challenge.
- The shrimp were challenged by sub-lethal dose of VP-AHPND i.e. log 6.
- The obtained result shows that Pondguard was able to reduce the green Vibrio load up to zero % in 24 hours of the challenge period.
- The trial results prove that Pondguard is able to reduce the Vibrio load in a controlled environment.



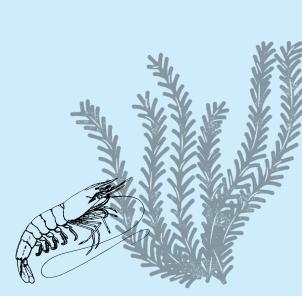


PRODUCT PACKAGING

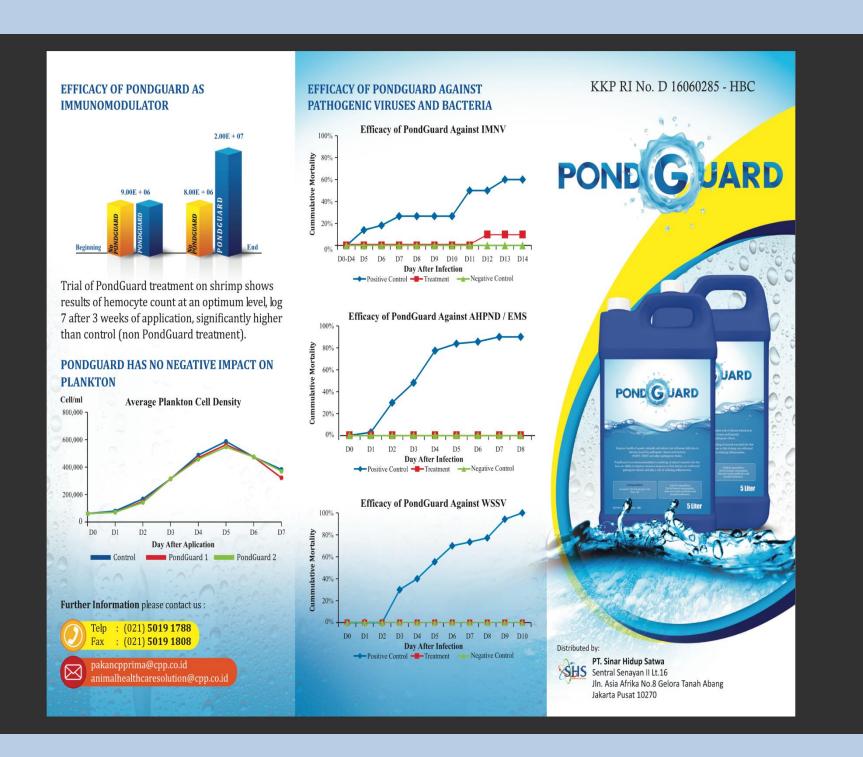














WHAT IS PONDGUARD?

PondGuard is an immunomodulator consisting of essential oils that have an ability to improve immune response so that shrimp can withstand pathogenic attacks and play a role in reducing inflammation.

PondGuard maintains healthy immune system of shrimp. If level of the immune system is low, it will be increased and maintained at its optimum level.

PONDGUARD CHARACTERISTICS

A liquid containing natural and harmless ingredients that have characters as such drinking water with a salinity of 0 ppt with a pH of 6.5 - 7.5.

PondGuard has no negative impact on plankton, probiotic bacteria or enzymes used during culture.

PONDGUARD BENEFITS

- Reduce load of harmful pathogens (viruses and bacteria) in pond environment.
- Maintain shrimp immunity to help protecting from infectious diseases.
- · Maintain metabolic functions of shrimp body.
- Reduce stress level of shrimp.

Composition

Mixture of lavender oil, eucalyptus oil and pine oil.

Packaging: 1 liter and 5 liters.

HOW TO USE Application on Feed

- Mix PondGuard according to the dosage into a soluble liquid (2 ml or 4 ml PondGuard + 150 ml water per kg of feed). Stir the PondGuard mixture homogenously.
- Apply the PondGuard mixture to feed evenly distributed. (Mix PondGuard into the feed in the shade).

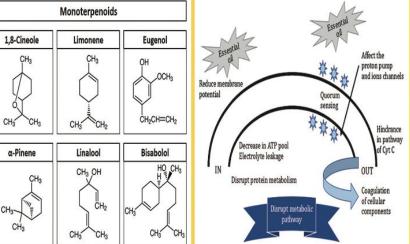
Application in Pond Water

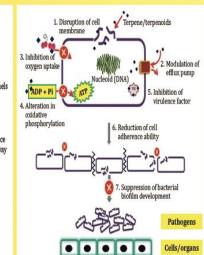
- Mix PondGuard according to the dosage into 10 liters of water. Stir the PondGuard mixture homogenously (2 - 3 minutes).
- Apply the PondGuard mixture into culture/ treatment pond water and ensure aerators are functioning to distribute evenly.

DOSAGE AND FREQUENCY OF USE

Culture Stag	e	Application on Feed	Application in Pond Water			
Stocking Preparation		Not applicable	Dose of 0.4 ppm applied on the 7 th and 3 rd day before fry stocking.			
During Culture	Normal Condition	 Dose of 2 ml (DOC ≤ 60) and 4 ml (DOC > 60) per kg of daily feed Apply daily at 2 highest feeding times (highest amount of feed) 	 Dose of 0.4 ppm per application Apply at least once a week 			
culture	Stress Condition (any signs or symptoms of disease infection, environmental	 Dose of 4 ml per kg of daily feed Apply daily at 2 highest feeding times (highest amount of feed) 	 Dose of 0.4 ppm per application 1 2 times a day Apply for 3 – 7 consecutive days 			

PONDGUARD MECHANISM OF ACTIONS





PUBLICATIONS











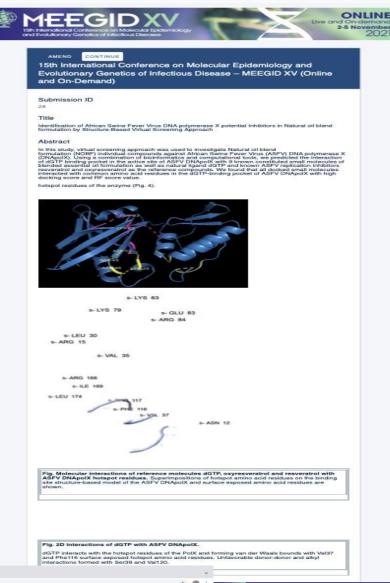


15th International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases

ONLINE
Live and On-demand
2-5 November
2021









AMEND CONTINUE

15th International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Disease – MEEGID XV (Online and On-Demand)

Submission ID

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MEEG 2021

Title

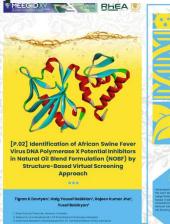
Development of essential oil blend formulation as a disinfectant against African Swine fever virus (ASFV)agent in PAM cells of Swine

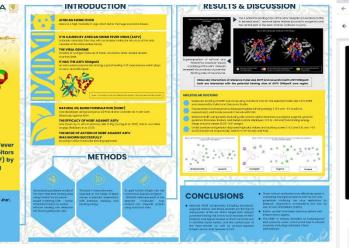
Abstract

📃 🛕 🥬 Rajeev Kumar Ji

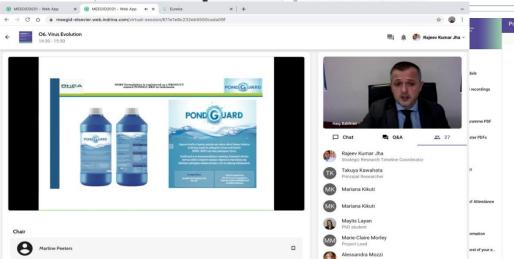
African swine fever virus has a significant impact on swine production and the economics of the swineproducing countries. The role of strict biosecurity measures becomes critical when there are no known remedies yet. Disinfection is considered a significant part of biosecurity measures and plays a vital role in reducing the risk of contaminating the environment.

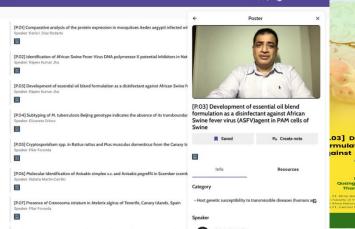
Successful efforts have been made to optimize a formulation Essential Oil Blend (EOB) product to determine its efficacy against the African swine fever virus (ASFV) in *in vitro* conditions. The Essential Oil Blend (EOB) comprises a blend of, i.e., Eucalyptus oil, Gardenia oil, and Jasmine oil. The *in vitro* trial results demonstrated that EOB up to dilution ten could degenerate the lethal dose log 5 of ASFV. The work was shown by observing hemadsorption (Rosetta formation) and conducting a real-time PCR test. There was no Rosetta formation up to dilution 11 of EOB. The Ct value of the EOB group at 96 hours post-infection (hpi) was the same as the initial value or lower (25) than it, whereas the Ct value of positive control increased several folds (17.84). It is a potential water supplement to work against ASFV and enhance pig immunity to fight against common pathogens.



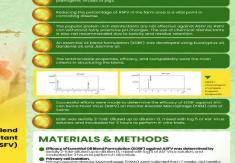


properties that reduce WSSV load.









- sectorly 7- feet of influence up to distance in meed with log 37 of 48 ff View solution, and concluded on the program of the p



RESULTS AND DISCUSSION

The reol-time PCR results indicated that virulent ASFV stroin could not replacet or denoture in PAMs cultures.
The concept was further proven by chollenging the FCBE incubated ASF Virus in Pig.
The accord as of in vive tricks showed that FCBE could insibit the hardworld transmission or obvioused pigs did not get infected by the ASFV chollenged pigs in the presence of ECBE distriction.







BIT's 5th Annual World Congress of Aquaculture and Fisheries-2016

Qingdao, China

Title: Development of Natural Herbal Oil Formulation as an Anti-White Spot Syndrome Virus Agent in Penaeusvannamei

Dr. Rajeev K. Jha*, Benjamin Jiaravanon, Yousef H. Babikian, Haig Y. Babikian, Sarayut Srisombat, Soy D. Wisoyo, and Yuli Asih

PT. Central Proteina Prima

Abstract

The Natural Herbal Oil Formulation (NHOF) have been designed to work against White Spot Syndrome Virus in Shrimp. The essential oil blend from the following plants, Lavandullatifolia, Pinussylvestrisa, Jasminum Officinale, C. Limon, Prunusavium, Viola odorata, Gardenia jasminoides, Cocosnucifera, Rosa damascene and Eucalyptus globulus. The product were added in the feed, as feed additive and also applied in tank water as liquid product named as "Pondguard". A bioassay trial was conducted to determine the efficacy of developed formulation as an anti-WSSV candidate. The treatment was fed on treatment feed and "Pondguard" applied in the tank water whereas control fed on the regular feed throughout the experiment. The shrimp of both treatment and control were challenged by WSSV on day 8. Two methods of WSSV challenge was applied, per os challenge and immersion challenge. The cumulative mortality in positive control reached up to 100% at dpi 10 whereas mortality appeared in treatment and in negative control. The trial results show that the developed anti-WSSV formulation significant effect against WSSV in a controlled



Yusef Babikyan PT Central Proteina Prima

Performance of natural oil blend formulation (NOBF) against white spot syndrome virus (WSSV) agent in Penaeus vannamei boone, 1931

Haig Babikian, Yusef Babikyan, Rajeev Kumar Jha, Soy Daniel Wisoyo, Yuli Asih and Sarayut Srisombat

Abstract

The white spot syndrome virus (WSSV) is lethal in penaeid shrimp. Successful efforts made to develop a natural oil blend formulation (NOBF) by blending Eucalyptus globulus, Pinus sylvestris, and Lavandula latifolia in different compositions with anti-WSSV properties. A bioassay challenge trial conducted using 1 g of specific pathogen-free Penaeus vannamei Boone, 1931 shrimp in 4 replicates of aquaria for each group. The NOBF dose of 0.4 ppm applied throughout the trial period by mixing in the aquariar water daily, starting seven days before the challenge. The efficacy of NOBF against WSSV was measured using the per os method of a challenge. The cumulative mortality in the positive control group reached 100 % after ten days of the challenge. The NOBF was applied in the commercial shrimp ponds to study its efficacy and palatability. A study in 8 ponds in a WSSV prone area in Indonesia, demonstrated no significant difference in crucial water quality parameters, especially on algae as natural food. The NOBF applied ponds had higher productivity (1.28 Kg of shrimp per m3) as compared to control (1.18 Kg of shrimp per m3). The FCR of NOBF group was lower (1.41) than control (1.33). The survival rate of the NOBF group was higher (86.96%) than control (80.36). The trial outcomes show that NOBF is safe and user-friendly, with properties to reduce pathogen load.

Keywords: White spot syndrome viral disease, natural oil, per os challenge, shrimp pond culture, shrimp productivity, natural food

The white spot syndrome virus (WSSV) is the most lethal pathogen of culture shrimp worldwide [1]. It is an enveloped non-occluded DNA virus of the family Nimaviridae under the

worldwide [15]. It is an enveloped non-occluded DNA virus of the family Nimaviridae under the new genus Whispovirus and is also the most devastating shrimp pathogen ever isolated and studied. This virus is extremely virulent and causes up to 100 % mortality in 3-7 days in all the cultured species of penaeid shrimp [12,3,4]. Some successful efforts made previously using natural herbs against shrimp viruses [5,6,7,8]. The plants, such as Eucalyptus globulus, Pinus sylvestris, and Lavandula latifolia are well documented for their anti-viral properties. Eucalyptus extract shows strong anti-viral and anti-bacterial properties [12, 13]. Pinus species have potent anti-viral, anti-bacterial, and immune-modulating properties [12, 13]. The Lavandula have shown strong anti-viral [14] as well as immunomodulatory [15] effects.

A successful effort made to blend the oils of similar properties to fight against WSSV more vehemently. The efforts made to test the palatability and its impact on critical water quality parameters in commercial shrimp ponds.

The materials and methods divided into two sections, section 1 as laboratory level trials and section 2 as field trials.

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NOBF preparation and composition

Eucalyptus globulus, Pinus sylvestris and Lavandula latifolia oils obtained from vendors who comply with the strictest industry practices:

Aquaculture & Fisheries

Abioassay trial was conducted to determine the efficacy of developed feed as an anti-AHPND candidate. The treatment was fed on anti-AHPND feed whereas control fed on the regular feed throughout the experiment. The shrimp of both treatment and control were challenged by per os method on day 8. The cumulative AHPND-gross sign appearance in positive control reached up to 95% at dpi 8 whereas no gross sign appeared in treatment and in negative control. The cumulative mortality reached up to 90% at dpi 8 in positive control whereas no mortality recorded in treatment and negative control. The Vibrio parahaemolyticus isolated from the hepatopancreas of infected shrimp were matched 100% with the existing AHPND strain. The trial results show that the developed anti-AHPND feed has significant effect against AHPND pathogen in a controlled condition.

Efficacy of Anti-AHPND formulated feed in vannamei shrimp in Vietnam

Benjamin Jiaravanon, Yousef Haig Babikian, Haig YousefBabikian, Le Van Khoa, Iswadi, Rajeev Kumar Jha*

Effectiveness of Natural Herbal Oil Formulation against White Spot

Pharmacognosy & Natural Products

up to 100% in 4-7 days in lab and 10-14 days in the ponds [3-6]. The up to 100% in 4-7 days in lab and 10-14 days in the points [3-6]. The intensity of infection and severity depends on the viral load. The stress factors, like, fluctuation in critical water quality and environmental parameters, like temperature, pH and Dissolved Oxygen parameters stimulates the process of infection.

Syndrome Virus in Penaeus vannamei

een made to identify the anti-WSSV properties in the plants, such s, Cynodon dactylon [9,10], Durio zibethinus Murr (Durian) [11] and Ceriops tagal [12] and Eucalyptus spp. and Plelargonium roseum [13].

latural Herbal Oil formulation and Pondguard against White Spo Syndrome Virus (WSSV) in Penaeus vannamei [14].

Materials and Methods

Bioassay lab and glass aquaria

A bioassay trial was set-up using 10 aquaria each for experimental groups as well as for control groups. The Specific Pathogen Free (SPF)-juvenile shrimp of average weight 0.25 g, 10 in number were maintained in each aquarium. The glass aquaria were cleaned, sun dried and disinfected with 70% alcohol. Each aquarium van filled with

The seawater utilized during the experiment was gone through the process of sedimentation, filtration and disinfected with 30 ppm active chlorine and finally treated with Ultra Violet radiation (SS-L75W. Matala Water Technology, Taiwan). The water quality parameters maintained during the trial were as followed, Dissolved Oxygen (DO) (>5 ppm), Temperature (27 ± 1°C), Salinity (20 ppt), pH (7.5-8.15) and alkalinity (80-120 ppm).

The duration of trial was of 30 days i.e., 5 days acclimatization, 14 days feeding, day 15 challenge and from day 16 onwards continues feeding and observation for 10 days. Shrimp of treatment group were fed on experimental feed whereas regular feed was fed to the control. There was no change in feed quality and the rate of feeding throughout the experiment. The liquid supplement, Pondguard was applied on daily basis in experimental tanks throughout the experiment. The the experiment. The liquid supplement, Pondguard was applied or daily basis in experimental tanks throughout the experiment. In shrimp were observed intensively for their behavior and feeding rat and cumulative mortality during the trial.

VETERINARY MEDICINE

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Research

Efficacy of Natural Herbal Formulation against Acute Hepatopancreatic Necrosis Disease (AHPND) causing Vibrio parahaemolyticus in Penaeus vannamei

Rajeev Kumar Jha, PhD'; Yousep Haig Babikian, PhD'; Haig Yousep Babikian, MSc³, Le Van Khoa, PhD³; Daniel Wisoyo, BSc¹; Sarayut Srisombat, MSc¹; Benjamin Jiaravanon, MSc¹

¹PT Central Proteina Prima, Indonesia ²Panacea Natural Sciences, Indonesia

Department of Agriculture and Rural Development, Vietnam

A formulation was developed using combination of blended natural essential oils as an anti
thrio parahemolyticus causing acute hepatopancreatic necrosis disease (AHPND) candidate.

Lavandula latifolia, Pinus sylvestris, Jasminum officinale, Citrus limon, Prunus avium, Viola

odorata. Gardenia jasminoides, Cocos nucifera, Rosa damascene and Eucalyptus globulus,

mixed together to develop as anti-V. parahemolyticus product. The treatment group was fed

on essential oil mixed feed whereas control group were fed on the regular feed throughout the

experiment. The shrimp of both treatment and control were challenged by immersion method

at day 8. The cumulative AHPND-gross sign appearance in positive control reached up to 95%

at dpi 10 whereas no gross sign appeared in treatment and in negative control. The cumulative

mortality reached up to 46.7% at dpi 10 in positive controls whereas no mortality recorded in

treatment and in negative control. The V. parahaemolyticus isolated from the hepatopancreas

of infected shrimp matched 100% with the existing AHPND strain. The trial results show that

the developed natural herbal formulation has significant effect against AHPND in a controlled

condition.

A formulation was developed using combination of blended natural essential oils as an ant

ABBREVIATIONS: AHPND: Acute Hepatopancreatic Necrosis Disease; SPF: Specific Pathogen Free; DO: Dissolved Oxygen; WSSV: White Spot Syndrome Virus; IMNV: Infectious Myonecrosis Virus; IHHNV: Infectious Hypodermal and Haematopoietic Necrosis Virus; TSV: Taura Syndrome Virus; YHV: Vellow Head Virus; PCR: Polymerase Chain Reaction; BLAST: Basic Local Alignment Search Tool.

ountries, like, Vietnam, Malaysia, Thailand, Mexico and in Philipines. 1-6 The unique symp toms and characteristics of this disease consisting of massive sloughing of hepatopancreas cynthesia of the disease consisting of the sloughing of hepatopancreas cynthelial cells. The external symptoms in infected shrimp like, empty stomach, bluish body color and shrunken hepatopancreas could be observed. The rate of mortality is significantly higher on the 1st 3 days of infection. The AHPND appear in the culture ponds from 8s-45 days of stocking.



Study on the Efficacy of Pondguard in Improving Clinical Performance of White Leg Shrimp (Penaeus Vannamei) in an AHPND Bacterial Challenge Model

Haig Yousef Babikian¹*, Rajeev Kumar Jha¹, Dang Thi Hoang Oanh² and Truong Quoc Phu²

*Corresponding author: Haig Yousef Babikian, PT Central Proteina Prima, Indonesia.

To Cite This Article: Haig Yousef Babikian. Study on the Efficacy of Pondguard in Improving Clinic Vannamed in an AHPND Ractorial Challenae Model. Am I Biomed Sci & Res. 2019 - 5(3). AIBSR MS.ID.00

Received:

September 19, 2019; Published:

September 25, 2019

Keywords: AHPND: Vibrio parahemolyticus: Essential Oil Blend; Pondguard

ly Mortaliy Syndrome (EMS) have been reported in several shrimp oducing countries, like, Vietnam, Malaysia, Thailand, Mexico and in Philipines [1-5]. There is significant impact on the world shrimp Materials and Methods production [1.6], were successfully able to identify and isolate the production [1,6], were successfully able to identify and isolate the causative agent Vibrio parahaemolyticus of AHPND. The unique symptoms and characteristics of this disease include severe atrophy of the shrimp hepatopancreas (HP) consisting of massive sloughing of HP epithelial cells [1]. The external symptoms in infected shrimp like, empty stomach, bluish body color and shrunken hepatopancreas, could be observed in the outbreak ponds. AHPND could transmit experimentally by horizontal transmission, co-habitation, immersion and by reverse gavage (Personal observation).

were used to conduct the trial. The bioassay trial was conducted by the researchers of Department of Aquaculture and Fisheries, Can tho University, Cantho, Vietnam,

Experimental shrimp

White leg shrimp larvae (PL10) were spawned and nursed to could transmit experimentally by horizontal transmission, co-habitation, immersion and by reverse gavage (Personal observation).

The AHPND appear in the culture ponds from 8-45 days of stocking. The shrimp cultured in earthen bottom ponds were reported more susceptible to AHPND as compared to the HDPE-lined ponds. The proposed trial was designed with the objective to conduct a laboratory scale trial by applying the developed anti-AHPND product i.e. Pondguard to determine its efficacy against the AHPND causative agent. The pre-determined doses, 40 ppm and 80 ppm

Aquaculture 2016

J Pharmacogn Nat Prod, an open access journal ISSN: 2472-0992

C H A P T E R - 14

Farming System Adaptation and Natural Herbal Oil Formulations against Various **Emerging Diseases of Shrimp**

Rajeev Kumar Jha², Haig Yousef Babikian¹, Kristina¹, and Sarayut Srisombat²

> ¹Asclepius Pharmaceutical Sciences, Singapore ²PT Central Proteina Prima, Indonesia

Organizing Secretary, ClimFishcon 2020

Our book titled "Impact of Climate Change on Hydrological Cycle, Ecosystem, Fisheries and Food Security" has been published. Please follow the link to order the copies.

https://www.nphindia.com/book/9789390309115/impact-of-climate-change-on-hydrology-and-fisheries

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DOCUMENTARY INDONESIA- SHRIMP









DOCUMENTARY INDIA











TRIALS DOCUMENTARY CHINA









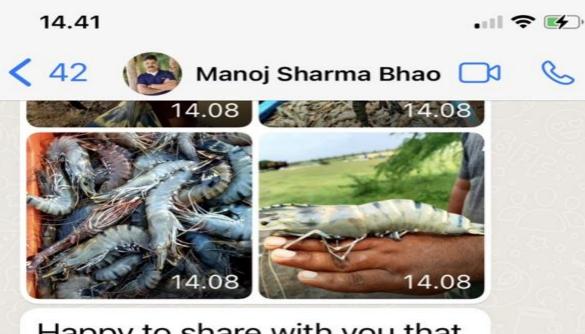








TRIALS DOCUMENTARY MONODON CULTURE - INDIA



Happy to share with you that at our farm we have harvested 10 count monodon in 160 days.

Other ponds harvested 13 to 17 count with 65 percent survival.

Regards

+

14.08

Pond guard administrated 10 ml per kg feed one meal a day through out the culture











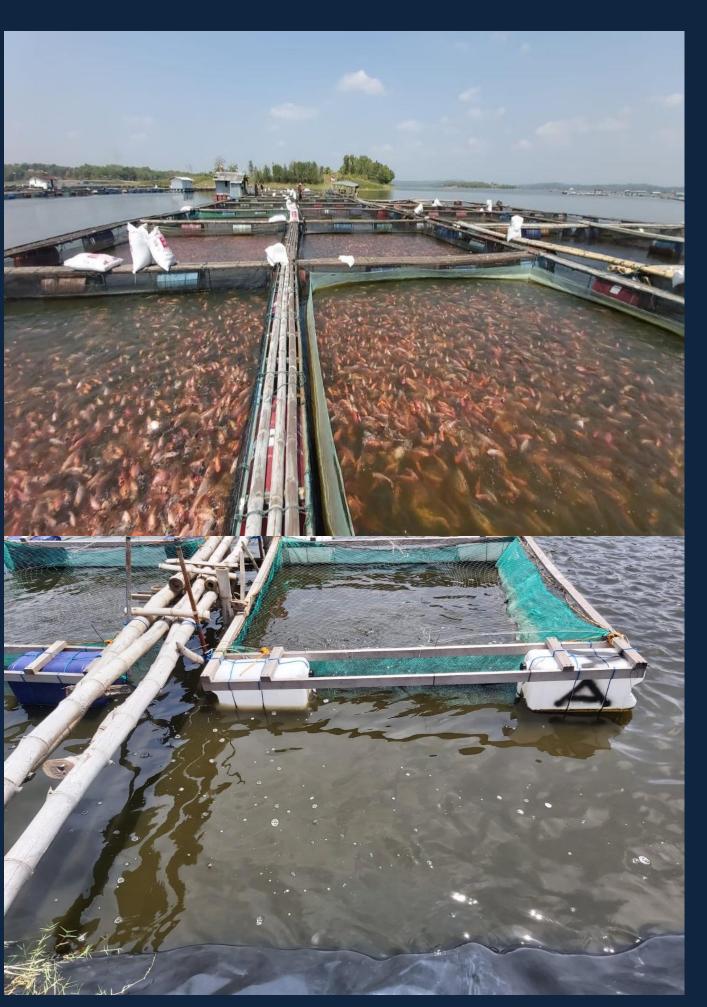








TRIALS DOCUMENTARY FISH CULTURE-INDONESIA











Biofloc System advance technique on Tilapia Fish Farming



THANK YOU



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