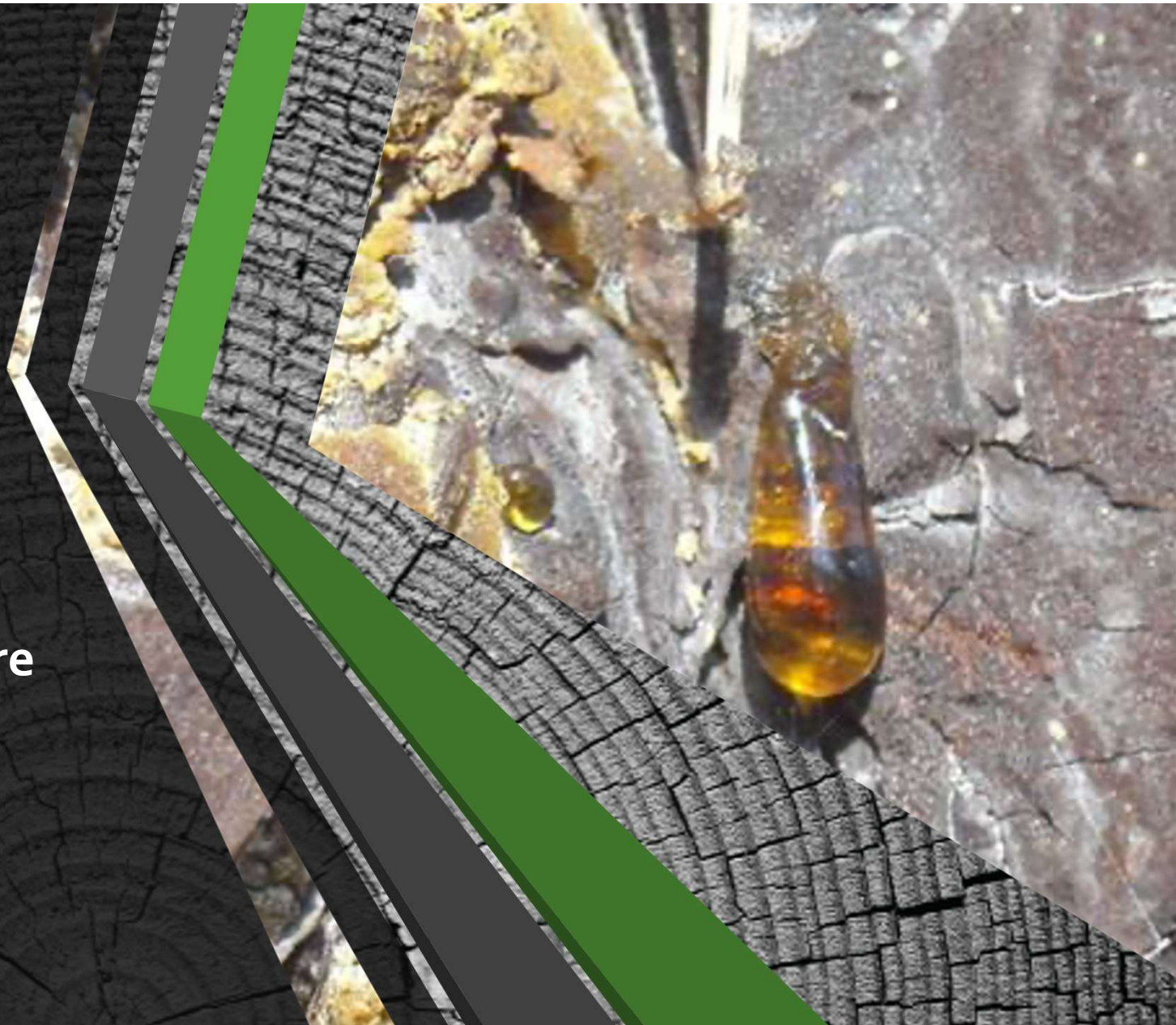


OxiFish

The secret of your future
success



An Overview

**What is
OxiFish:
An introduction**

Shrimp Trial

Conclusions



**Green
Innovation**

OxiFish: a mixture of Polyphenols and Terpens from pine

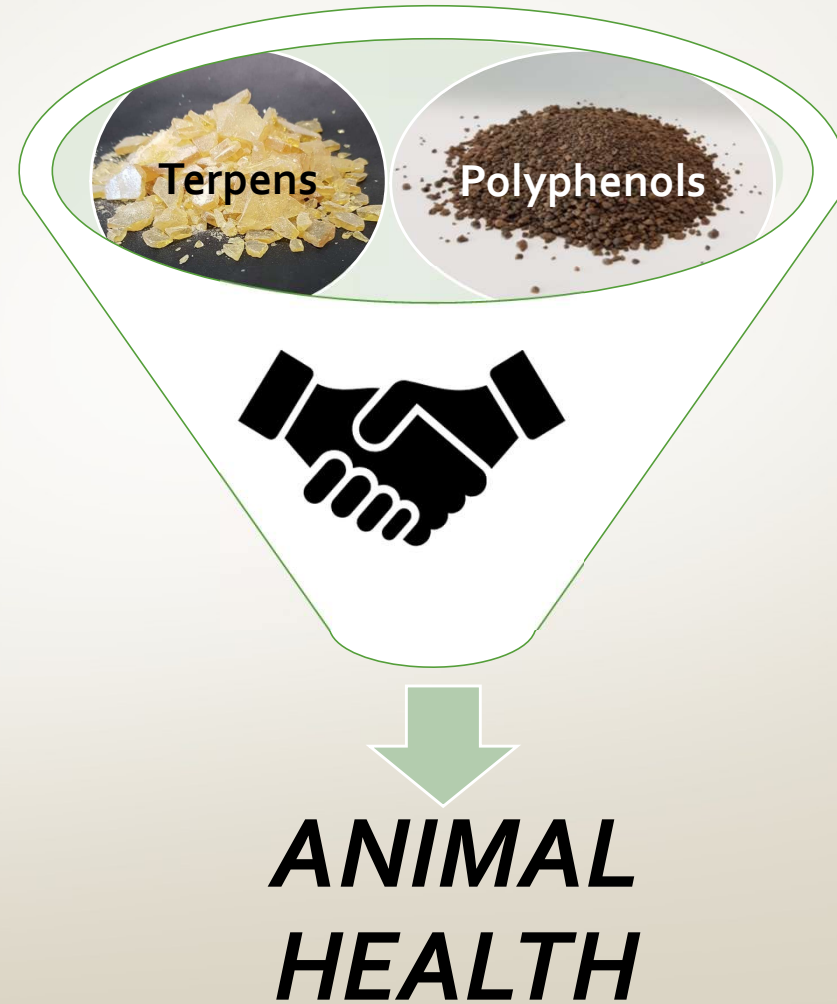
Technology developed by



Features:

- * 100% from plants
- * Made in Europe
- * Environmentally friendly
- *Terpens and polyphenols: two ingredients, one goal*
- * GMO free
- * For all animal species

Green
Innovation





Metabolic antioxidant

Antiinflammatory

Antibacterial

Prebiotic – gut modulator

Active towards Gram negative & Gram positive

OxiFish

White Leg Shrimp Trial



Green
Innovation



Kasetsart University
Bangkok Thailand
Dr. Orapin Jintasatporn

อรพินท์ จินตสภาพร



OxiFish Shrimp Trial

Objective

- ***Objective:*** to determine the effect of **OXIFISH** as a feed additive in White Leg shrimp diets on growth and health performance, and in response to a disease challenge against *Vibrio parahaemolyticus*.



OxiFish Shrimp Trial Protocol



- ***Design:*** Completely Randomized (CRD).
- **OXIFISH** added to a standard, nutrient balanced shrimp diets at 0.0, 1.0 or 3.0 kg/tonne.
- The 0.0 kg **OXIFISH**/tonne was the **CONTROL** diet.
- ***Each Treatment:*** six (6) replications for growth performance; four (4) replicates for the challenge test, for a total of ten (10) replicates (30 tanks).

OxiFish Shrimp Trial

Protocol

- *Thirty (30) tanks*: 3 treatments, 6 replicates growth + 4 replicates challenge.
- *Each tank*: 500 L capacity, but held 300 L 15 ppt saline water, pH 7.7-8.2, DO > 5 ppm, T=27-30 °C, total ammonia <1.0 ppm.
- *Juvenile Shrimp (~2 g)*: stocked at 80 shrimp/m³ (25 individuals/tank); feed given to shrimp 3 x's/day @ 3-4% body weight for 8 weeks.
- *Shrimp*: cultured in clear water system; 15-20% batch water-exchange every two (2) days.



OXIFISH Shrimp Trial

Diets*

Ingredients	Control, %	OXIF, 0.1%	OXIF, 0.3%
Fishmeal	20.0	20.0	20.0
Soybean meal	30.0	30.0	30.0
Squid and squid liver meal	5.0	5.0	5.0
Wheat gluten	6.0	6.0	6.0
Wheat flour	27.7	27.6	27.4
Tuna fish oil	1.5	1.5	1.5
Soya oil	1.0	1.0	1.0
Soy lecithin	2.0	2.0	2.0
Vitamin C	0.05	0.05	0.05
Monocalcium phosphate	2.0	2.0	2.0
Polymethyl carbamide	0.5	0.5	0.5
Vitamin-mineral premix	4.25	4.25	4.25
OXIFISH	0	0.1	0.3
Total	100	100	100
Crude protein	38.0	38.0	38.0
Crude lipid	7.0	7.0	7.0

*Shrimp feed materials were ground to 150-250 micron, mixed-together, and then water was added to 25% before passing through a Hobart mincer to form pellets, and these were then dried in a hot air oven.

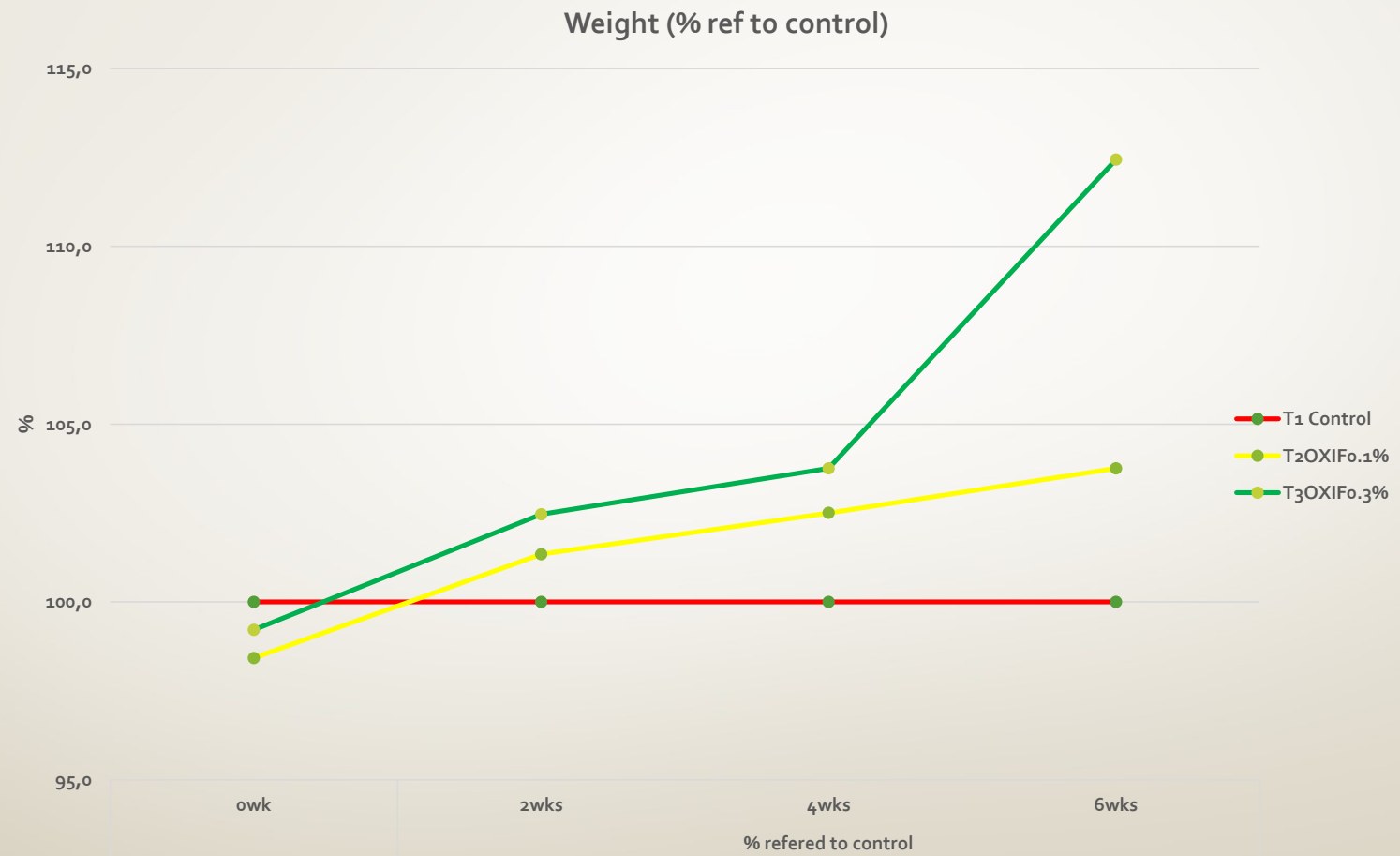
OXIFISH Shrimp Trial Growth Performance

Growth Performance	Period (wk)	T1: Control	T2: OXIF, 0.1%	T3: OXIF, 0.3%	P-value
Weight (g/ind)	0	2.54	2.50	2.52	0.658
	2	4.46	4.52	4.57	0.494
	4	6.39	6.55	6.63	0.341
	6	8.52^b	8.84^b	9.58^a	0.002
	8	12.44	12.78	13.18	0.133
Weight Gain (g/ind)	2	1.92	2.02	2.05	0.525
	4	3.85	4.05	4.11	0.335
	6	5.98^b	6.35^b	7.06^a	0.001
	8	9.89	10.28	10.66	0.123
Average Daily Gain (g/ind/d)	2	0.14	0.14	0.15	0.421
	4	0.14	0.14	0.15	0.306
	6	0.14^b	0.15^b	0.17^a	0.004
	8	0.18	0.18	0.19	0.128
Specific Growth Rate (%/d)	2	4.01	4.22	4.26	0.551
	4	3.29	3.44	3.45	0.367
	6	2.88^b	3.01^b	3.18^a	0.001
	8	2.83	2.91	2.95	0.147

a, b, c: mean values with different superscripts in a row are significantly different (p < 0.05).

Graf

OXIFISH Shrimp Trial



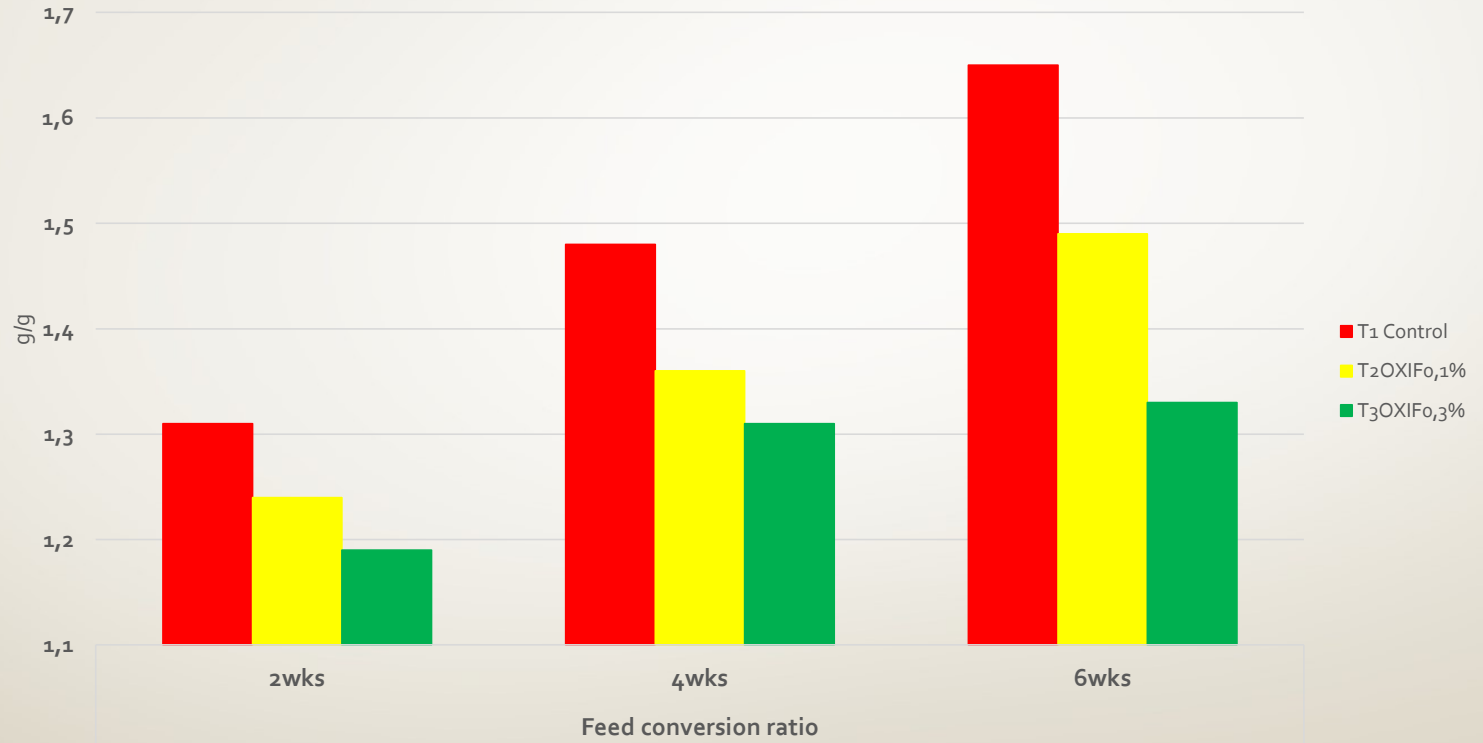
OXIFISH Shrimp Trial Growth Performance

Growth Performance	Periods (wk)	T1: Control	T2: OXIF, 0.1%	T3: OXIF, 0.3%	P-value
Feed Consumed (g/ind)	2	2.47	2.47	2.43	0.658
	4	5.63	5.50	5.35	0.235
	6	9.81	9.43	9.35	0.353
	8	14.54	14.37	13.91	0.704
Feed Conversion Ratio	2	1.31	1.24	1.19	0.411
	4	1.48	1.36	1.31	0.187
	6	1.65 ^c	1.49 ^b	1.33 ^a	<0.001
	8	1.47	1.40	1.31	0.237
Survival Rate (%)	2	97.33	97.33	98.67	0.667
	4	92.67	94.67	97.33	0.238
	6	86.00	89.33	90.00	0.37
	8	83.33	84.00	86.67	0.72

a, b, c: mean values with different superscripts in a row are significantly different (p < 0.05).

OXIFISH Shrimp Trial

Growth Performance: FCR



OXIFISH Shrimp Trial

Survival rate: 8 wks



- T1 Control
- T2OXIFo,1%
- T3OXIFo,3%

OXIFISH Shrimp Trial

Immunity – Normal Conditions

Parameters	Period (wk)	T1: Control	T2: OXIF, 0.1%	T3: OXIF, 0.3%	P-value
Haemocyte Count ($\times 10^5$ cell/ m^3)	4	29.00 ^b	32.67 ^{ab}	36.33 ^a	0.046
Hemolymph Protein (mg/dl)	4	4.80 ^b	5.16 ^a	5.16 ^a	0.010
Phenoloxidase Activity (unit/min/mg Protein)	4	157.75	159.74	163.61	0.901
Hepatopancreas (Log cfu/ml)	4	3.63 ^a	3.42 ^b	3.40 ^b	0.022
Intestine (Log cfu/ml)	4	4.79 ^a	4.55 ^b	4.43 ^b	0.002
Haemocyte Count ($\times 10^5$ cell/ m^3)	8	19.83	20.33	20.17	0.912
Hemolymph Protein (mg/dl)	8	17.86	18.67	21.27	0.422
Phenoloxidase Activity (unit/min/mg Protein)	8	74.32	93.59	82.12	0.084
Hepatopancreas (Log cfu/ml)	8	3.57 ^a	2.68 ^b	2.69 ^b	<0.001
Intestine (Log cfu/ml)	8	4.27 ^a	3.46 ^b	3.34 ^b	<0.001

a, b, c: mean values with different superscripts in a row are significantly different ($p < 0.05$).

OXIFISH Shrimp Trial

Proximate Composition

Proximate composition of white shrimp fed different levels of OXIFISH for 8 weeks under normal condition

Proximate Composition	T1: Initial*	T1: Control	T2: OXIF, 0.1%	T3: OXIF, 0.3%	P- value
Moisture (%)	76.71	74.47 ^a	73.58 ^b	72.68 ^c	<0.001
Dry Matter (%)	23.29	25.53 ^c	26.42 ^b	27.32 ^a	<0.001
Protein (%)	15.36	18.47 ^b	18.53 ^b	18.99 ^a	0.021
Lipid (%)	1.02	0.89 ^c	1.17 ^b	1.29 ^a	<0.001
Fiber (%)	1.32	2.34	2.58	2.36	0.130
Ash (%)	3.74	3.65 ^b	3.71 ^a	3.56 ^c	<0.001
Calcium (%)	0.49	0.86 ^b	0.90 ^a	0.84 ^b	<0.008
Phosphorus (%)	0.37	0.32 ^a	0.30 ^b	0.30 ^b	<0.001

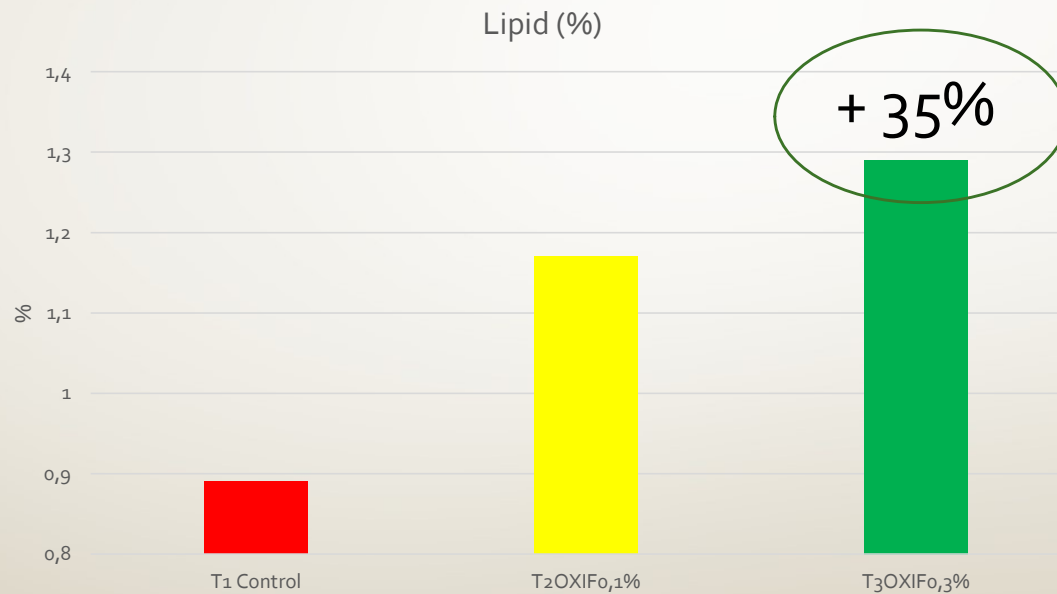
a, b, c: mean values with different superscripts in a row are significantly different (p < 0.05).

* Data do not compare to other data.

OXIFISH Shrimp Trial

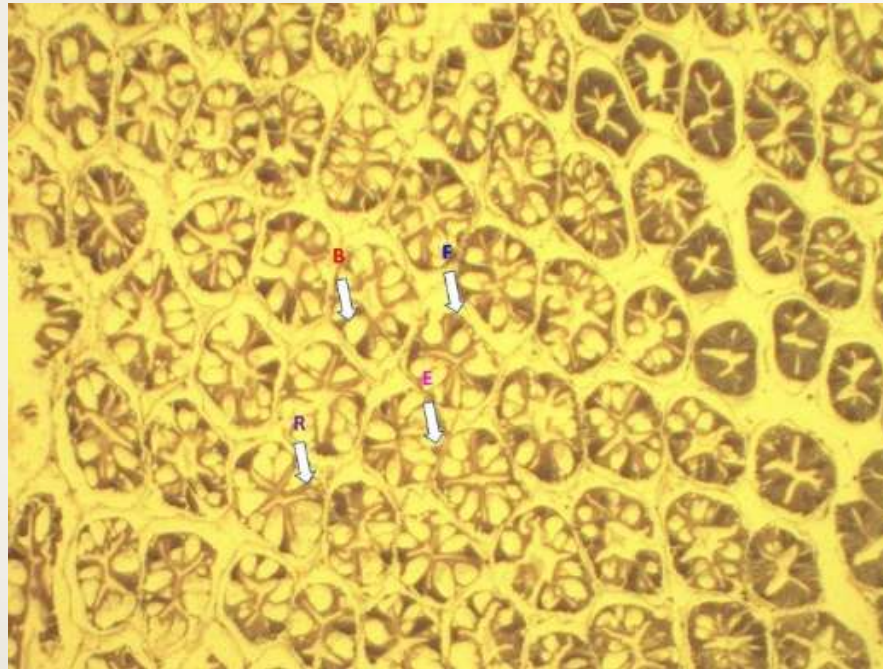
Proximate Composition

Proximate composition of white shrimp fed different levels of OXIFISH for 8 weeks under normal condition



OxiFish Shrimp Trial

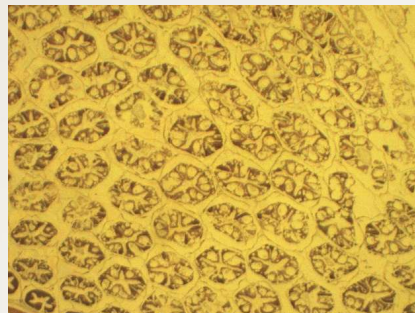
Histology (x 100)



E-cells (embryonic): found at distal tips of each tubule with proximal nuclei and conspicuous nuclear bodies; give rise to other three cell types of digestive glands. **R-cells (resorptive):** in hepatopancreas epithelia; main site of lipid storage; can be used to monitor nutritional condition in crustaceans. **F-cells (fibrillar):** responsible for protein synthesis and storage of minerals. **B-cells (blister-like):** main site of digestive enzyme synthesis; antioxidant enzymes accelerate the mobilization of nutrients in the hepatopancreas tubules.

OXIFISH Shrimp Trial

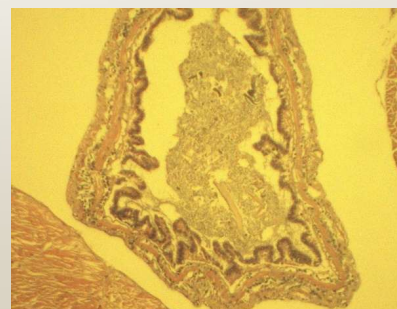
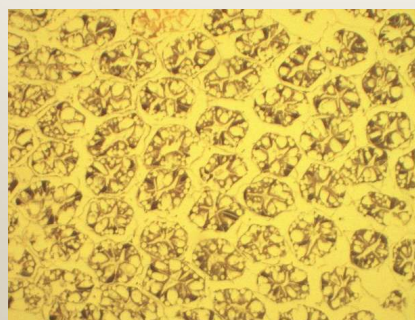
Histology (x 100)



T1: OXIFISH-0.0 Kg/tonne



T2: OXIFISH-1.0 Kg/tonne



T3: OXIFISH-3.0 Kg/tonne

OXIFISH Shrimp Trial Histological Findings

- **B-cells** in the hepatopancreas of shrimp fed **OXIFISH** were larger than those in shrimp fed the **T₁-control diet**.
- Moreover, a high lipid accumulation in **R-cells** was shown in shrimp fed **OXIFISH**, especially in those fed **OXIFISH @ 3.0 Kg/tonne**.
- In the intestine, shrimp fed **OXIFISH** displayed high villi density and greater villi fold and length in those shrimp fed **OXIFISH @ 1.0 and 3.0 Kg/tonne** than in the **T₁-control fed shrimp**.

OXIFISH Shrimp Trial

Survivability

Survival rate of white shrimp fed different levels of OXIFISH after immersion with 7.8×10^4 cfu/ml *Vibrio parahemolyticus* (EMS) for 15 days

Days after Immersion	T1: Control	T2: OXIF, 0.1%	T3: OXIF, 0.3%	P-value
0	100.00	100.00	100.00
3	87.50	95.00	97.50	0.060
6	72.50	85.00	85.00	0.059
9	65.00	70.00	75.00	0.500
12	55.00	65.00	70.00	0.228
15	47.50	57.50	67.50	0.075

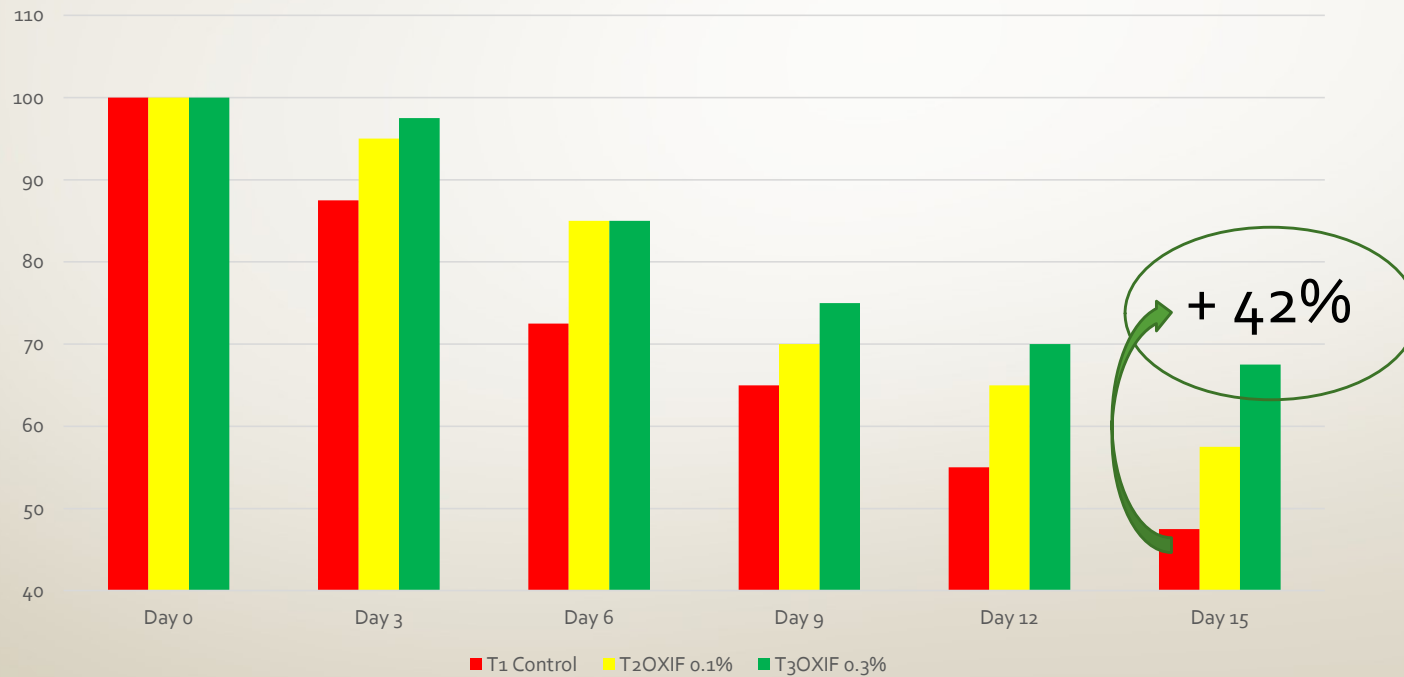
a, b, c: mean values with different superscripts in a row are significantly different ($p < 0.05$).

OXIFISH Shrimp Trial

Immune Challenge

Immunity of white shrimp fed different levels of OXIFISH after immersion with 7.8×10^4 cfu/ml *Vibrio parahaemolyticus* (EMS) for 15 days

Survival rate



OXIFISH Shrimp Trial

Conclusions

- Under normal conditions, **OXIFISH @ 0.1-0.3% (1-3 Kg/tonne)** promotes growth ($p < 0.05$) and improves feed utilization ($p < 0.05$) up to 6 weeks of age.
- Shrimp exhibited a better immune response @ 4 and 8 weeks in the presence of **OXIFISH** ($p < 0.05$), and they also exhibited improved ability to reduce the pathogen load in the digestive tract, especially *Vibrio spp.* ($p < 0.05$).
- When stressed by low concentrations of *V. parahaemolyticus* (EMS), shrimp fed **OXIFISH @ 0.1 and 0.3%** trended toward improved survival ($p = 0.059-0.075$) and showed better clearance ability ($p < 0.05$) by decreasing *Vibrio* counts in the hemolymph, the hepatopancreas, and in the intestine.

OXIFISH Shrimp Trial

Conclusions

- **OXIFISH** at 0.1 to 0.3% (1-3Kg/tonne) can improve growth performance and immunity under normal conditions and the stress imposed by a disease challenge from *Vibrio parahaemolyticus* (EMS).
- For better performance, the higher dosage of **OXIFISH** of 0.3% is recommended, and the application period should be ~6 weeks.

GENERAL CONCLUSIONS:

*polyphenols and terpens:
two ingredients, one goal*

*Effect towards Gram negative & Gram
positive bacteria*

Low dosage

Unique synergic effect & high value

**Thank you
for your attention**



*Let us produce
healthy food
together*



**Green Innovation
Team**