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ORIGINAL ARTICLE

Modulation of the gut microbiota of Pacific white shrimp (*Penaeus vannamei* Boone, 1931) by dietary inclusion of a functional yeast cell wall-based additive

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Abstract

Several eco-friendly natural substances can enhance the shrimp immune defence system therby acting as a prophylactic agent in feed additives. Agents such as (1, 3)-(1, 6)-D β-glucan and complex mannan-oligosaccharides located in yeast cell walls present such immunomodulatory and potential prebiotic properties. The aim of our study was to evaluate the effect of a commercial yeast cell wall extract (YCW) on shrimp performance and health status, and influence on gut microbiota. Juvenile *Penaeus vannamei* (Boone, 1931) were raised at an intensive shrimp farm and fed with two different diet inclusions levels of YCW, that is, 0.5% and 1.0%, in addition to a yeast free control group. After 102 days, animals were sampled, and standard nutrition performance parameters were measured. Additionally, the phylogenetic profile and composition of shrimp gut microbiota were evaluated. Animal performance, including growth and survival, was significantly better on animals fed with YCW than the control group. Furthermore, beneficial bacteria phylotypes were stimulated by the presence of YCW, positively modulating the gut microbiota, with emphasis on 1.0% YCW treatment. Therefore, YCW can be regarded as a prophylactic functional agent in the intensive rearing of juvenile *P. vannamei* thus improving animal performance and contributing to a healthy intestinal microbiota.

KEYWORDS
feed additive, gut microbiota modulation, nutrition, shrimp growth, shrimp health, Yeast extract

1 | INTRODUCTION

Aquaculture is the most rapidly growing animal production sector and shrimp production already exceeds the capture fishery yield (Flegel, 2019). However, intensive shrimp production is facing many global challenges, particularly with respect to infectious diseases such as Acute Hepatopancreatic Necrosis Disease (AHPND) and White Spot Disease (WSD). This scenario has led to the assessment that future sustainable shrimp aquaculture will depend on the development of more efficient bio-secure production systems that rear specific pathogen-free shrimp. This along with genetically improved stock for growth and disease tolerance or resistance will be an increasing trend (Castillo-Juárez et al., 2015).

It is also becoming recognized that nutrition and animal health are intrinsically related and are now playing a major part in animal production including aquatic species such as fish and shrimp with

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major studies directed to this field (Davies et al., 2019). An optimal nutritional condition leads to a satisfactory immune response when animals face an infectious or non-infectious challenge such as stress and environmental pressures. Nowadays, aiming at an eco-friendly and sustainable shrimp farming industry, researchers and farmers are searching for new prophylactic treatments to prevent and protect animals against pathogens and increase resistance during stressful situations (e.g. disease conditions, environmental disturbances, inadequate nutrition, handling) (Thitamadee et al., 2016). The concept is to develop nutritional strategies to enhance animal health and production, without the use of pharmaceuticals and antibiotics, the latter being of particular concern due to antimicrobial resistance and other emerging pathogens.

Natural compounds (nutritional or non-nutritional) can positively modulate the animal immune system, resulting in increased resistance against diseases and/or pathogens (Zhang & Mai, 2014). Also, they promote better growth and production efficiency. Many of the natural compounds are termed as prebiotics and consist of defined carbohydrate cell wall structures mostly originating from fungi, bacteria, plants, microalgae and macroalgae (Ringø et al., 2010; Song et al., 2014). These have proved to be most attractive for aquaculture as they are derived from natural sources and meet consumer expectations for safety and environmental concerns. The yeast cell wall, for example, is composed by different polysaccharides (Ringø et al., 2010), which may have prebiotic effects. Polysaccharides are polymers of simple sugars with two main biological functions: energy storage and extracellular structural integrity (Nelson & Cox, 2004). The mechanism of action of polysaccharides is related to specific binding proteins, opsonins and other defence proteins that activate the cellular function when reacting with β-glucans or LPS, that is, when the β-glucans bind to the receptors on the haemocytes the stimulation of the immune response may be initiated (Karunasagar et al., 2014; Meena et al., 2013; Smith et al., 2014).

The gut microbiota has been described as the 'new organ' in the animal imparting major systemic effects (Baquero & Nombela, 2012; McFall-Ngai et al., 2013), with an important role in digestion (physiology), nutritional metabolism and influencing immune components in disease and inflammatory responses (Akhter et al., 2015). The factors to be considered in conducting microbiome research were described comprehensively by Goodrich et al., (2014) in terms of design, execution and interpretation of data analysis for animal studies. To date, less work has been undertaken on fish and shrimp compared to other species and humans.

The relevance of the microbiome for the Pacific white shrimp has been evaluated by Cornejo-Granados et al., (2017) showing differential bacterial community composition between wild, aquaculture raised and shrimp with AHPND/Early Mortality Syndrome outbreak conditions. Wild shrimp presented larger biodiversity gut microbiota than shrimp raised under controlled conditions. Likewise, AHPND/Early Mortality Syndrome led to a loss of hepatopancreas microbiota diversity. These authors have stated that little is known about shrimp microbiota remodelling. With all the different scenarios that shrimp will encounter, there is a need to relate the microbiota to the diet composition and potential to modulate favourably the defence barrier mechanisms in shrimp. There is now much importance given to the effect of functional feed additives in aquaculture and particularly in fish but also gaining momentum for shrimp (Gainza & Romero, 2020). Thus, there is much interest in comprehending the links between diet immune stimulants and the microbial ecology of the gastrointestinal tract.

It has been stated by Dai et al., (2020) that the gut keystone taxa disproportionally affect the function and stability of their resident community and thus are candidate targets for improving host health. However, exactly how disease progression changes the assembly of gut bacterial community remains unclear requiring more elucidation. Their influence on shrimp gut microbiota can be a great asset to understand patterns in bacteria and environmental changes. Thus, the search to include sustainable feed supplements into shrimp diets could lead to the stabilization of potential beneficial bacteria in the digestive system and act as effective control agents to mitigate against disease and infection. This has been recently comprehensively reviewed by Holt at al. (2020). These authors have stated how gut microbiome manipulation may offer an attractive option for aquaculture (e.g. improved digestion, immunity and health as well as an alternative to antibiotics). It has been suggested as a possible alternative to the use of broad-spectrum antibiotics in the management of disease processes in shrimp.

Therefore, the purpose of our study was to identify the biological potential and environmental and microbial ecological aspects relating to the composition of gut microbiota in cultured shrimp when fed with a commercial complex of brewer's yeast cell wall extract with supportive proteic by-products (enzymes) from fungus (powder from *Trichoderma longibrachiatum* spp) (PAQ-Gro™ Phibro, Teaneck, USA). PAQ-Gro™ is a unique, patented propriety feed additive product for fish and shrimp diets. It has been stated that it can improve growth performance, FCR, survival rates and the overall health status of shrimp and fish during the critical hatchery, nursery and grow-out phase. We examine this potential in juvenile Pacific white shrimp (*Penaeus vannamei* Boone, 1931) under typical intensive farming conditions employing a commercial propriety feed.

2 | MATERIALS AND METHODS

2.1 | Experimental design

A total of 1,200 juvenile Pacific white shrimp (*P. vannamei*), 3 g ± 0.25 g, were raised for 102 days in an intensive system with seawater with minimum water exchange (<10% cycle), in the experimental station of Laguna Aquaculture, Colima city, Mexico. Animals were kept in twelve floating cages randomly allocated in a 1500 m^2 shrimp pond with HDPE liner with sand bottom. Floating cages dimensions were as follows: a) length: 1.60 m; b) width: 1.65 m; depth: 0.60 m; bottom area: 2.56 m^2; volume: 1.58 m^3. Floating cages were preferred rather than free on the pond due to better control of the trial conditions. A single anchoring kept the cages flowing around the ponds. No solid mud or

soil was inside the cages, as they were 0.6 m depth. Cages were covered with 5 mm mess to keep shrimp inside the cages and to avoid predation, especially from birds. Shrimp were randomly distributed in twelve cages, 100 animals per cage and four cages per treatment. Initial shrimp density was 63 shrimp per m^3. The same stocking density was used outside the cages; thus, the standard farm protocol and commercial conditions were maintained. Water was pumped from an artesian well with oceanic salinity. Constant aeration was provided by two horsepower (hp) paddlewheel aerators located outside the floating cages, with total capacity of 16 hp per hectare.

The water quality parameters were monitored daily throughout the duration of the trial. The minimum and maximum values observed during the study period were as follows: salinity (33–35 g L^{-1}), ammonia (0.1–0.3 mg L^{-1}), nitrite (0.2–0.5 mg L^{-1}), pH (8.5–9.6), water temperature (29–34°C) and dissolved oxygen (4–10 mg L^{-1}). Biomass measurements occurred weekly, with 20 shrimp per cage, that is, 20% of the total population were randomly selected with the use of nets and individually weighed at the site.

Growth performance and feed efficiency were determined with the indexes described below. Statistical analyses were calculated using one-way ANOVA, followed by Tukey post hoc test, with a *p*-value < .05 considered statistically significant.

- Survival (%) = [(initial number of shrimp in the cage - final number of shrimp in the cage) / initial number of shrimp in the cage] × 100
- Weight gain (g) = final body weight – initial body weight
- Specific growth rate (SGR, g.week^{-1}) = [(ln final shrimp weight – ln initial shrimp weight) / days of feeding trial)] x 100
- Feed conversion ratio (FCR) = weight of total feed provided / weight gain
- Final yield (g.cage^{-1}) = final net biomass – initial net biomass

2.2 | Experimental diets

Two different doses of a commercial shrimp feed additive, containing primarily yeast cell wall components, that is, β-glucans and mannans from *Saccharomyces cerevisiae* (YCW, i.e., PAQ-Gro™ Phibro, Teaneck, USA), were added 'on top' of the shrimp feed formula mixture, and compared to a control group (without YCW supplement). Four cages were fed with 5 kg of YCW per tonnes of feed (0.5%), four cages were fed with 10 kg of YCW per tonnes of feed (1.0%), while the remaining four cages were fed with commercial diet without supplement (control group). A typical shrimp commercial diet, with standard commercial nutrient levels, was used as basal diet (Table 1). The proximate composition of each protein ingredient is presented in Table S1.

Feed was formulated by Azteca Nutrimentos Acuícolas, (Tlaquepaque, Guadalajara, Mexico), isonitrogenous (30%) and isoenergetic (7%), and were prepared using a 250-hp California Pellet Mill (Crawfordsville, USA) batches of five tonnes at 2 mm size pellet. In Mexico, the current use of wheat flour in shrimp feed ranges from 30% to 50%. Particularly due to established milling techniques (e.g. double preconditioner plus conditioner), it is possible to use a die of 1.5 mm and achieve a good pellet stabilization. Similarly, kelp hydrolysate was used as a binder as it helps in the stabilization of the feed pellet with the presence of alginates. Also, the starch from wheat flour gelatinization supports the feed stabilization for a minimum of two hours under the water. The YCW supplement was added 'on top' of the commercial formula mixture. It was included with micro ingredients in the mash, prepared by mixing the feed in a mixer with the YCW prior to standard pelleting. As we have used a standard shrimp commercial diet rather than an experimental diet, the tested additive was included in addition to the feed blend. It is frequent practice under realistic commercial feed mill conditions for testing a new product without altering the primary specification.

TABLE 1 Feed formulation, ingredient profile and nutrient analysis of the experimental diets for the trial with *Penaeus vannamei* supplemented with 0.5 and 1.0% (PAQ-Gro™ Phibro, Teaneck, USA)

Ingredients (g kg^{-1})	Control	0.5%	1.0%
Fish meal[a]	100	100	100
Soybean meal[b]	220	220	220
Poultry meal[c]	180	180	180
Wheat flour[d]	430	430	430
Kelp hydrolysate (Binder)[e]	6	6	6
Lecithin[f]	10	10	10
Fish oil[g]	30	30	30
Soybean oil[h]	10	10	10
Vitamin premix b[i]	10	10	10
Mineral premix c[j]	10	10	10
Vitamin C[l]	3	3	3
Antifungal[m]	1	1	1
Yeast cell wall (PAQ-Gro™)	0	5	10
Analysis (g 100 g^{-1}; as fed basis)			
Moisture (%)	6	6	6
Crude protein (%)	35	35	35
Crude lipid (%)	8	8	8
Ash (%)	15	15	15

Vitamin PREMIX: Folic Acid 5000 mg kg^{-1}, Biotin 500 mg kg^{-1}, Magnesium 150 mg kg^{-1}, Riboflavin 200 mg kg^{-1}, Vitamin D: 1.500 mg kg^{-1}, Vitamin E: 90 mg kg^{-1}, Vitamin C: 1000 mg kg^{-1}. Mineral PREMIX: Total Calcium 1.846%, Zinc 50 g kg^{-1}, Selenium 200 g kg^{-1}.

[a]Fish Meal, Sonora, Mexico,

[b]Soybean meal, Arkansas, USA,

[c]Poultry meal, Arkansas, USA

[d]Wheat Flour, Sonora Mexico,

[e]Kelp hydrolysates, BCN, Mexico

[f]Lecithin, Jalisco, Mexico

[g]Sardine Fish Oil: Guaymas, Mexico

[h]Soy oil: Arkansas, USA

[i]Vitamin Premix: Jalisco Mexico,

jVitamin C: Jalisco, Mexico

kAntifungal, USA

Feeding rate was adjusted with the use of feeding trays and based on the farm's feeding chart recommendations for body percentage weight, occurring twice a day. Feed quantity was adjusted according to the biomass in the cages, water temperature, and dissolved oxygen, and supported by a daily monitoring to check if feed remained on trays.

2.3 | Sample collection

After the 102 days of the feeding trial, the cages were harvested. A total of 21 shrimp were randomly sampled for intestine collection, that is, 7 shrimp per treatment (2 shrimp from the first three replicas and 1 shrimp from the fourth replica). Shrimp were euthanized by thermal shock (33–9°C), carcass surfaces were cleaned with 70% (v/v) ethanol and the midgut with its content was collected, using sterilized forceps and scissors. As shrimp were constantly fed, with continuous feeding activity, at the moment of gut collection, the midgut was filled with the provided feed without natural food. Samples were immediately fixed in 70% (v/v) molecular grade ethanol, stored in sterile 2 ml microtube, and kept under −20°C for subsequent analysis. Transportation for the microbiology laboratory at University of Plymouth, UK occurred under the UK Home Office importation Licence #TARP/2015/2019.

2.4 | DNA extraction and 16S rRNA gene amplification

DNA extraction was performed with the 21 shrimp gut samples (n = 7 per treatment) (10 mg ±1.5 mg), using the QIAamp Stool Mini Kit (Qiagen®, Hilden, Germany), and following manufacturer's instructions. In order to enhance the lysis of Gram-positive bacteria, an initial incubation with 50 mg mL^{-1} of lysozyme for 30 min at 37°C was added to the protocol. Extracted DNA purity and quantity were measured using a UV spectrophotometer (NanoDrop™ 2000 Spectrophotometer, ThermoFischer Scientific®, Waltham, USA), based on the ratio of absorbance at 260/280 nm and 260/230 nm.

A fragment of 350 bp from the hypervariable V1-V2 regions, from bacterial 16S ribosomal RNA (16S rRNA gene), was amplified through a *touchdown*-polymerase chain reaction (PCR) assay, using the primers 27F (5' AGA GTT TGA TCM TGG CTC AG 3') and a pool of primers 338R-I (5'GCW GCC TCC CGT AGG AGT 3') and 338R-II (5' GCW GCC ACC CGT AGG TCT 3') (Gajardo et al., 2016). For the *touchdown*-PCR, 1 µl of the DNA template (1 ng μl^{-1}) was added to the PCR mix solution containing 25 µl of MyTaq™ Red Mix (Bioline®), 1 µl of each primer (25 pM) and ultrapure DNase-free water for a final volume of 50 µl. The amplification cycling profile is presented in Table 2. To demonstrate an accurate PCR performance, positive (*Escherichia coli* DNA) and negative (ultrapure water) controls were used in each amplification reaction. Subsequently, the amplified products were analysed by 1.5% agarose gel electrophoresis, with SYBR Safe DNA gel stain (ThermoFischer Scientific®, Waltham, USA), in TAE buffer at constant voltage (80 V) for ~40 min and visualized under UV light (300 nm transillumination).

TABLE 2 Cycling profile of the *touchdown*-PCR amplification

Phase	Temperature (°C)	Time	Cycles
Initial denaturation	94°	7'	
Denaturation	94°	30"	10× touchdown
Hybridization	63°–53°	30"	
Elongation	72°	30"	
Denaturation	94°	30"	25×
Hybridization	53°	30"	
Elongation	72°	30"	
Final Elongation	72°	10'	
Finalization	10°	Until end	

PCR products were purified using AMPure XP (Beckman Coulter®), based on the magnetic bead's technology. Finally, purified PCR products were sent to Systems Biology Centre of University of Plymouth, UK, Genomics Facilities, for the High Throughput Sequencing, utilizing Life Technologies Ion Torrent™ Personal Genome Machine™ System (ThermoScientific®). All other described molecular biology techniques were performed at the Microbiology laboratory at the University of Plymouth, UK.

2.5 | Analysis of high throughput sequencing results

The main focus of our study was to address the modulation of the gut microbiota with regards to the stabilization of a desirable, symbiotic microbiota profile. We adopted the protocols for conducting a microbiome study by Goodrich et al., (2014) in terms of design, execution and data analysis of the gut microbiome in *P. vannamei* fed the respective treatments.

Raw sequence data were trimmed using FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/), and sequences with low-quality scores (Q < 20) were filtered out. Data were then assessed using Quantitative Insights Into Microbial Ecology (Qiime 1.8.0) (Gajardo et al., 2016). Sequences were analysed using QIIME 1.8.0, and Operational Taxonomic Units (OTUs) were sorted and filtered with 97% of sequence identity. Ribosomal Database Project tool was used to assign taxonomic affiliation, with 0.8 of confidence. Alpha and beta diversity were calculated with ape, vegan and R. Bacterial richness and diversity were determined with indexes such as Chao1, Observed Species and Phylogenetic diversity. Good's coverage was also identified. Additionally, Weighted and Unweighted UniFrac distances were used to estimate similarity and dissimilarity and confirmed with Principal Coordinates Analysis (PCoA). The taxonomic analysis was estimated with relative abundance graphs at phylum and genus level. LEfSe (Linear discriminant analysis effect size) tool was used to determine differentially abundant taxa between treatments, and significantly different taxa were used to calculate LDA (linear

discriminant analysis) effect size (Segata et al., 2012), with a significant *p*-value < .05 and effect size threshold of 2. Finally, the Venn diagram was built to identify the core microbiota, as well as unique and shared OTUs between treatments, using Venny 2.1 software (http://bioinfogp.cnb.csic.es/tools/venny/, Oliveros, 2007-2015). Data are presented as mean ±SD. The *p*-value < .05 was considered statistically significant.

2.6 | Data availability

The 16S rRNA gene raw sequence data are deposited and publicly available in NCBI Sequence Read Archive database. BioProject ID PRJNA634676, SRA Study SRP263439. Biosamples from SAMN15004973 to SAMN15004993. (https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA634676).

2.7 | Ethics statement

The research was monitored and listed under the guidelines of the relevant institutional animal care and guidance committee for all projects involving animal work. In the UK, crustaceans including decapods and shrimp are not included in the United Kingdom Animal Scientific Procedures Act of 1986. This investigation on Pacific white shrimp was conducted on a remote commercial farm facility in Mexico and conformed to the local state regulations and standards for the welfare of shrimp, using propriety feeds and met with the full compliance of the farm management committee.

3 | RESULTS

3.1 | Growth performance and feed efficiency

Growth performance results are summarized in Table 3. Final shrimp survival was statistically different between experimental groups. Both treatment groups fed with YCW inclusion in diet displayed significantly better survival compared to the control diet. The 0.5% and 1.0% of YCW groups presented increased survival by 19% and 23%, respectively, in comparison to the control group. All experimental groups presented a survival rate above the commercial breaking point at the moment of the trial, that is, 45% (calculated based on the price of feed and postlarvae). Animals that received 1.0% of YCW showed 4.19% higher final average weight, 4.59% higher weight gain and 1.60% higher SGR, in comparison to non YCW fed shrimp. These parameters were statistically different from the control group. Regarding the FCR, animals from both experimental groups, that is, 0.5% and 1.0% of YCW showed better result (43.80% and 47.78% lower in comparison to control group, respectively). In both treatments with YCW, that is, 0.5% and 1.0%, the final yield was significantly most advantageous in comparison to the control group (51.22% and 66.96% higher than control group, respectively). No disease was recorded during the whole trial.

3.2 | High throughput sequencing results

The microbiota profile of *P. vannamei* intestine was analysed based on the sequencing of the 16S rRNA target gene, using Ion Torrent™ technology, in two different feed doses of YCW, that is, 0.5% and 1.0%, and a control group. Sequencing resulted in a total of 1,983,557 raw sequences. After removing singletons (OTUs observed fewer than two times) and Streptophyta phylum (chloroplast diet associated), 1,238,780 reads were qualified as high quality. Good´s estimator of coverage presented values above 0.992, demonstrating that almost the entire bacterial diversity was identified. The rarefaction curves revealed that a satisfactory sequencing coverage was achieved, with signs of saturation for all experimental groups (Figure S1). Table 4 summarizes the High Throughput Sequencing results.

3.3 | Relative abundance at phylum and genus levels

Among the five most abundant phyla in the taxonomic analysis (Figure 1a), the phyla Fusobacteria and Proteobacteria were the most prominent. Fusobacteria phylum was the most abundant in the gut

TABLE 3 Growth performance and feed efficiency of *Penaeus vannamei* fed with two feed doses of Yeast Cell Wall (YCW) and control group diets

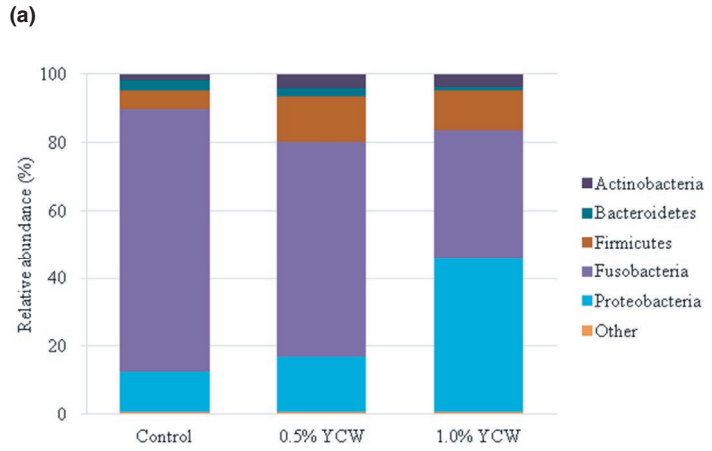
	Control	0.5% YCW	1.0% YCW	*p*-value
Survival (%)	46.00 ± 6.21^{b}	65.50 ± 7.54^{a}	69.00 ± 4.39^{a}	0.0043**
IAW (g shrimp^{-1})	1.79 ± 0.10	1.79 ± 0.10	1.78 ± 0.10	0.6477
FAW (g shrimp^{-1})	22.89 ± 0.37^{b}	23.41 ± 0.44^{ab}	23.85 ± 0.45^{a}	0.0318*
Weight gain (g)	21.09 ± 0.36^{b}	21.61 ± 0.43^{ab}	22.06 ± 0.46^{a}	0.0290*
SGR (g.week^{-1})	2.50 ± 0.01^{b}	2.52 ± 0.01^{ab}	2.54 ± 0.02^{a}	0.0180*
FCR	4.27 ± 0.86^{a}	2.40 ± 1.04^{b}	2.23 ± 1.43^{b}	0.0006***
Final yield (g.cage^{-1})	878.50 ± 148.53^{b}	1328.50 ± 159.30^{a}	1466.75 ± 133.15^{a}	0.0008***

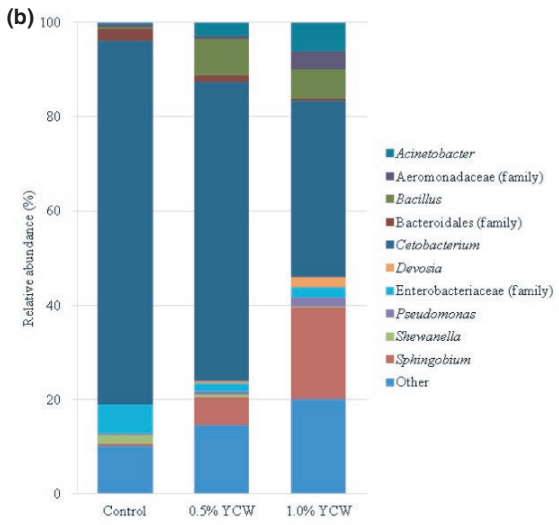
Abbreviations: FAW, Final average weight; FCR, Feed Conversion Ratio; IAW, Initial average weight; SGR, Specific Growth Rate.
Different superscript letters indicate significant differences. Data presented as mean ±SD. (ANOVA, Post hoc Tukey test, $p < .05$).

	Control	0.5% YCW	1.0% YCW	*p*-value
Reads after trimming	451,475	392,770	394,535	-
OTUs—Phylum level	15	15	16	-
OTUs—Genus level	194	202	206	-
Indexes				
Chao 1	419.53 ± 20.09	426.36 ± 26.89	452.84 ± 44.29	0.6733
Observed Species	368.67 ± 17.00	375.98 ± 39.04	407.45 ± 50.45	0.6784
Phylogenetic diversity	12.24 ± 0.70	12.40 ± 0.75	12.87 ± 1.30	0.4456

TABLE 4 Summary of High Throughput Sequencing result, showing the alpha diversity indexes of *Penaeus vannamei* intestinal microbiota, fed with two doses of Yeast Cell Wall (YCW) and control group diets

Indexes in samples at a dissimilarity level of 3% (ANOVA, Post hoc Tukey test, $p < .05$).





Phylum name	Control	0.5% YCW	1.0% YCW	*p*-value
Actinobacteria	1.74%	4.29%	3.64%	0.540
Bacteroidetes	3.15% ^{a}	2.11% ^{ab}	1.31% ^{b}	0.001**
Firmicutes	5.29%	13.25%	11.57%	0.170
Fusobacteria	77.34% ^{a}	63.65% ^{ab}	37.48% ^{b}	0.002**
Proteobacteria	11.89% ^{b}	16.11% ^{b}	45.44% ^{a}	0.007**
Other	0.59%	0.59%	0.56%	-

Different superscript letters indicate significant differences. Data presented as mean (n=7). ANOVA, Post-hoc Tukey test, $p<0.05$.

Genus name	Control	0.5% YCW	1.0% YCW	*p*-value
Actinetobacter	0.37% ^{b}	2.79% ^{ab}	6.06% ^{a}	0.010*
Aeromonadaceae†	0.64% ^{b}	0.75% ^{b}	3.86% ^{a}	0.027*
Bacillus	0.43% ^{b}	7.74% ^{a}	6.37% ^{a}	0.025*
Bacteroidales†	2.30% ^{a}	1.25% ^{b}	0.23% ^{c}	<0.001***
Cetobacterium	77.31% ^{a}	63.62% ^{ab}	37.46% ^{b}	0.002**
Devosia	0.01% ^{b}	0.32% ^{b}	2.24% ^{a}	0.010*
Enterobacteriaceae†	6.27%	1.87%	2.28%	0.297
Pseudomonas	0.24% ^{b}	0.60% ^{b}	1.77% ^{a}	0.003**
Shewanella	1.78% ^{a}	0.60% ^{ab}	0.26% ^{b}	0.016*
Sphingobium	0.37% ^{b}	5.89% ^{a}	19.38% ^{a}	0.005**
Other	10.36%	14.55%	20.09%	-

†Family. Different superscript letters indicate significant differences. Data presented as mean (n=7). ANOVA, Post-hoc Tukey test, $p<0.05$.

FIGURE 1 Relative abundance of gut microbiota composition of *Penaeus vannamei* receiving two different doses of Yeast Cell Wall (YCW) and control group diets, describing the distribution (%) of bacteria, (a) at the phylum level and (b) at the genus level. ANOVA +Tukey test ($p < 0.05$)

of shrimp that received 0.5% of YCW and in those from the control group, the latter group being statistically significantly higher than 1.0% treatment ($p = 0.0029$). The intestinal microbiota of animals that received 1.0% of YCW presented a high relative abundance of phyla Proteobacteria and Fusobacteria, being Proteobacteria relative abundance significantly higher than 0.5% and control groups ($p = 0.0073$). These two bacterial phyla comprised had relative abundances greater than 80% in all the three analysed groups, that is, 80%, 82% and 89% in 0.5% and 1% treatment, and control group, respectively. Finally, the relative abundance of Bacteroidetes was statistically lower in 1% treatment than in control group ($p = 0.001$).

Similarly, among the ten most abundant genera, the three genera *Cetobacterium*, *Sphingobium* and *Bacillus* were more representative (Figure 1b) and, summed, represent 77%, 63% and 78% of the total relative abundance at genus level on 0.5%, 1.0% and control groups, respectively. Moreover, main statistical differences were observed in the 1.0% treatment. *Cetobacterium* was the most abundant genus of all and in all the treatments (63.62%, 37.46% and 77.31% for 0.5%, 1.0% and control groups, respectively), and the 1.0% treatment showed a statistically lower relative abundance of this genus in comparison to the two other treatments ($p = 0.0029$). Then, *Sphingobium* was the second most abundant genus in the gut microbiota of animals from 1.0% treatment, which presented a higher relative abundance in comparison to the control group. *Sphingobium* was significantly higher in both treatments with YCW, in comparison to the control group ($p = 00058$) (5.89%, 19.38% and 0.37% for 0.5%,

1.0%, and control groups, respectively). Lastly, *Bacillus* was significantly higher in both treatments with YCW, in comparison to the control group (p = 0.0253) (7.74%, 6.37% and 0.43% for 0.5%, 1.0% and control groups, respectively).

3.4 | Similarities and dissimilarities

Concerning the similarities and dissimilarities of the bacterial population, the PCoA revealed a spatial separation between the categories, mainly between control and 1.0% treatment. Weighted UniFrac distance (Figure 2a) showed that 1.0% treatment samples clustered all together, with one exception, in the opposite direction of all control samples, while 0.5% treatment samples were dispersed. Unweighted UniFrac distance (Figure 2b) displayed similar results, with spatial differentiation between control and 1.0% treatment, and with five of seven 0.5% samples clustering close to control.

3.5 | Linear discriminant analysis Effect Size.

Regarding possible distinct taxa with statistical significance and biological relevance, the linear discriminant analysis effect size (LEfSe) identified 25 distinct taxa in control group, three on 0.5% YCW treatment and 22 on 1.0% YCW treatment that could explain differences among treatments (Figure 3). The logarithmic LDA score revealed the effect size of each factor, with positive scores ranging between 2.0 and 5.0. The gut microbiota of 1.0% YCW treatment showed the highest LDA score (5.0) with *Sphingobium* genus, also notable on the relative abundance at the genus level. Further, this treatment showed the smallest LDA score, though always above 2.0. Control group displayed the greatest quantity of distinct taxa, 25 in total, all above or close to 3.0 LDA score, highlighting *Shewanella* and *Clostridium* genera. Finally, 0.5% YCW treatment revealed only three distinct taxa, with LDA score between 3.0 and 4.0.

3.6 | Venn diagram and shared OTUs

In order to define the intersection list of OTUs between treatments, as the core gut microbiota of *P. vannamei*, as well as to determine the unique OTUs of each group, a Venn diagram was constructed, at the genus level (Figure 4). Supplementary Material 1 lists the OTUs presented in Venn diagram sections. The core gut microbiota comprised 57.5% of all identified OTUs, including lactic acid bacteria, such as *Lactobacillus* and *Lactococcus*, and well-established potential probiotics, like *Bacillus*, *Shewanella*, *Pseudomonas* and *Halomonas*. *Cetobacterium* was also observed in all treatments. Notably, the core

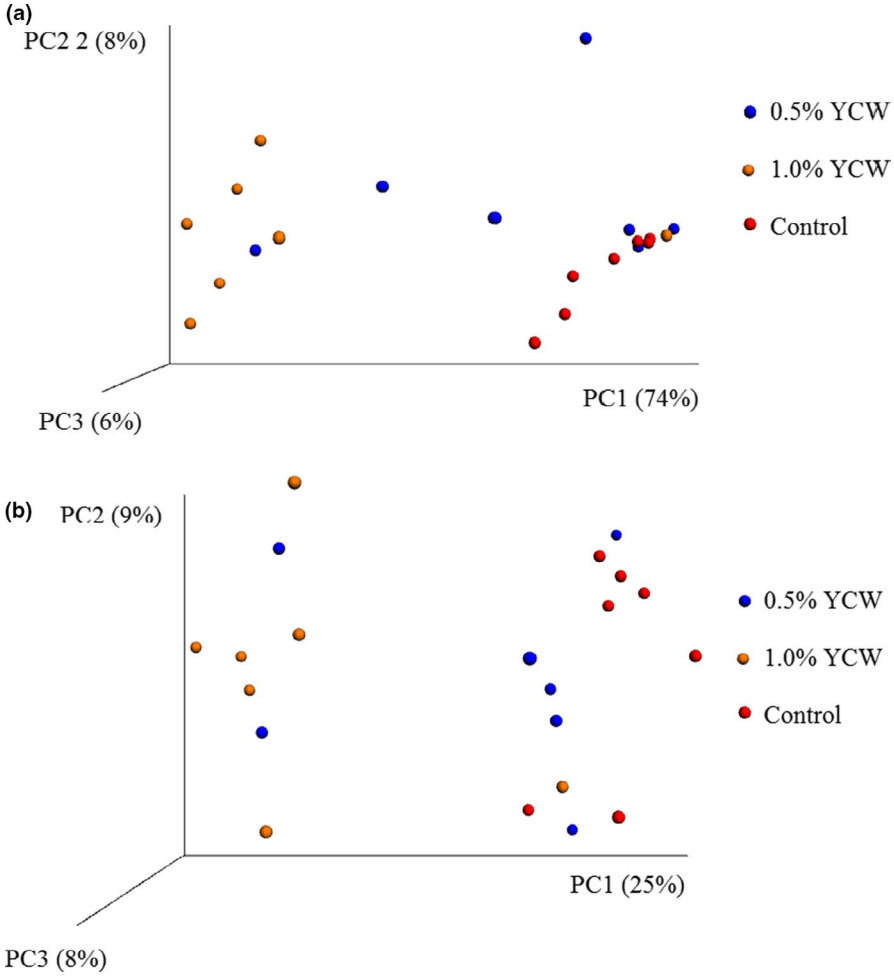
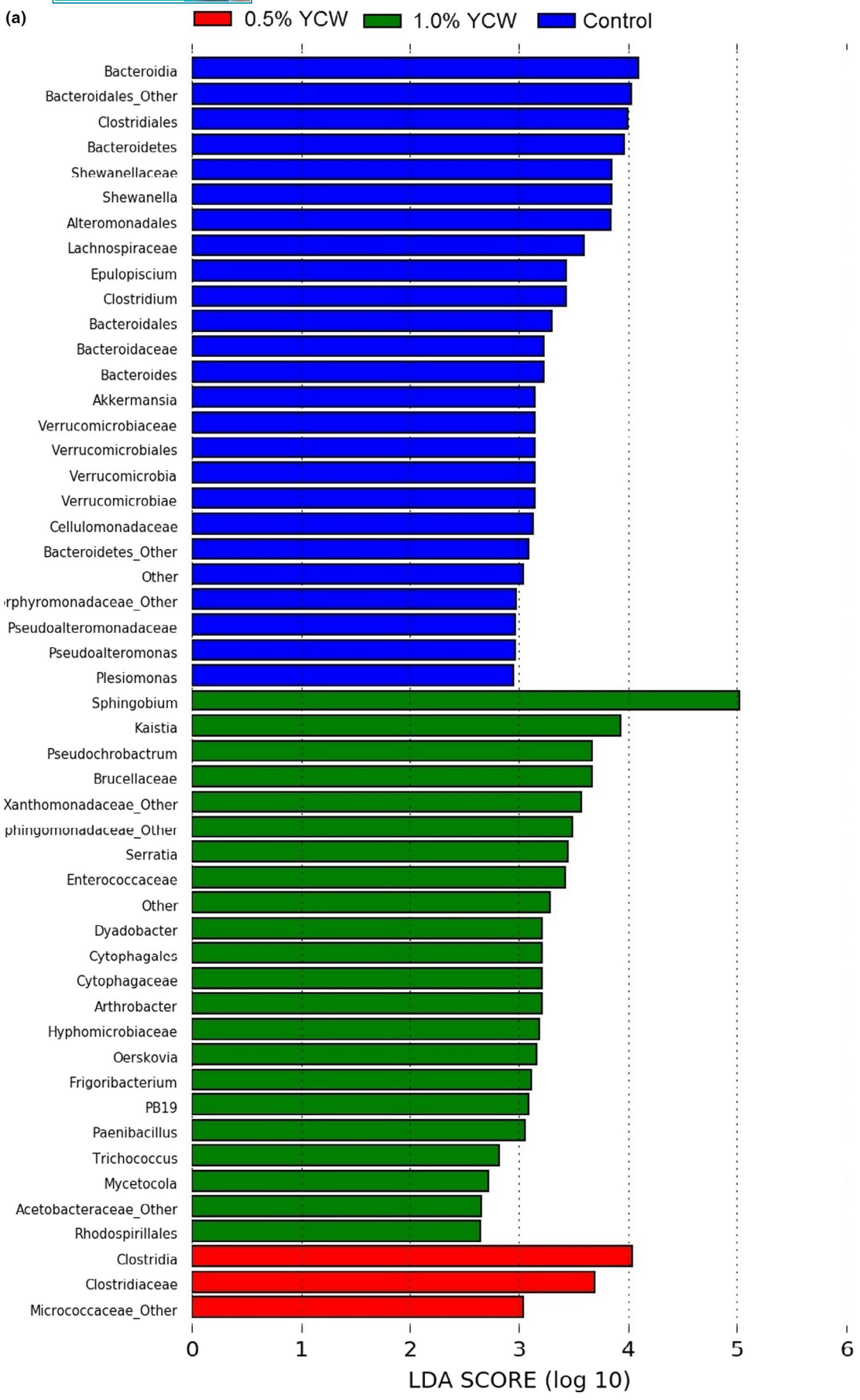


FIGURE 2 Similarities and dissimilarities of gut microbiota composition of *Penaeus vannamei* receiving two different doses of Yeast Cell Wall (YCW) and control group diets, based on (a) Weighted (the first, second and third principal component accounting for 88% of the sample variation) and (b) Unweighted (per cent variation explained 42%). UniFrac distances


(a)
0.5% YCW
1.0% YCW
Control
Bacteroidia
Bacteroidales_Other
Clostridiales
Bacteroidetes
Shewanellaceae
Shewanella
Alteromonadales
Lachnospiraceae
Epulopiscium
Clostridium
Bacteroidales
Bacteroidaceae
Bacteroides
Akkermansia
Verrucomicrobiaceae
Verrucomicrobiales
Verrucomicrobia
Verrucomicrobiae
Cellulomonadaceae
Bacteroidetes_Other
Other
rphyromonadaceae_Other
Pseudoalteromonadaceae
Pseudoalteromonas
Plesiomonas
Sphingobium
Kaistia
Pseudochrobactrum
Brucellaceae
Xanthomonadaceae_Other
phingomonadaceae_Other
Serratia
Enterococcaceae
Other
Dyadobacter
Cytophagales
Cytophagaceae
Arthrobacter
Hyphomicrobiaceae
Oerskovia
Frigoribacterium
PB19
Paenibacillus
Trichococcus
Mycetocola
Acetobacteraceae_Other
Rhodospirillales
Clostridia
Clostridiaceae
Micrococcaceae_Other
0
1
2
3
4
5
6
LDA SCORE (log 10)

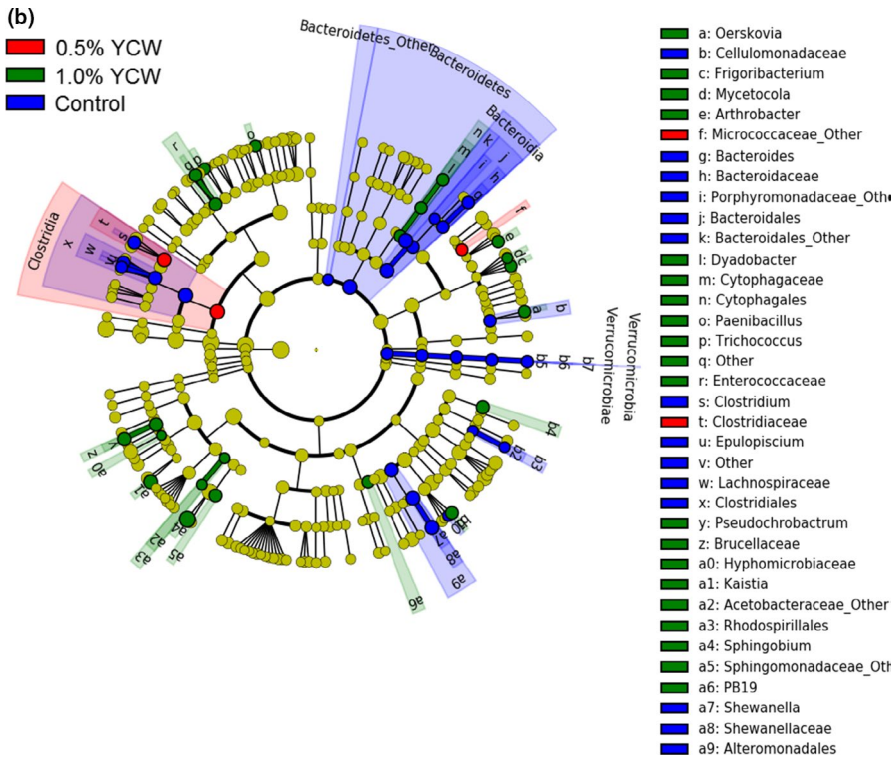


FIGURE 3 Distinct enriched taxa in the gut microbiota of *Penaeus vannamei*, with two different doses of Yeast Cell Wall (YCW), that is, 0.5% and 1.0%, and control group, at genus level. (a) LDA score indicating the scale of difference among taxa. (b) Relative abundance of the five most abundant bacteria, at genus level, in order to support LefSe results. $p < 0.05$; LDA threshold of 2

microbiota can be understood as the persistent microorganisms of the intestinal microbial community, that is, the genera cited above were permanent and stable, regardless with or without the presence of YCW in the shrimp diet.

Conversely, a number of unique OTUs were observed in each treatment. The 1% YCW treatment had the highest quantity, accounting for 9.2% of the total OTUs within the treatment. The control group presented 1.9% unique OTUs and the 0.5% presented 3.4% unique OTUs. Additionally, when observing the intersection (∩) between the 0.5% and 1.0% YCW treatments (0.5% ∩1.0%), two genera were distinct due to their potential and well-known probiotic effects, respectively *Exiguobacterium* and *Vibrio*. Lastly, 31.9% of the OTUs were found exclusively on the gut microbiota of shrimp feed with YCW, that is, 66 OTUs were influenced and selected by the inclusion of YCW 'on top' of shrimp feed.

4 | DISCUSSION

A high priority for contemporary aquaculture is to find efficient and safe technologies for prophylaxis and stimulation of the animal immune system with the associated mechanism of the gut mucosal interface and the role of the gut microbiota. This complex relationship has been well described by many authors and more recently by Holt at al. (2020).

The present study evaluated the influence of a commercial yeast cell wall extract (YCW) on the growth performance and feed efficiency of *Penaeus vannamei*. It also analysed the gut microbiota composition of *P. vannamei* raised on an intensive system, simulating commercial shrimp farming conditions (e.g. variability in water temperature, large production, and open pond culture) and thus providing realistic scenario.

All the growth performance and feed utilization parameters analysed (growth rate, feed conversion efficiency and survival) were deemed to be adequate for the established standards for shrimp farming. Currently, the standard growth parameters in Mexican shrimp farm are growth rate 1 g week$^{-1,}$ FCR 1.6 to 2.0, and survival rates 40% to 65%. Moreover, animals that received YCW inclusion, especially those that were fed with 1.0% YCW, presented higher overall performance than the control group. Both treatments groups fed diets containing YCW displayed significantly better survival compared to the control diet. Additionally, when analysing the growth parameters, that is, the growth rate and the final weight, animals that received 1.0% YCW feed showed improved performance than those that received 0.5% YCW or from the control group. It is recognized that the dose and the frequency that various prebiotics and immunostimulants are administrated can notably influence animal response (Zhang & Mai, 2014), and a better performance in our investigation was observed in animals fed with 1.0% YCW.

It is worth noting that when doing evaluations under commercial conditions, ideal results are rare. Moreover, the economic relevance should be the main objective. In the trial described in this article, the survival rates and animal performance were significantly superior compared to the control group and the farm inventory record

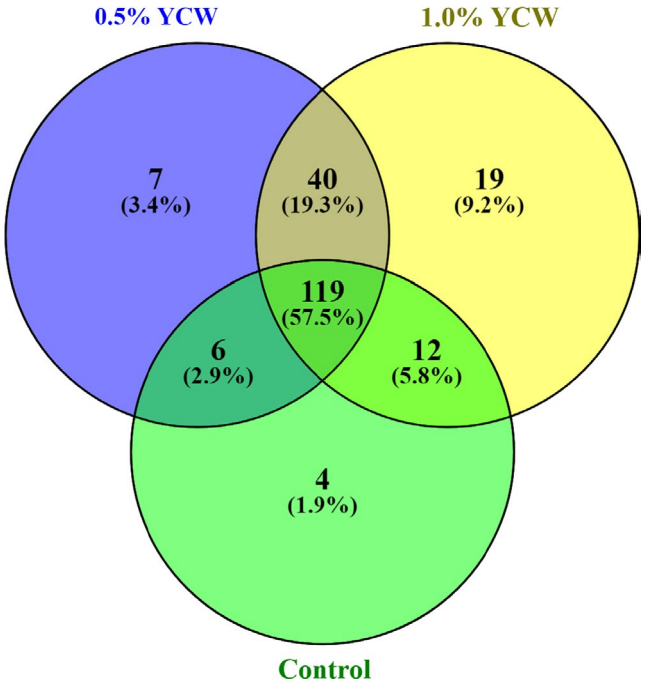


FIGURE 4 Venn diagram showing unique and shared OTUs (Operational Taxonomic Units) in the gut microbiome of *Penaeus vannamei*, with two different doses of Yeast Cell Wall (YCW), that is, 0.5% and 1.0%, and control group diets, at genus level

logs. Commercial aquaculture is a multi-diverse activity where each farm looks for profit or cost benefit rather than simply performance, adjusting the parameters according to their market, carrying capacity and productivity. Additionally, in regard to shrimp survival and compensatory growth, a typical scenario is when the shrimp population reduces, shrimp size increases. This is related to carrying capacity. At our site, typical capacity was 600 g m^{-3}. During our feeding trial, final biomass in each tank was maintained above the capacity (control group 624 g m^3; 0.5% YCW 965 g m^{-3}; 1.0% YCW 978 g m^{-3}). Our hypothesis in this regard is that the immune robustness, due to inclusion of the tested product, aids the shrimp to maintain adequate survival in both treatment groups, but not in the control group. Additionally, shrimp survivals are widely interpreted from the technical point of view, that is, economic standards and health management. The farm records survival rates fluctuate from 40 to 65% (exempting the trial, survivals range from 40% to 70%), which is considered to be a good trend. Our goal in this experiment was to link the performance results with the inclusion of the functional feed additive and the gut microbiota profile. Thus, we aimed at building a scientific case based on the hypothesis that beneficial bacterial can increase stress tolerance as well nutrition absorption increasing the robustness of the shrimp.

Regarding the growth parameters, the superior performance observed for shrimps that received the YCW may have resulted from the direct induction of the immune system by the polysaccharide-rich feed, that is, the β-glucan from the yeast cell wall along with other constituents such as proteoglycans and mannan-oligosaccharides (MOS). Particularly, in our study, the natural compounds found within the yeast cell wall matrix appeared to maximize the shrimp production in terms of weight gain, feed efficiency and survival.

It should be mentioned that Sajeevan et al., (2009) studied various doses and different feeding intervals of an insoluble glucan extracted from marine yeast in the diet of the Indian white prawn (*Penaeus indicus* Edwards, 1837) infected with white spot syndrome virus (WSSV). These authors concluded that a specific combination of dose +frequency was decisive for a better shrimp survival similarity. Bai et al., (2014) reported that *P. vannamei* fed with 0.1% of synthetic carboxymethylglucan in the diet showed the best immunity and survival in experimental infection of WSSV; authors tested 18 different diets, including different β-glucan derivatives and degrees of substitution. Moreover, shrimp immunity was kept at high levels for up one month when carboxymethylglucan was added in animal feed. Particularly, the effect of the immunostimulants in shrimp tends to be transitory; thus, special attention should be given to a regular frequency and the best dose for optimum results (Smith et al., 2014).

The main reason for the use of immunostimulants in aquaculture is to attain optimal production, through achieving growth stimulation and promoting animal health and survival as reported by other authors (Barman et al., 2013; Mohan et al., 2019; Wang et al., 2017). In order to estimate the influence of YCW on shrimp farming output, we measured the final shrimp yield. Indeed, the addition of YCW resulted in improved final yield, demonstrating that the inclusion of the yeast cell wall product in *P. vannamei* diet enhanced this important production parameter. Certainly, the continuous shrimp feeding management with YCW not only showed no negative effects on animal performance but also improved the growth parameters, particularly animals that received 1.0% of YCW dietary inclusion.

When analysing the inclusion of a new component to aquafeed, it is imperative that it does not lead to an intestinal dysbiosis under any circumstances. Moreover, if the new compound favours a beneficial bacteria selection, the greater is the advantage for healthy shrimp farming. Based on our results, it is possible to affirm that the dietary YCW inclusion for juvenile *P. vannamei* did not lead to any adverse intestinal microbiota imbalance. Moreover, the β-glucan-rich MOS product derived from the yeast cell wall also contributed to the increase of relevant probiotic genera and preserved the core microbiota.

The addition of 1.0% YCW was the treatment that had the most influence on the shrimp intestinal microbiota composition. This finding was also corroborated with the PCoA results that revealed a distinct separation and dissimilarity between 1.0% YCW treatment and control group fed shrimp. Animals that received this inclusion level of the commercial YCW product presented the phylum Proteobacteria as the most prevalent constituent. This phylum, in fact, has been reported by other studies as the most dominant in shrimp (Li et al., 2018; Xiong et al., 2017). Meanwhile, the Fusobacteria phylum was the most prevalent in the digestive tract among the animals that received 0.5% YCW and those from the control group. This phylum has also been described as one of the most ubiquitous phyla

in the intestine of the Pacific white shrimp (Souza Valente et al., 2020). In fact, these two phyla, among others, were described to be part of the autochthonous gut microbiota of *P. vannamei*, since larval until adult stage (Zeng et al., 2017). Moreover, these two phyla were reported to be upregulated by WSSV infection (Wang et al., 2019). Luis-Villaseñor et al., (2013) addressed that the phyla Proteobacteria, Fusobacteria, Sphingobacteria and Flavobacteria were the most prevalent in the gut of *P. vannamei* after being fed with a *Bacillus* probiotic mix.

Those findings can also be observed in the most dominant microbial genera. The genus *Cetobacterium* was the most predominant in all treatments, although significantly lower in 1% YCW. This genus, and specifically *C. somerae* species, is related to the production of vitamin B_{12} (cobalamin) in fish (Rodiles et al., 2018; Tsuchiya et al., 2008). In Chinese crabs, cobalamin is associated with the nonspecific immune responses (Wei et al., 2014). In shrimp, vitamin B_{12} is commonly supplemented as a form of cyanocobalamin, in optimal doses of 0.1 – 0.2 mg kg^{-1} in complete diets (Koshio, 2014). Although there is recent research showing the relation between *Cetobacterium* and cobalamin in fish, there is a lack of studies on this bacterium and its role in shrimp. As the most prevalent genera in the three analysed group of the present study, certainly this genus is worthy of future attention.

Similarly, the genus *Sphingobium* was higher in the 1.0% YCW group in comparison to the control group, indeed reaching a relevant percentage of relative abundance for that treatment. *Sphingobium* was isolated from fresh and treated water (Corre et al., 2019; Sheu et al.,2013) and, as some bacteria from this genus may degrade polycyclic aromatic hydrocarbons, they can be used for soil bioremediation (Chen et al., 2016). In the LEfSe analysis, this genus was enriched and presented the biggest LDA score, showing that this genus had great relevance, and could explain part of differences between this treatment and the other groups. It is a relatively new described genus, first proposed by Takeuchi et al., (2001) and, at the moment, it is only mentioned to be part of some intestinal shrimp microbiota (Hu et al., 2017), but its relevance is rarely discussed. The genus counts as the 40 most common taxa found in the arthropod gut microbiota, from soil and the aquatic environment (Esposti & Romero, 2017). It was isolated from the rhizosphere of an aquaponics system (Schmautz et al., 2017) and associated with an antibiotic resistance (glycopeptide resistance gene) in an experimental aquaculture facility (Colombo et al., 2016). The role of this genus on the gut microbiota of shrimp from the present study remains unclear.

Noteworthy, the inclusion of YCW within the experimental shrimp diets, both at 0.5%, and 1.0%, significantly increased the relative abundance of the genus *Bacillus* in the gut microbiota. This result is remarkable due to the significant probiotic importance of this genus. *Bacillus* is considered as an autochthonous member of crustacean's environment and is among the widely used probiotic bacteria for crustaceans (Castex et al., 2014), mainly due to its capacity to activate both cellular and humoral shrimp immune responses (Rengpipat et al., 2000) and to naturally produce antibiotic compounds (Van Hai & Fotedar, 2009). Thus, the *Bacillus* stimulation by the YCW diet inclusion is a favourable influence towards maintenance of a healthy shrimp gut microbiota.

Complementary, the inclusion of YCW led to almost no OTU suppression. In fact, only 1.9% of the identified OTUs were only found in the control group, and we may infer that the inclusion of the YCW into the shrimp diet did not suppress an expressive number of microorganisms. On the contrary, one-third of the OTUs were found exclusively within the gut microbiota of animals that received the YCW, including *Exiguobacterium* and *Vibrio*. *Exiguobacterium* has been proposed to be a potential probiotic for *P. vannamei* (Cong et al., 2017) and may increase shrimp survival and growth (Sombatjinda et al., 2014). Moreover, this genus has potential biotechnological use to industry and agriculture (Kasana & Pandey, 2018). Equally, although *Vibrio* genus also encompasses some opportunistic pathogens, others may act as probiotics for crustaceans (Castex et al., 2014), such as *Vibrio alginolyticus* (Austin et al., 1995). Furthermore, *Vibrio* may play a relevant role in enhancing shrimp digestion, due to its genes related to digestive enzymes (Gao et al., 2019). Therefore, shrimp aquafeed with YCW not only preserved relevant bacteria genera in the gut microbiota but also selected for two other beneficial ones (i.e. *Exiguobacterium* and *Vibrio*).

Lastly, even with changes observed in the gut microbiota composition due to the addition of YCW especially on 1.0% treatment that showed dissimilarity to the control group, we noticed a stable core microbiota. A stable and permanent bacterial community was preserved, regardless the addition of YCW in the shrimp diets tested in this study. The core microbiota, composed by LAB and recognizable or promising probiotic strains in this study, is intimately associated with healthy and diseased animals, being paramount on the host–bacteria interaction. A healthy shrimp gut microbiota is characterized by a high diversity with cooperative interactions, while diseased animals tend to have less diversity and simple gut microbiota (Yao et al., 2018). Moreover, when elucidating the core microbiota composition, it is easier to manipulate it in order to develop effective strategies to promote animal health and growth (Steinberg, 2018). Thus, the preservation of a healthy and balanced gut microbiota, as observed in the inclusion of YCW, may result in more resilient and stronger shrimp, likely to better respond to stressful situations.

In conclusion, the present study has implied that the dietary inclusion of a yeast cell wall extract complex (PAQ-Gro™ Phibro, Teaneck, USA) resulted in beneficial attributes for shrimp farming. Particularly, for juvenile *P. vannamei* raised on an intensive floating cage system. YCW can be supplied to shrimp as a prophylactic agent in order to promote animal performance and gut health, especially at a 1.0% inclusion level in a formulated diet.

The product we evaluated (PAQ-Gro™ Phibro, Teaneck, USA) is not a pure β-glucan but also contains other important constituents of the yeast cell wall such as MOS and proteoglycans and enzymes from fungal sources that could influence the gut health but confounding our understanding of the direct causative effect. As a

complex bioactive agent, it may maximize shrimp yield farming profitability, likely providing economic advantages to the producer.

Future studies should also be directed to assessing the cost benefit analyses of YCW under various production scenarios and within the context of disease challenge studies with specific pathogenic agents encountered in the shrimp farming industry (e.g. White Spot Disease and Acute Hepatopancreatic Necrosis Disease). These collectively offer much scope for the future of applying such yeast cell wall products as feed supplements in formulated diets for shrimp to achieve superior health and resilience. Further work should be directed towards metagenomic techniques to establish the functionality of the various microbial taxa and specific roles of commensal bacteria in relation to the shrimp immune competence.

Additionally, it was shown that supplementation of a propriety shrimp feed with a functional feed additive under practical farming conditions resulted in positive outcomes for production. This investigation will therefore allow us to better understand their applications under varying environmental and rearing conditions for more effective control and use of prebiotic type additives for attaining superior health status. This will be transformational to achieve a more sustainable industry and meet with consumer expectations for a superior quality end product.

5 | DATA STORAGE AND DOCUMENTATION

All data that support the findings presented in the manuscript are available within the manuscript and supplemental material.

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CONFLICT OF INTEREST

Phibro Aqua, Phibro Animal Health Corporation donated the commercial product analysed in this study. This did not alter, influence, or affect the development of the study, including trial design, sampling, analysis of results, interpretation of data or decision for publication. No patent or market product under development is related to this study. This study was part of the first author´s PhD thesis. KSA, CSV, GVP, BS and SJD declare no other conflict of interest.

AUTHOR'S CONTRIBUTION

KSA and SJD conceived and designed the research. KSA provided funding and conducted the trial. BS provided funding and the commercial product analysed in this study. KSA, CSV and GVP performed the analyses. CSV wrote the first draft of the manuscript. All authors participated in the interpretation of data, manuscript revisions and approved the final version of the article.

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REFERENCES

Akhter, N., Wu, B., Memon, A. M., & Mohsin, M. (2015). Probiotics and prebiotics associated with aquaculture: a review. *Fish & Shellfish Immunology*, *45*, 733–741.

Austin, B., Stuckey, L. F., Robertson, P. A. W., Effendi, I., & Griffith, D. R. W. (1995). A probiotic strain of *Vibrio alginolyticus* effective in reducing diseases caused by Aeromonas salmonicida, *Vibrio anguillarum* and *Vibrio ordalii*. *Journal of Fish Diseases*, *18*, 93–96.

Bai, N., Gu, M., Zhang, W., Xu, W., & Mai, K. (2014). Effects of β-glucan derivatives on the immunity of white shrimp *Penaeus vannamei* and its resistance against white spot syndrome virus infection. *Aquaculture*, *426*, 66–73.

Baquero, F., & Nombela, C. (2012). The microbiome as a human organ. *Clinical Microbiology and Infection*, *18*, 2–4.

Barman, D., Nen, P., Mandal, S. C., & Kumar, V. (2013). Immunostimulants for aquaculture health management. *Journal of Marine Science: Research and Development*, *3*(3).

Castex, M., Daniels, C., & Chim, L. (2014). Probiotics Application in Crustacea. In D. L. Merrifield, & E. Ringo (Ed.), *Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics* (pp. 290–327). John Wiley & Sons.

Castillo-Juárez, H., Campos-Montes, G. R., Caballero-Zamora, A., & Montaldo, H. H. (2015). Genetic improvement of Pacific white shrimp [*Penaeus* (*Litopenaeus*) *vannamei*]: perspectives for genomic selection. *Frontiers in Genetics*, *6*, 93.

Chen, C., Lei, W., Lu, M., Zhang, J., Zhang, Z., Luo, C., Chen, Y., Hong, Q., & Shen, Z. (2016). Characterization of Cu (II) and Cd (II) resistance mechanisms in *Sphingobium* sp. PHE-SPH and *Ochrobactrum* sp. PHE-OCH and their potential application in the bioremediation of heavy metal-phenanthrene co-contaminated sites. *Environmental Science and Pollution Research*, *23*, 6861–6872.

Colombo, S., Arioli, S., Guglielmetti, S., Lunelli, F., & Mora, D. (2016). Virome-associated antibiotic-resistance genes in an experimental aquaculture facility. *FEMS Microbiology Ecology*, *92*, fiw003.

Cong, M., Jiang, Q., Xu, X., Huang, L., Su, Y., & Yan, Q. (2017). The complete genome sequence of *Exiguobacterium arabatum* W-01 reveals potential probiotic functions. *MicrobiologyOpen*, *6*, e00496.

Cornejo-Granados, F., Lopez-Zavala, A. A., Gallardo-Becerra, L., Mendoza-Vargas, A., Sánchez, F., Vichido, R., & Ochoa-Leyva, A. (2017). Microbiome of Pacific Whiteleg shrimp reveals differential bacterial community composition between Wild, Aquacultured and AHPND/EMS outbreak conditions. *Scientific Reports*, *7*(1), 11783.

Corre, M. H., Delafont, V., Legrand, A., Berjeaud, J. M., & Verdon, J. (2019). Exploiting the richness of environmental waterborne bacterial species to find natural anti-Legionella active biomolecules. *Frontiers in Microbiology*, *9*, 3360.

Dai, W., Sheng, Z., Chen, J., & Xiong, J. (2020). Shrimp disease progression increases the gut bacterial network complexity and abundances of keystone taxa. *Aquaculture*, *517*, 734802.

Davies, S. J., Herath, T., & Bower, P. H. (2019). Nutritional diseases. In S. A. Smith (Ed.), *Fish diseases and medicine* (pp. 270–282). CRC Press.

Esposti, M. D., & Romero, E. M. (2017). The functional microbiome of arthropods. *PLoS One*, *12*(5), e0176573.

Flegel, T. W. (2019). A future vision for disease control in shrimp aquaculture. *Journal of the World Aquaculture Society*, *50*, 249–266.

Gainza, O., & Romero, J. (2020). Effect of mannan oligosaccharides on the microbiota and productivity parameters of *Litopenaeus vannamei* shrimp under intensive cultivation in Ecuador. *Scientific Reports*, *10*, 1–12.

Gajardo, K., Rodiles, A., Kortner, T. M., Krogdahl, Å., Bakke, A. M., Merrifield, D. L., & Sørum, H. (2016). A high-resolution map of the gut microbiota in Atlantic salmon (*Salmo salar*): a basis for comparative gut microbial research. *Scientific Reports*, *6*, 30893.

Gao, S., Pan, L., Huang, F., Song, M., Tian, C., & Zhang, M. (2019). Metagenomic insights into the structure and function of intestinal microbiota of the farmed Pacific white shrimp (*Penaeus vannamei*). *Aquaculture*, *499*, 109–111.

Goodrich, J. K., Di Rienzi, S. C., Poole, A. C., Koren, O., Walters, W. A., Caporaso, J. G., Knight, R., & Ley, R. E. (2014). Conducting a microbiome study. *Cell*, *158*(2), 250–262.

Holt, C. C., Bass, D., Stentiford, G. D., & van der Giezen, M. (2020). Understanding the role of the shrimp gut microbiome in health and disease. *Journal of Invertebrate Pathology*, *107387*.

Hu, X., Cao, Y., Wen, G., Zhang, X., Xu, Y., Xu, W., & Li, Z. (2017). Effect of combined use of *Bacillus* and molasses on microbial communities in shrimp cultural enclosure systems. *Aquaculture Research*, *48*(6), 2691–2705.

Karunasagar, I., Karunasager, I., & Alday-Sanz, V. (2014). Immunostimulants, probiotics and phage therapy: alternatives to antibioitics. In V. Alday-Sanz (Ed.), *The shrimp book* (pp. 695–711). Nottingham University Press.

Kasana, R. C., & Pandey, C. B. (2018). *Exiguobacterium*: an overview of a versatile genus with potential in industry and agriculture. *Critical Reviews in Biotechnology*, *38*(1), 141–156.

Koshio, S. (2014). Vitamin requirements of shrimp and prawns. In V. Alday-Sanz (Ed.), *The shrimp book* (pp. 491–496). Nottingham University Press.

Li, E., Xu, C., Wang, X., Wang, S., Zhao, Q., Zhang, M., Qin, J. G., & Chen, L. (2018). Gut microbiota and its modulation for healthy farming of Pacific white shrimp *Penaeus vannamei*. *Reviews in Fisheries Science & Aquaculture*, *26*(3), 381–399.

Luis-Villasenor, I. E., Castellanos-Cervantes, T., Gomez-Gil, B., Carrillo-García, Á. E., Campa-Córdova, A. I., & Ascencio, F. (2013). Probiotics in the intestinal tract of juvenile whiteleg shrimp *Penaeus vannamei*: modulation of the bacterial community. *World Journal of Microbiology and Biotechnology*, *29*(2), 257–265.

McFall-Ngai, M., Hadfield, M. G., Bosch, T. C., Carey, H. V., Domazet-Lošo, T., Douglas, A. E., Dubilier, N., Eberl, G., Fukami, T., Gilbert, S. F., Hentschel, U., King, N., Kjelleberg, S., Knoll, A. H., Kremer, N., Mazmanian, S. K., Metcalf, J. L., Nealson, K., Pierce, N. E., … Wernegreen, J. J. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences*, *110*(9), 3229–3236.

Meena, D. K., Das, P., Kumar, S., Mandal, S. C., Prusty, A. K., Singh, S. K., Akhtar, M. S., Behera, B. K., Kumar, K., & Mukherjee, S. C. (2013). Beta-glucan: an ideal immunostimulant in aquaculture (a review). *Fish Physiology and Biochemistry*, *39*(3), 431–457.

Mohan, K., Ravichandran, S., Muralisankar, T., Uthayakumar, V., Chandirasekar, R., Seedevi, P., Abirami, R. G., & Rajan, D. K. (2019). Application of marine-derived polysaccharides as immunostimulants in aquaculture: A review of current knowledge and further perspectives. *Fish & Shellfish Immunology*, *86*, 1177–1193.

Nelson, D. L., & Cox, M. M. (2004). The Foundations of Biochemistry In: *Lehninger Principles of Biochemistry*, 4th ed. (pp. 1–44). WH Freeman & Co.

Oliveros, J. C. (2007- 2015) Venny. An interactive tool for comparing lists with Venn´s diagram. Retrieved from: http://bioinfogp.cnb.csic.es/tools/venny/index.html

Rengpipat, S., Rukpratanporn, S., Piyatiratitivorakul, S., & Menasaveta, P. (2000). Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiont bacterium (*Bacillus* S11). *Aquaculture*, *191*(4), 271–288.

Ringø, E., Olsen, R. E., Gifstad, T. Ø., Dalmo, R. A., Amlund, H., Hemre, G. I., & Bakke, A. M. (2010). Prebiotics in aquaculture: a review. *Aquaculture Nutrition*, *16*(2), 117–136.

Rodiles, A., Rawling, M. D., Peggs, D. L., Pereira, G. D. V., Voller, S., Yomla, R., Standen, B. T., Bowyer, P., & Merrifield, D. L. (2018). Probiotic Applications for Finfish Aquaculture. In D. Gioia, & B. Biavati (Eds.), *Probiotics and Prebiotics in Animal Health and Food Safety* (pp. 197–217). Springer.

Sajeevan, T. P., Philip, R., & Singh, I. B. (2009). Dose/frequency: a critical factor in the administration of glucan as immunostimulant to Indian white shrimp *Fenneropenaeus indicus*. *Aquaculture*, *287*(3–4), 248–252.

Schmautz, Z., Graber, A., Jaenicke, S., Goesmann, A., Junge, R., & Smits, T. H. (2017). Microbial diversity in different compartments of an aquaponics system. *Archives of Microbiology*, *199*(4), 613–620.

Segata, N., Waldron, L., Ballarini, A., Narasimhan, V., Jousson, O., & Huttenhower, C. (2012). Metagenomic microbial community profiling using unique clade-specific marker genes. *Nature Methods*, *9*(8), 811–814.

Sheu, S. Y., Shiau, Y. W., & Chen, W. M. (2013). *Sphingobium sufflavum* sp. nov., isolated from a freshwater lake. *International Journal of Systematic and Evolutionary Microbiology*, *63*(9), 3444–3450.

Smith, V. J., Roulston, C., & Dyrynda, E. (2014). The shrimp immune system. In V. Alday-Sanz (Ed.), *The shrimp book* (pp. 89–148). Nottingham University Press.

Sombatjinda, S., Wantawin, C., Techkarnjanaruk, S., Withyachumnarnkul, B., & Ruengjitchatchawalya, M. (2014). Water quality control in a closed re-circulating system of Pacific white shrimp (*Penaeus vannamei*) postlarvae co-cultured with immobilized *Spirulina* mat. *Aquaculture International*, *22*(3), 1181–1195.

Song, S. K., Beck, B. R., Kim, D., Park, J., Kim, J., Kim, H. D., & Ringø, E. (2014). Prebiotics as immunostimulants in aquaculture: a review. *Fish & Shellfish Immunology*, *40*(1), 40–48.

Souza Valente, C., Rodiles, A., Marques, M. R. F., & Merrifield, D. L. (2020). White spot syndrome virus (WSSV) disturbs the intestinal microbiota of shrimp (*Penaeus vannamei*) reared in biofloc and clear seawater. *Applied Microbiology and Biotechnology*, *104*(18), 8007–8023.

Steinberg, C. E. W. (2018). The Intestinal Microbiota – 'Your Eating Feeds a Plethora of Guests' and 'This Plethora of Guests Determines Who You Are and How Well You Do'. In ___. *Aquatic Animal Nutrition* (pp. 61–136). Springer International Publishing.

Takeuchi, M., Hamana, K., & Hiraishi, A. (2001). Proposal of the genus *Sphingomonas* sensu stricto and three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, on the basis of phylogenetic and chemotaxonomic analyses. *International Journal of Systematic and Evolutionary Microbiology*, *51*(4), 1405–1417.

Thitamadee, S., Prachumwat, A., Srisala, J., Jaroenlak, P., Salachan, P. V., Sritunyalucksana, K., Fleges, T. W., & Itsathitphaisarn, O. (2016). Review of current disease threats for cultivated penaeid shrimp in Asia. *Aquaculture*, *452*, 69–87.

Tsuchiya, C., Sakata, T., & Sugita, H. (2008). Novel ecological niche of *Cetobacterium somerae*, an anaerobic bacterium in the intestinal tracts of freshwater fish. *Letters of Applied Microbiology*, *46*(1), 43–48.

Van Hai, N., & Fotedar, R. (2009). Comparison of the effects of the prebiotics (Bio-Mos® and β-1, 3-D-glucan) and the customised probiotics (*Pseudomonas synxantha* and *P. aeruginosa*) on the culture of juvenile western king prawns (*Penaeus latisulcatus* Kishinouye. 1896). *Aquaculture*, *289*, 3-4, 310-316.

Wang, J., Huang, Y., Xu, K., Zhang, X., Sun, H., Fan, L., & Yan, M. (2019). White spot syndrome virus (WSSV) infection impacts intestinal microbiota composition and function in *Penaeus vannamei*. *Fish & Shellfish Immunology*, *84*, 130–137.

Wang, W., Sun, J., Liu, C., & Xue, Z. (2017). Application of immunostimulants in aquaculture: current knowledge and future perspectives. *Aquaculture Research*, *48*(1), 1–23.

Wei, J., Yu, N., Tian, W., Zhang, F., Wu, Q., Li, E., Zhang, M., Du, Z., Qin, J., & Chen, L. (2014). Dietary vitamin B12 requirement and its effect on non-specific immunity and disease resistance in juvenile Chinese mitten crab *Eriocheir sinensis*. *Aquaculture*, *434*, 179–183.

Xiong, J., Zhu, J., Dai, W., Dong, C., Qiu, Q., & Li, C. (2017). Integrating gut microbiota immaturity and disease-discriminatory taxa to diagnose the initiation and severity of shrimp disease. *Environmental Microbiology*, *19*(4), 1490–1501.

Yao, Z., Yang, K., Huang, L., Huang, X., Qiuqian, L., Wang, K., & Zhang, D. (2018). Disease outbreak accompanies the dispersive structure of shrimp gut bacterial community with a simple core microbiota. *AMB Express*, *8*(1), 120.

Zeng, S., Huang, Z., Hou, D., Liu, J., Weng, S., & He, J. (2017). Composition, diversity and function of intestinal microbiota in Pacific white shrimp (*Penaeus vannamei*) at different culture stages. *PeerJ*, *5*, e3986.

Zhang, W., & Mai, K. (2014). Nutrition and shrimp health. In V. Alday-Sanz (Ed.), *The shrimp book* (pp. 497–516). Nottingham University Press.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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