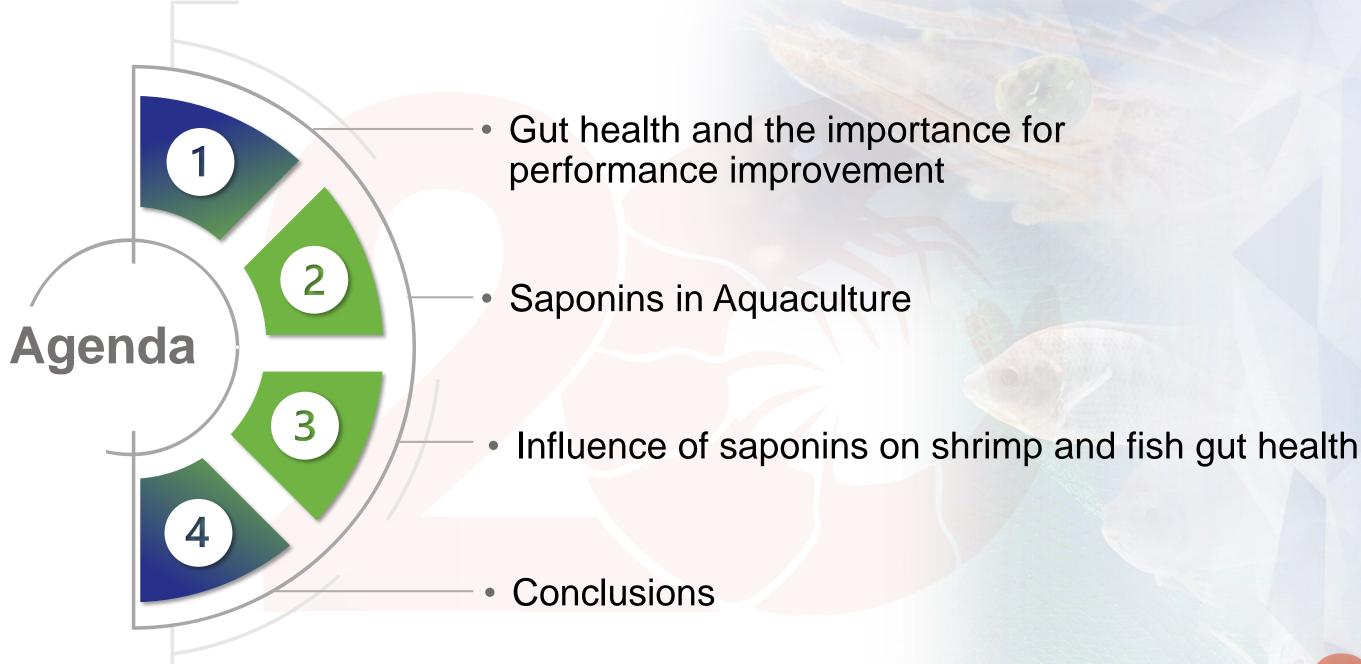
## Unlock the growth potential: The role of saponins in enhancing shrimp gut health



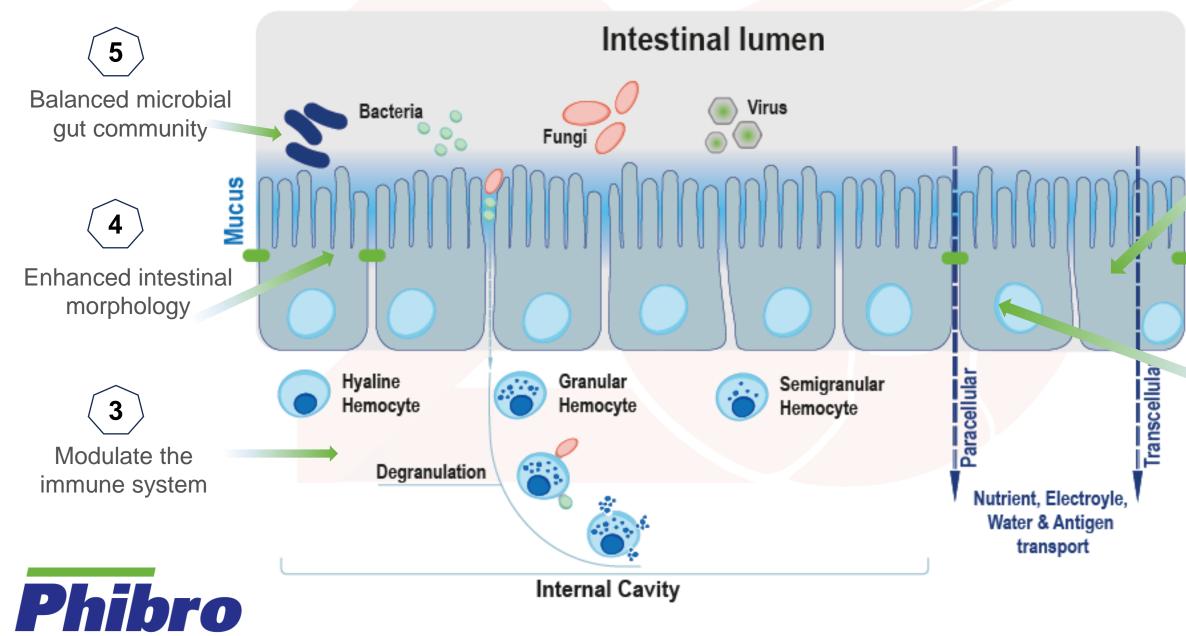
















#### Activity of digestive enzymes

Mucosal Layer

Tight junction



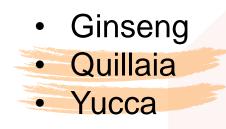


#### Nutrient digestion and absorption



## What are Saponins?

- Secondary plant compounds. •
- Contain lipophilic part & hydrophilic sugar side ulletchains.
- Can be found in several molecular forms. •
- Known for their hemolytic and piscicidal effects. •
- Can be found in several plant sources: ullet



- Soy •
- Asparagus
- Alfalfa •





OH

## Literature review of benefit of Saponin in aquafeed

search Article	quality, growth performance, blood health histomorphology of Nile tilapia, Oreochron		Research Article
The effects of <i>Yucca</i> s and enzymes a	Abmed M Abozeid <sup>1</sup> Mohamed M Abdel-Rahim <sup>4</sup>	ma Abouelenien <sup>3</sup>	The effects of <i>Yucca schidigera</i> and <i>Quillaja saponaria</i> on and enzymes activities of juvenile shrimp <i>Litopen</i>
The biol	ogical action of saponins in animal systems: a review		<i>Quillaja</i> saponins—a natural growth promoter for fish
<sup>1</sup> Department of Aquacul <sup>2</sup> Institute of Biochemistr	<ul> <li>Francis<sup>1</sup>, Zohar Kerem<sup>2</sup>, Harinder P. S. Makkar<sup>3</sup> and Klaus Becker<sup>1</sup>*</li> <li>ture Systems and Animal Nutrition, Institute for Animal Production in the Tropics and Subtropics, University of Hohenheim (480), D 70593 Stuttgart, Germany</li> <li>y, Food Science and Nutrition, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, P.O.B. 12, Rehovot 76100, Israel</li> <li>id Health Section, International Atomic Energy Agency, P.O. Box 100, Wagramerstr. 5, A-1400 Vienna, Austria</li> <li>(Received 4 December 2001 – Revised 19 June 2002 – Accepted 11 August 2002)</li> </ul>	fects of <i>Quillaja</i> rformance and h mly distributed in th received basal ml/m <sup>3</sup> per week tal diet suppleme eived basal diet s	George Francis <sup>a</sup> , Harinder P.S. Makkar <sup>b</sup> , Klaus Becker <sup>a,*</sup> <sup>a</sup> Department of Animal Nutrition and Aquaculture, Institute for Animal Production in the Tropics and Subtropics, University of Hohenheim (480), D-70593 Stuttgart, Germany <sup>b</sup> Animal Production and Health Section, Joint FAO/LAEA Division, International Atomic Energy Agency, P.O. Box 100, Wagramerstr: 5, A-1400 Vienna, Austria Abstract
g produ ascrib v they l C mals. r mollu v and r agent	ins are steroid or triterpenoid glycosides, common in a large number of plants and plant ts that are important in human and animal nutrition. Several biological effects have been ed to saponins. Extensive research has been carried out into the membrane-permeabilis- nmunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins and ave also been found to significantly affect growth, feed intake and reproduction in ani- These structurally diverse compounds have also been observed to kill protozoans and tes, to be antioxidants, to impair the digestion of protein and the uptake of vitamins unerals in the gut, to cause hypoglycaemia, and to act as antifungal and antiviral . These compounds can thus affect animals in a host of different ways both positive ggative. Saponins: Steroids: Triterpenoids: Biological activity	tract in water. R (YS, YS and QS) gher growth perfi- best findings bei ased intestinal vil hocytes, total pr rol, triglycerides, S compared with id/or YS supplem hile reduced make ng recorded in Q rgistic effects th	Environmental concerns and the forthcoming ban on antibiotics in the European Union have re- newed interest in renewable and non-persistent plant-based growth promoters in fish feeds. A review of the published literature and unpublished recent results following addition of a <i>Quillaja</i> saponin mixture in the diets of common carp and Nile tilapia are presented. It emerges that <i>Quillaja</i> saponins have the potential to increase growth in culture fish species, reduce their metabolic rate and suppress reproduction in tilapia. The current study is the first to have demonstrated beneficial effects of a <i>Quillaja</i> saponin-rich fraction when used as a feed additive in fish diet. It is hoped that this collation of information and synthesis of results, discussion and conclusions will act as a catalyst for future research on isolation of the active fraction, its optimum concentration for obtaining the desired effects, and physiological mechanisms of action for the diverse biological effects of these compounds. © 2005 Elsevier B.V. All rights reserved. <i>Keywords:</i> Saponins; Fish; Carp; Tilapia; Performance; Reproduction
0.25 g kg <sup>-1</sup> of NTF treatment       quality, growth performance, immune-oxid         the control group and treatment       intestine histomorphology.         detected in present study sug       KEY W O R D S         Keywords: Litopenaeus van       ammonia, blood health, feed efficiency, gills and			<ul> <li>b.25 g kg of 1011 treatment. However, any significant differences in enzyme activity for a control group and treatments. The increase effect in shrimp growth and any dedicated in present study suggest that NTF shows potential as a feed additive for s</li> <li>Keywords: <i>Litopenaeus vannamei</i>, growth, enzyme activity, low-salinity, aquac</li> </ul>

ANIMAL HEALTH CORPORATION

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#### owth performance <u>s vannamei</u>

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aquatic organisms ) with low-salinity eight) feeding with cultured in a close 1 parameters (body enzyme activities ere evaluated after nd feed conversion compared to other nrimp feeding with were detected with were detected between effect in enzyme activity

cultured at low-salinity.

#### /or lineard ail improved growth mmunecus fingerlings

#### ed<sup>1</sup>⊙

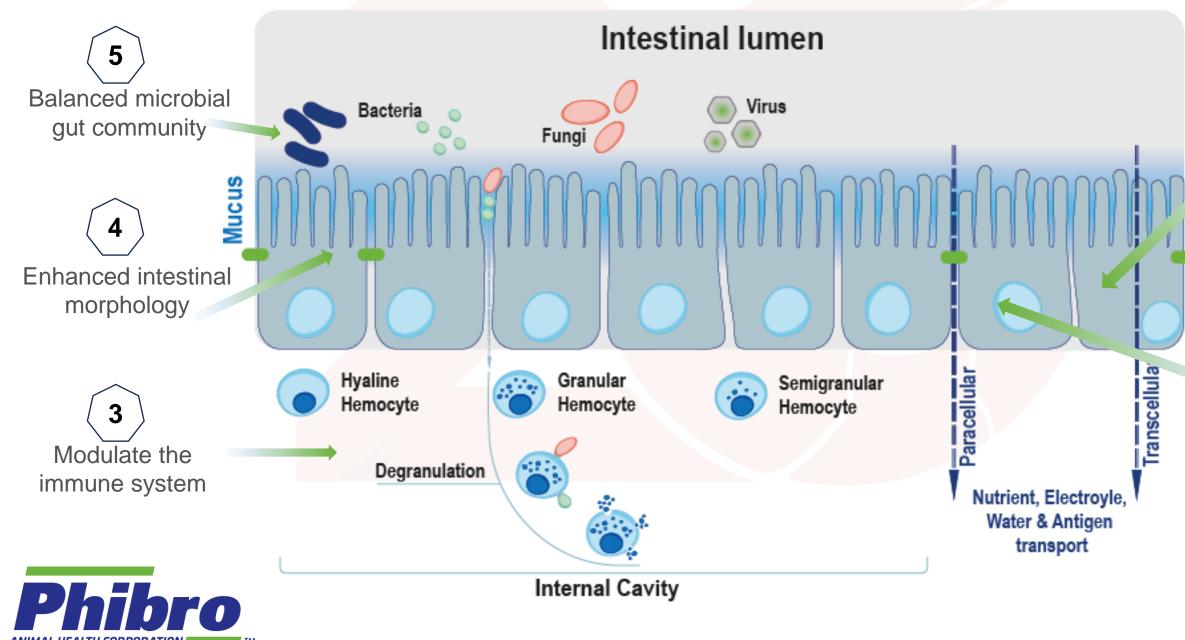
Quillaja Saponaria (QS) and elfare profile, blood health niloticus fingerlings. Fish 2 aguaria in triplicate. Fish liet (control group, CG), (2) ria (QS), (3) basal diet supmented with 300 mg kg<sup>-1</sup> aled a significant (p < 0.05) , specific growth rate and trogen and unionized ammage) in fish received QS O. Dietary inclusion of QS and reduced the cholesthe best findings in QS/LO. itase and reduced malonalve gene expression (insulin ctor-alpha) were improved QS/LO. In conclusion, dierformance, water quality, fingerlings. The best find-

atus, Quillaja Saponaria,

Fitzsimmons, 2016). Egypt is the on the African continent, with ,501,457 tonnes in 2018 (FAO, Africa, it was introduced to many cical, subtropical and temperate a-Sayed, 2006). Tilapia fish poswhich enable their culture under

© 2021 John Wiley & Sons Ltd 1









#### Activity of digestive enzymes

Mucosal Layer

Tight junction

Epithelial cell



#### Nutrient digestion and absorption



# Evaluation of saponin-based supplement (SBS) on the enzymatic activity of Pacific white shrimp, *Litopenaeus vannamei*

Production of digestive enzymes

	Species	Shrimp	
Ċ	Treatment	Control vs SBS	
	Number Replicas	4	
	Duration	28 days	
	Stocking	30 Shrimps/tank	
	Initial Wei <mark>ght</mark>	2g	
	Inclusion of SBS	2kg/MT	







## **Enhanced enzyme activity**

#### Enzyme activity in the hepatopancreas

### **Enzyme activity in the gut**

Enzyme (Ul/g)	Control	SBS	Enzyme (IU/g)	Control
Amylase	311.79 ±175	378.63 ±129*	Amylase	1242±384
Lipase	58 ± 28	57.03 ± 42	Lipase	104±25



Results are in line with Hernandez-Acosta et al. (2016)



#### SBS

#### 1811±312\*

#### 123±50\*



## **Enhanced enzyme activity**

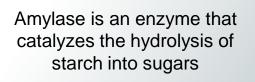
Amylase activity

Saponin supplement: Yucca (15%) and Quillaia (85%): ≥ 3.0 % w/w of saponin

Higher amylase activity leads to better chitin synthesis = induce growth

Shrimp molting is made of chitin that made of mono-sugars

Phibro





#### Saponins = glucosides that consist of a polycyclic aglycones

#### Polycyclic aglycones trigger amylase activity



## Lipase activity

Lipid requirement in shrimp is limited to 5-8%.

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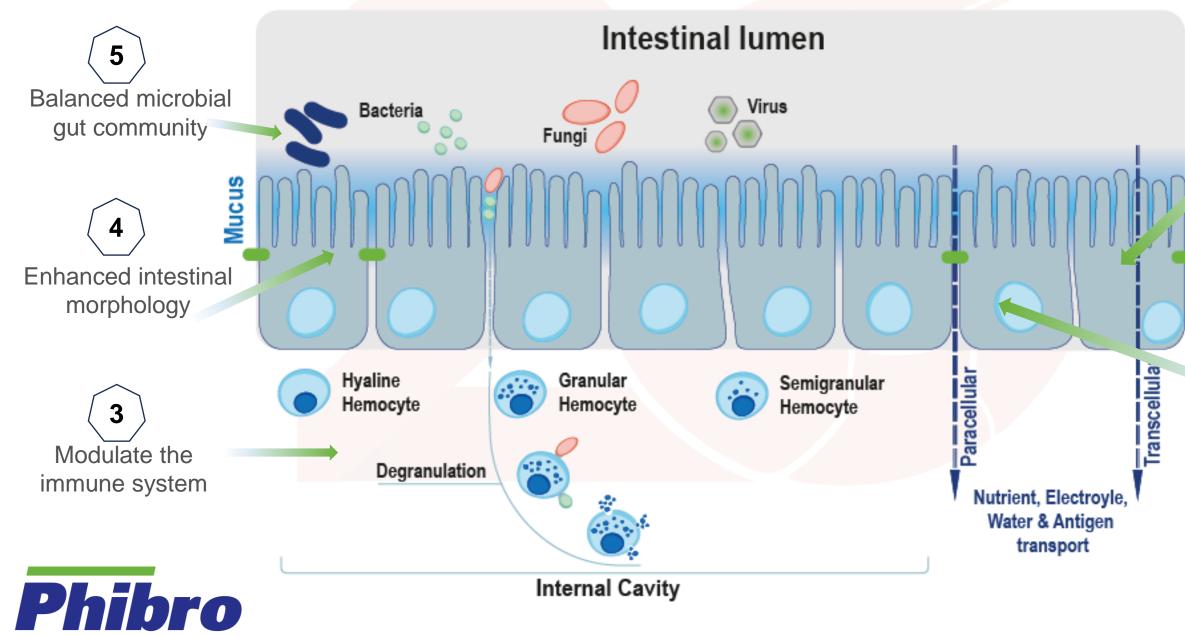
Enhanced lipase activity enable better extraction of fatty acids. Inducing lipase activity can lead to optimum growth by improving digestibility of fatty acids within the lipid constraint.



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HC





12

## Activity of digestive enzymes

Mucosal Layer

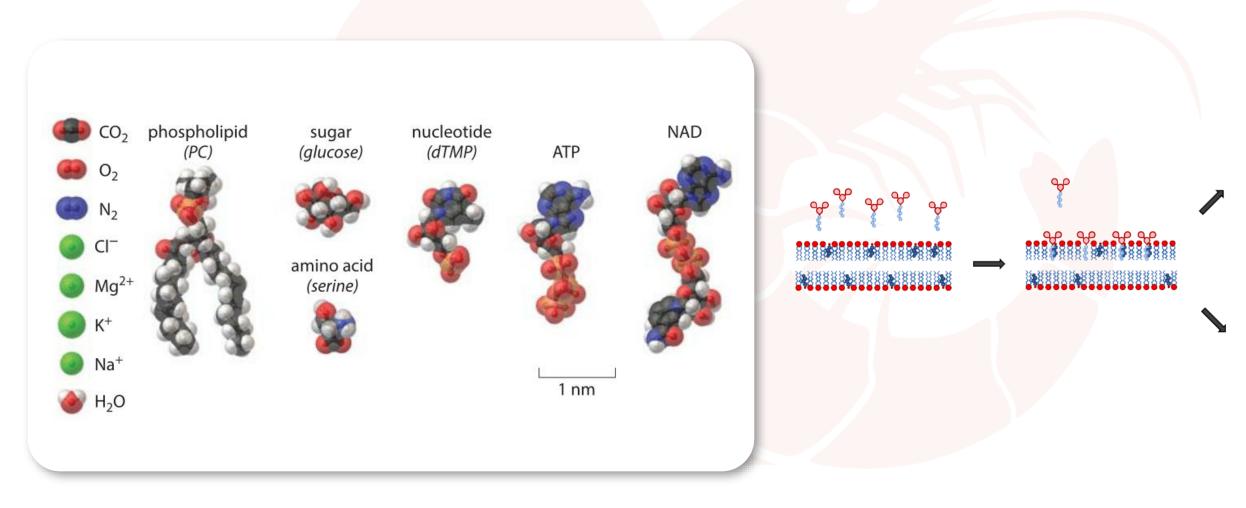
**Tight junction** 







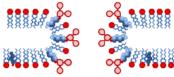
### The ability of Saponins to form pores and membrane elasticity



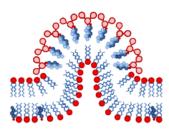


J.M. Augustin et al. / Phytochemistry 72 (2011) 435–457





(A) pore formation



(B) vesiculation



### Effect of SBS on digestibility of soybean-meal-based diet in *L. vannamei*

Improvement in digestion and absorption

Species	Shrimp	
Treatment	<ul> <li>Control vs SBS</li> <li>Control (9% fishmeal inclusion) without SBS</li> <li>Negative control (0% fishmeal inclusion Soybean based meal) without SBS</li> <li>Negative control with SBS 1kg/MT</li> </ul>	
Number Replicas	4	
Duration	60 days	
Stocking	100 Shrimps/tank	
Initial Weight	$0.51 \pm 0.10g$	

**Body** composition

Analysis:

Proteolytic enzymes activity and in vitro protein digestibility; protease, trypsin and chymotrypsin

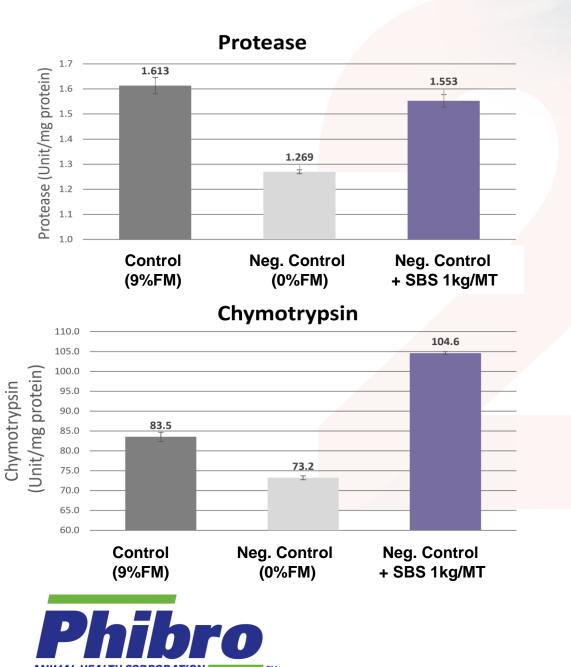




#### Growth & survival



### Effect of SBS on digestibility of high soybean meal diet with L.vannamei



	Control (9%FM)	Neg. control (0%FM)	Neg. control + SBS 1kg/MT
Protein	75.8±1.1	75.0±0.7	75.3±2.1
Arginine	7.74	7.93	8.95
Histidine	0.71	0.73	0.74
Isolucine	1.2	1.3	1.59
Leucine	1.99	1.93	2.44
Lysine	3.69	3.76	4.44
Methionine	0.79	0.81	0.85
Phenylalanine	1.37	1.34	1.17
Threonine	1.21	1.36	1.07
Tryptophan	0.53	0.39	0.42
Valine	1.21	0.35	1.32
Sum EAA	20.4	19.9	22.9

\*EAA (Essential Amino Acids)

1





### SBS Metabolic analyses of shrimp – protein level and retention

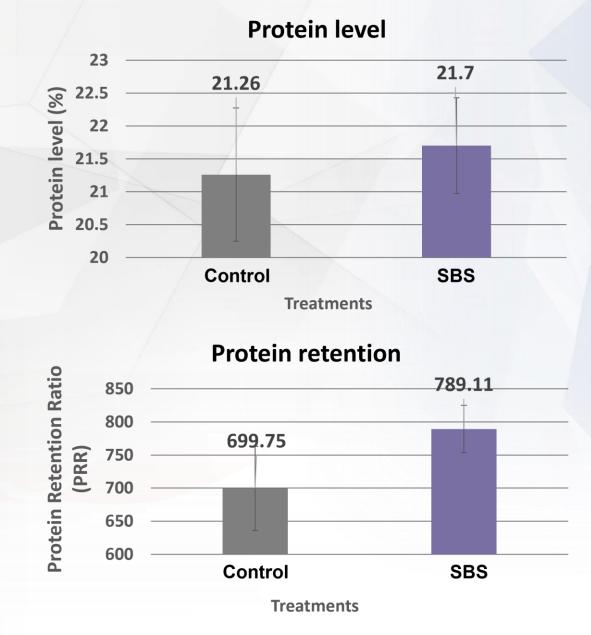
Batam Dae Hae Seng research station, Indonesia

	Species	Shrimp
Ø	Treatment	Control vs SBS
	Number Replicas	6
	Duration	60 days
	Stocking	180 Shrimps/tank
	Initial Weight	Size- 4.24±0,03 g initial weight with <i>L. vannamei</i>
	Inclusion of SBS	2kg/MT

#### • Analysis:

- Protein level %: Kjeldahl method
- Protein retention ratio (PRR)

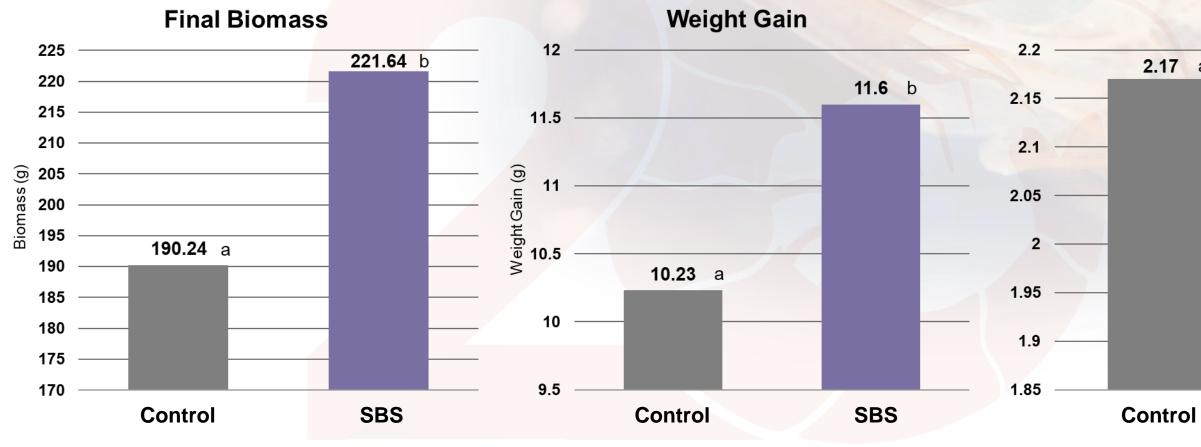








### **Results** Performance





\* Indicates significantly difference p<0.05

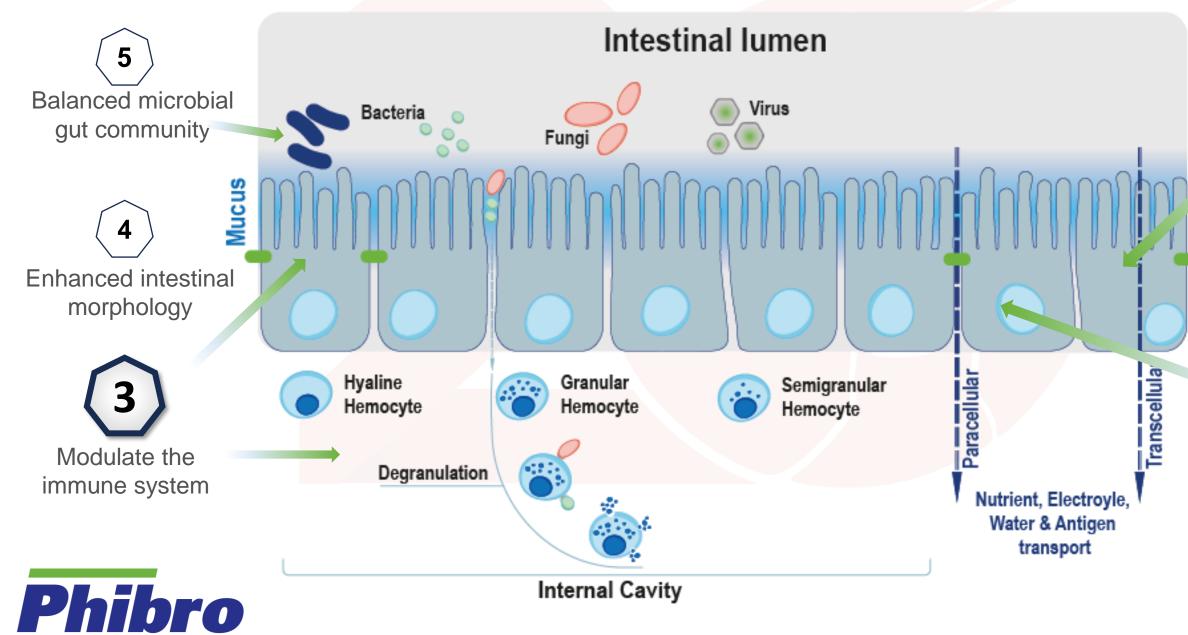


#### FCR

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## Activity of digestive enzymes

**Mucosal Layer** 

**Tight junction** 

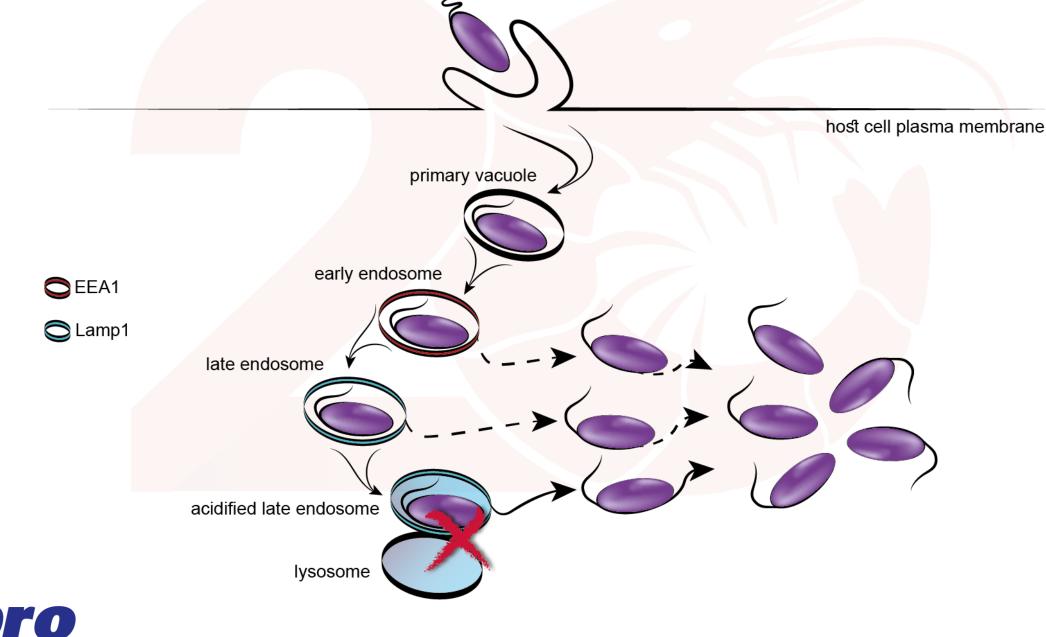




Nutrient digestion and absorption



### Intracellular Vibrio parahaemolyticus escapes the vacuole and establishes a replicative niche in the cytosol of epithelial cells

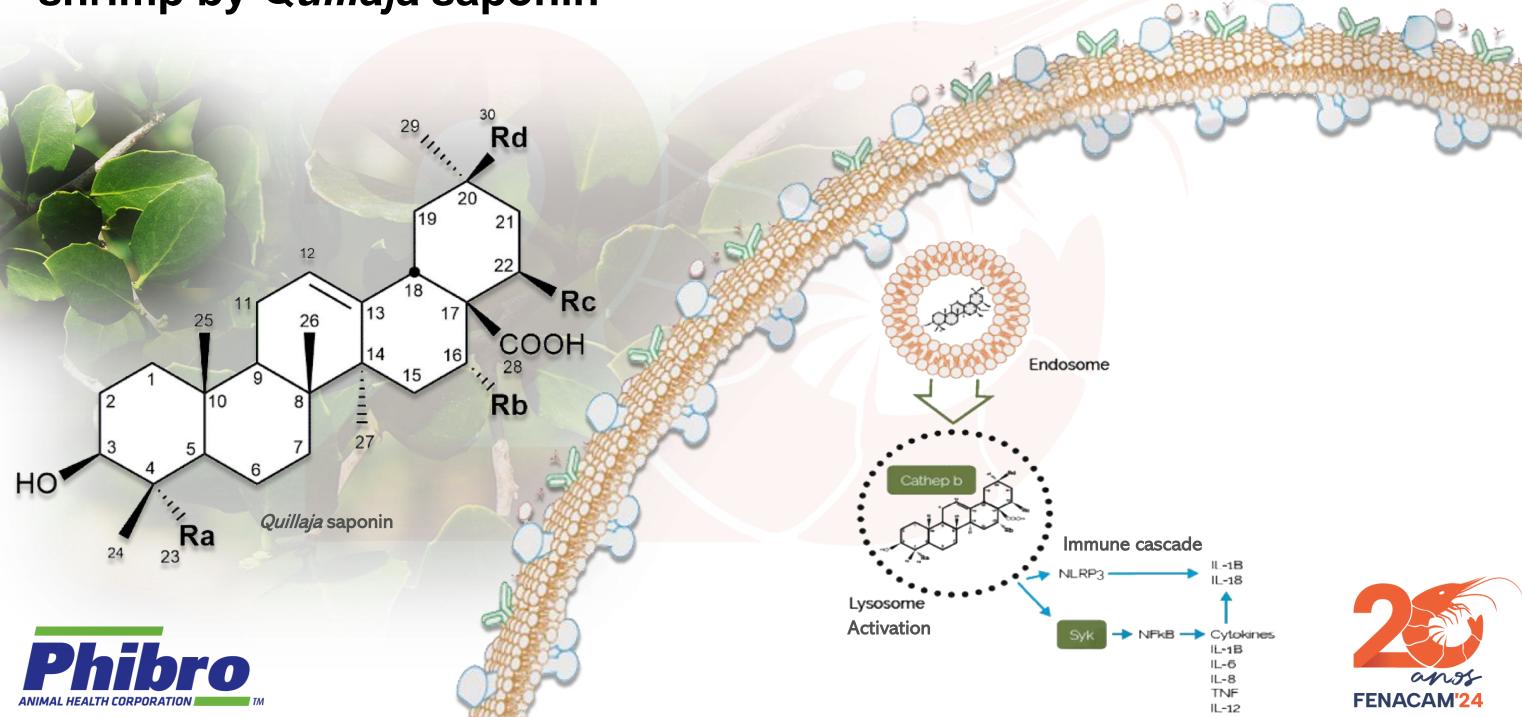








### Proposed mechanism of enhancement of immune response in shrimp by Quillaja saponin







# Evaluating the effect of SBS on growth performance and overall health status of *Litopenaeus vannamei* culture under normal and challenge conditions

Kasetsart University, Thailand - 2021

	Species	Shrimp <i>L. vannamei</i>
Ø	Treatment	Control vs SBS 3 Kg/MT feed
	Number Replicas	6
	Duration	8 weeks
	Stocking	25 Shrimps/tank
	Initial Weight	Size 2±0.05g
	Challenge	Vibrio parahemolyticus

#### Analysis:

- Growth performance
- Blood and biochemistry
- Bacteria count

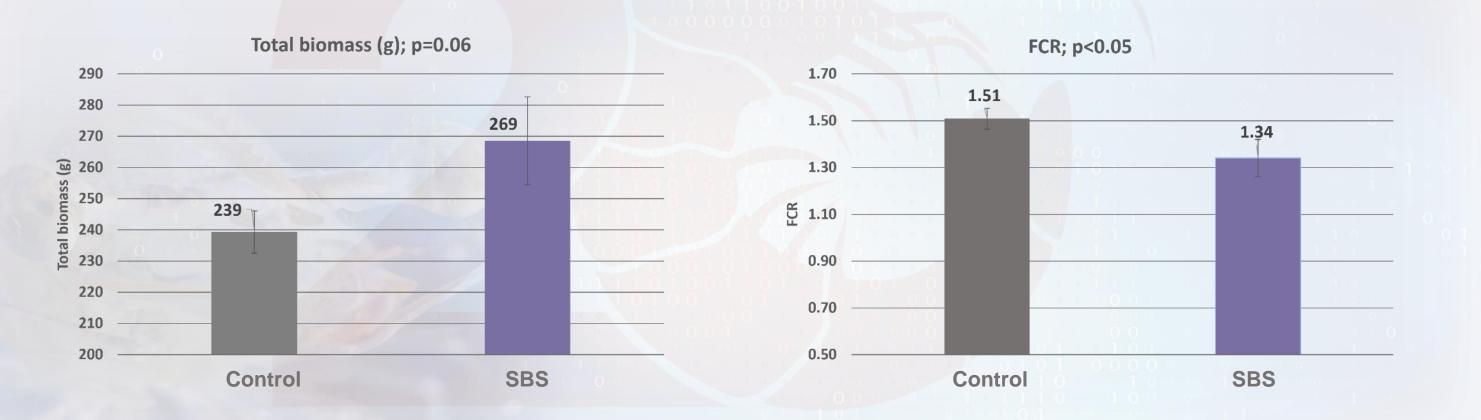






## Evaluating the effect of SBS on growth performance and overall health status of *Litopenaeus vannamei* culture under challenge conditions

Growth performance normal conditions



Survival was higher with SBS







### Evaluating the effect of SBS on growth performance and overall health status of *Litopenaeus vannamei* culture under challenge conditions

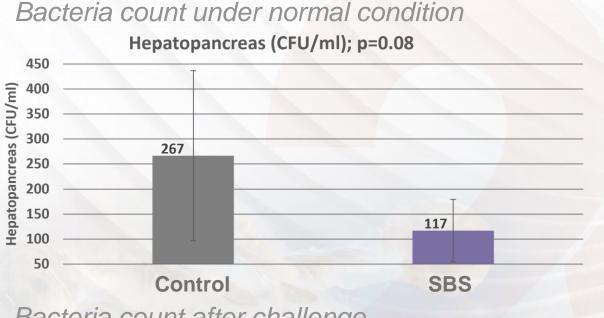
Immune parameters under challenge, one week after challenge

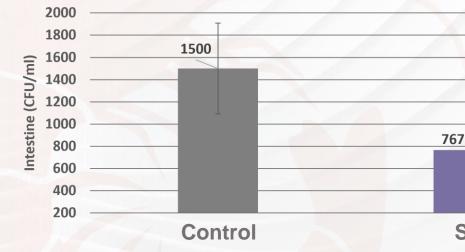
	Hemocyte count (x10 <sup>5</sup> cell/ml)	Hemolymph Protein (g/dL)	Phenoloxidase activity(unit/min/ mg Protein)	Lysosyme	Superoxide dismutase (SODN)	Glutathionine
Control	29.7	1.0	250.6	30.0	7.4	30.3
SBS	32.7	2.1	284.3	43.3	11.1	32.4
P value	0.02	0.31	0.21	0.001	0.04	0.01



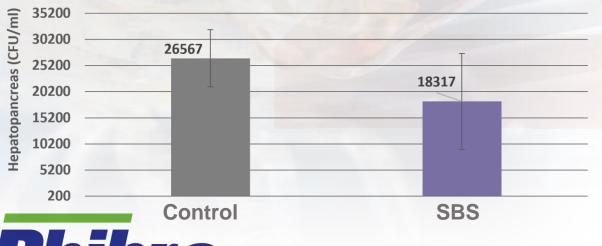


## Evaluating the effect of SBS on growth performance and overall health status of *Litopenaeus vannamei* culture under normal and challenge conditions

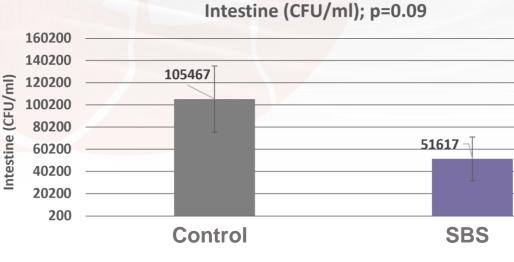




#### Bacteria count after challenge Hepatopancreas (CFU/ml); p=0.3







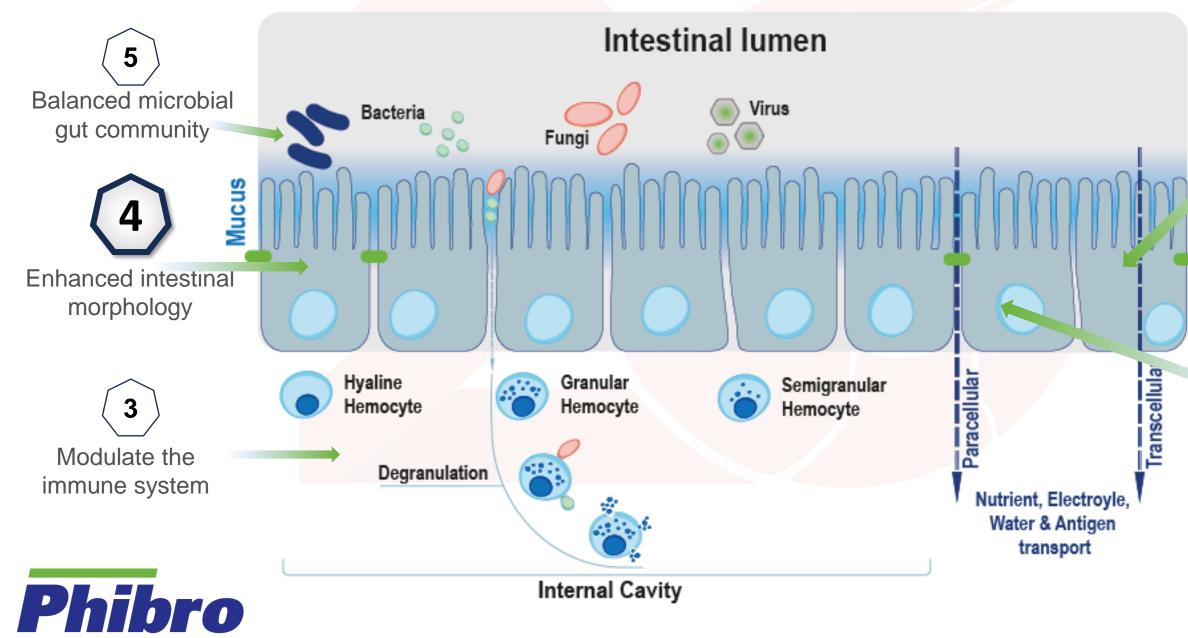
Intestine (CFU/ml); p=0.3





SBS







## Activity of digestive enzymes

**Mucosal Layer** 

**Tight junction** 





Nutrient digestion and absorption

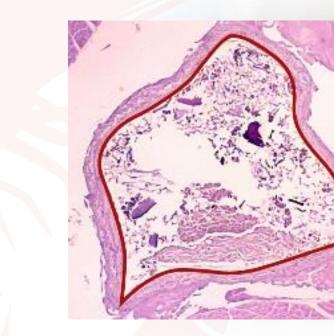


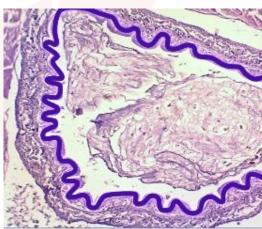
## Inclusion effect of SBS in plant-based diet on histomorphology conditions of white-leg shrimp, *Litopenaeus vannamei*

Ċ	Treatment	<ul> <li>Commercial feed</li> <li>Commercial feed + 2kg/MT of SBS</li> </ul>	
	Animals	Litopenaeus vannamei, 2g (initial weight) 15 shrimp/aquarium	
	Duration	90 days	
	Facility	24 aquariums tanks: 75 x 40 x 40 cm (100 L each)	
	Тетр	26 °c	
	Replicates	8	
	Location	Center for marine and fisheries, Jakarta Technical University of Fisheries located in Serang, Banten, Indonesia.	

Growth performance was significantly better at the SBS treatment









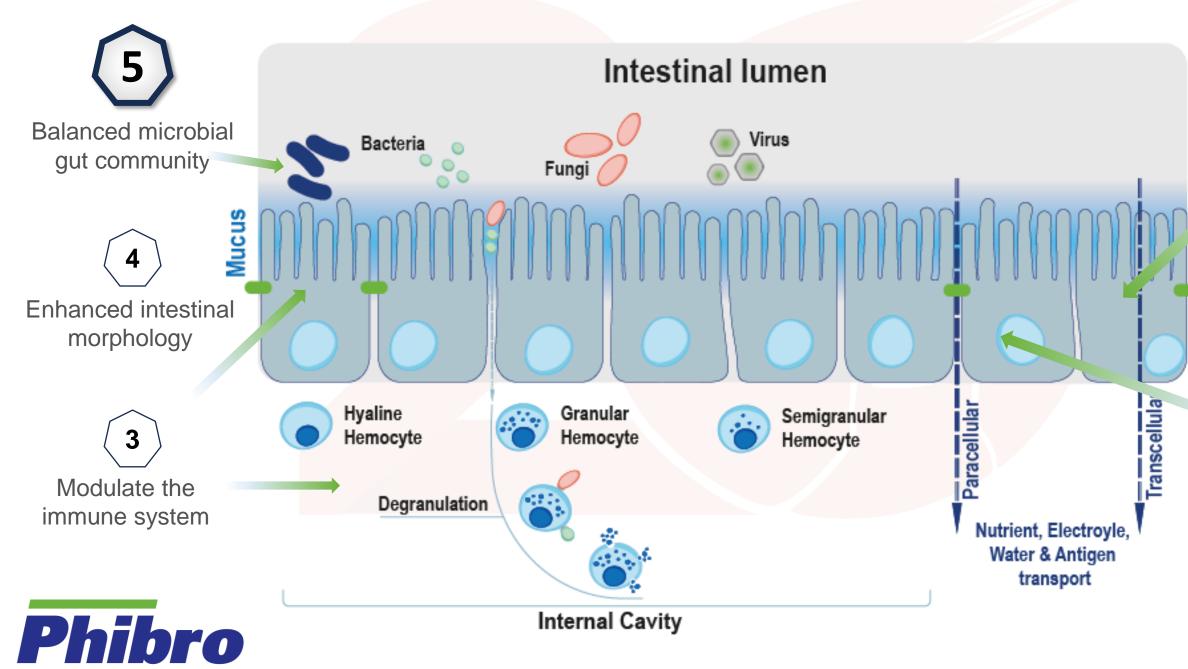
#### Grade 0 – Low level to no intestinal folds



 Commercial feed + 2kg/MT of SBS

Grade 4 – High level of intestinal folds







## Activity of digestive enzymes

**Mucosal Layer** 

**Tight junction** 



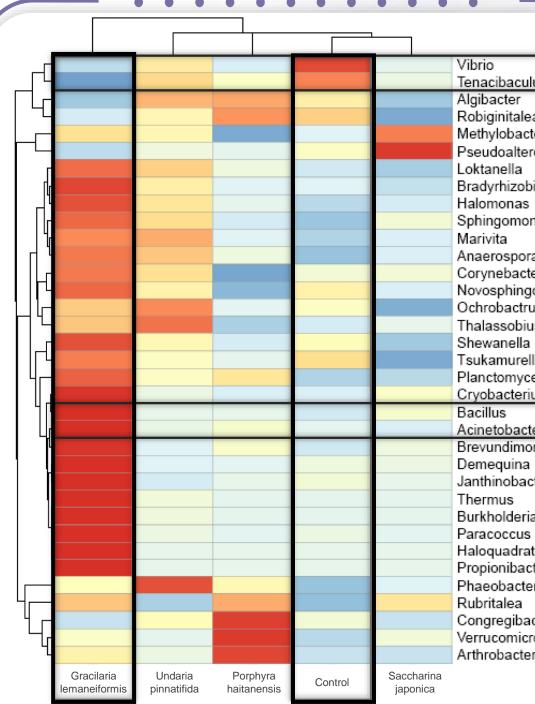


Nutrient digestion and absorption



## The Effect of Phytogenic on Microbiome in Shrimp Gut

- Intestinal microbiota supplies the host with nutritional and energy, acts as a pathogenic barrier, and exerts great influence on the maintenance of immune homeostasis
- Gracilaria lemaneiformis contains triterpenic saponins.
- *Gracilaria lemaneiformis* increased α-diversity of microbes in the intestine.
- We see significant impact on the microbial community vs control

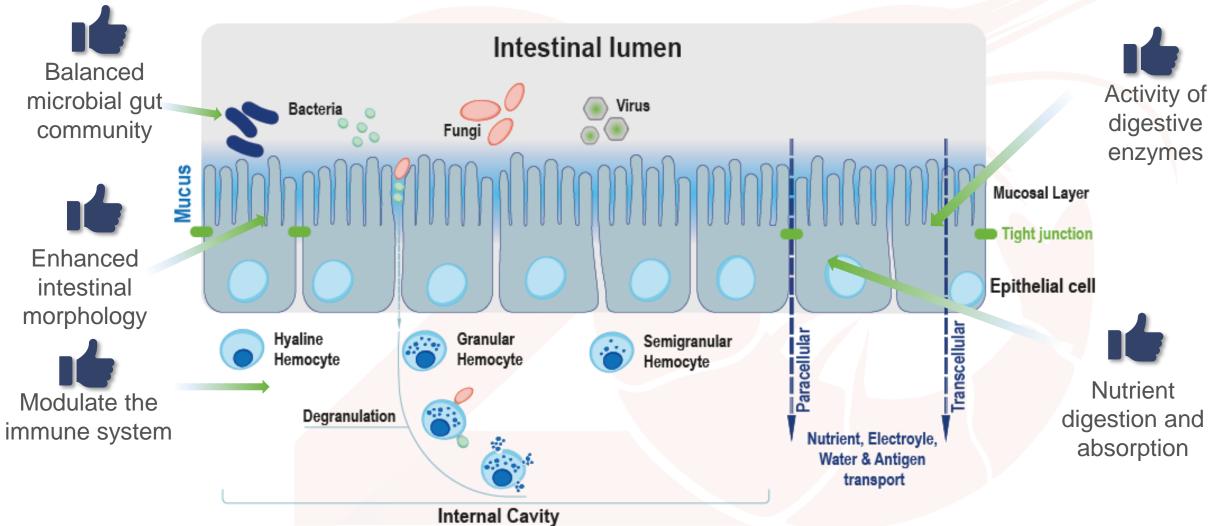




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#### (Quillaia sanonaria e Yucca schidiger

Indicação do Produt Aditivo melhorador de desempenho para melhora da integridade intestinal e aumento do ganho de peso eficiência alimentar e imunidade de peixes e camarões. Composição Básica do Produto: Quilaia (*Quillaja saponaria*) 85 % e Yucca schidigera 15 %. Níveis de Garantia: Quillaja saponaria (mín) 850 g/kg e Yucca schidigera (mín) 150 g/kg Dosagem e Modo de Usar: Misturar a ração de forma contínua nas do nutricionista Recomenda-se uma pré mistura do produto com milho me fino ou premix, antes da mistura final Restrições de Uso. Contraindicações e Cuidado Restruções de Uso, Loitranancações e Luídados: Assim como qualquer aditivo para ração, PAQ-Protex<sup>®</sup> deve ser mantido sob os cuidados comuns de segurança. Pode causar intrações nos olhos, pele, sistema respiratório ou reações alergicas devido ao contato com o podudo. Ao manojadar o produto, usar equipamentos de proteção individual (roupa proteíbras, diculos, luvas impermeáveis e máscara antipó). Os operadores devem tomar banho após a jornada de trabalho. Se acontecer oblicito mentiamente, levindo o penís lavar com gua abundante. Em caso de ingestão addenta procurar o relácito mentiamente, levindo o penís lavar com gua abundante. Em caso de ingestão addenta procurar o relácito mentiamente, levindo o penís lavar com penístico de poteção addenta procurar o lagos, cursos de água ou camais com produto ou recipientes. Condições de Armazenamento Conservar na embalagem original, em lugar fresco e seco, a temperatura ambiente. Evitar a luz solar. Mante fora do alcance de crianças. O DA AC Fabricado por: Desert King Chile S.A. - LEEAA: VA-07-001 Fundo El Refugio No Lote A-17-4 Camino 3, Parcelacion Fundo El Refugio, Casablanca, Region De Valparaiso, Chile Aditivo formulado. Exclusivo para uso em ri 'BRASIL ESTABELECIMENTO hos - SP - BRASI REGISTRADO SP 000347-6

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## Thank you!

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