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RESEARCH PAPER

From concept to impact of a multispecies synbiotic for sustainable shrimp farming

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Abstract

Aquaculture is the fastest growing food producing sector in the world. India is second largest seafood producer in the world. Disease management is the big challenge in shrimp aquaculture. Even with the availability of specific pathogen-free (SPF) seeds, Vibriosis and *Enterocytozoon hepatopenaei* (EHP) still affect the culture causing substantial loss. Controlling pathogens in an ecofriendly and sustainable method is the desirable direction. In this context numerous research reports pointed out options such as probiotic, prebiotic, phytobiotic, phage therapy for disease management but synbiotic related reports are still limited. Synbiotic applications provide numerous beneficial effects for shrimp culture such as improving the immune system, disease resistance, pathogen control, maintenance of gut microbial population, and increased digestive enzymes. Synbiotic role and activity is effective and efficient than individual probiotic and prebiotic supplements. It is the more effective alternative for antibiotics and chemotherapeutics. This paper presents insights into the concept of synbiotic applications, performs a review of the literature, and discusses the efficacy of a multispecies synbiotic product Ecoforce in growth promotion and disease control in the shrimp farming industry.

Introduction

India has emerged as one of the top farmed shrimp producers with a production of 902,525 MT in 2022 (SAP, 2023). Indian aquaculture industry attained this through better disease control and management. With the harmful effects and concerns about safety, antibiotics face bans and rejection from potential buyers. In a recent and extensive review of the approach for sustainable aquaculture, overuse of antibiotics, disinfectants and other chemicals for disease control is listed among the serious concerns in aquaculture. The study listed the use of probiotics and prebiotics among the practices for sustainable aquaculture (Austin et al., 2022). Promising research established that pathogens need not be killed using antibiotics, but rather controlled with the use of beneficial bacteria that can eliminate the pathogens. Probiotics play a crucial role with their versatile potential in preventing pathogens by competition, antagonistic functions, promoting shrimp health and growth with gut activity and immunomodulatory effects (Van Hai & Fotedar, 2010; Zorriehzahra et al., 2016; Pérez-Sánchez et al., 2018; Madhana et al., 2021). The interest for bacterial probiotics also stems from the possibility of genetic engineering aiming at tailoring their metabolism and antagonism resulting in fine-tuned properties. Supplementing prebiotics enables the probiotic bacteria to better compete with the pathogens in the gut. Strong presence of probiotic bacteria intervenes with the quorum sensing ability of the pathogens and disrupts the biofilm formation in the villi of the shrimp thus making possible for better feed intake by the host. Ecoforce (a commercial product of Tablets India Limited, Chennai, Tamilnadu, India), a blend of probiotics and prebiotics has served the Indian shrimp farming as a synbiotic product for almost two decades. This review summarizes the research evaluating the efficacy of Ecoforce against Vibriosis and in growth promotion of shrimp in laboratory as well as in farms.

Probiotics for aquaculture

Selected single or multiple strains of organisms can contribute to a probiotic product to promote the health of the host or to benefit the environment. In aquaculture, probiotics can be generally divided into gut probiotics, water probiotics and soil probiotics. Gram-positive bacteria are the dominant probiotics used in aquaculture. Among the yeasts *Saccharomyces cerevisiae* and *Saccharomyces boulardii* are most found in probiotic products. If a probiotic strain yields good results in the laboratory, it may be regarded safe and may have potency for large scale industrial production. The product form should retain its character when introduced into the host or the environment for a prescribed shelf-life period so that it can be a viable product. When it comes to multispecies probiotics the participant strains should not have antagonistic properties against each other and compete among themselves. Preferably they should establish a relation with each other and colonize in the gut together (Austin et al., 2022; Gomez-Gil & Roque, 1998; Timmerman et al., 2004; Sahu et al., 2008; Kesarcodi et al., 2008; Ringo, 2020).

Gut probiotics, prebiotics and synbiotics

The gut of the crustacean shrimp can be thought of as a riverine/stream ecosystem since both have ecological principles applied in a similar means. Unique and niche microbial species inhabit both systems. The gut is a biotic substratum unlike a riverbed which is abiotic (Charalampopoulos & Rastall, 2009). Abiotic substratum of the riverbed doesn't get affected by the microbial consortia whereas the gut has epithelium cells which can be directly affected by the microbiota in the gut. Pathogens affect this ecosystem destructively leading to morbidity and mortality of the host. Supplementing probiotics enable establishing a supportive microbiota in the gut.

Hippocrates, the first historically recognized physician, noted that "bad digestion is the root of all evil" and that "death sits in the bowels" (Gasbarrini et al., 2016). Researchers have established the scientific aspects of this note through their work. Gut probiotics when fed along with the feed establish in the gut and support the host animal health through settling on the villi (Figure 1), improving nutrient absorption, helping in digestion, stimulating innate immunity and preventing pathogenic microorganisms by competitive inhibition (Zorriehzahra et al., 2016; Madhana et al., 2021). Probiotics also help shrimps overcome stress conditions (Van Hai &

Fotedar, 2010). Gut probiotics are used in aquaculture as an effective tool in disease management. Increased awareness in the bioaccumulation and biomagnification consequences of the use of antibiotic and the stringent regulations have emphasized the importance to the use of probiotic as a feed supplement in the aquaculture. Apart from disease management, now the applications of probiotic in Indian shrimp and fish farming have grown to maintain water quality and soil fertility.

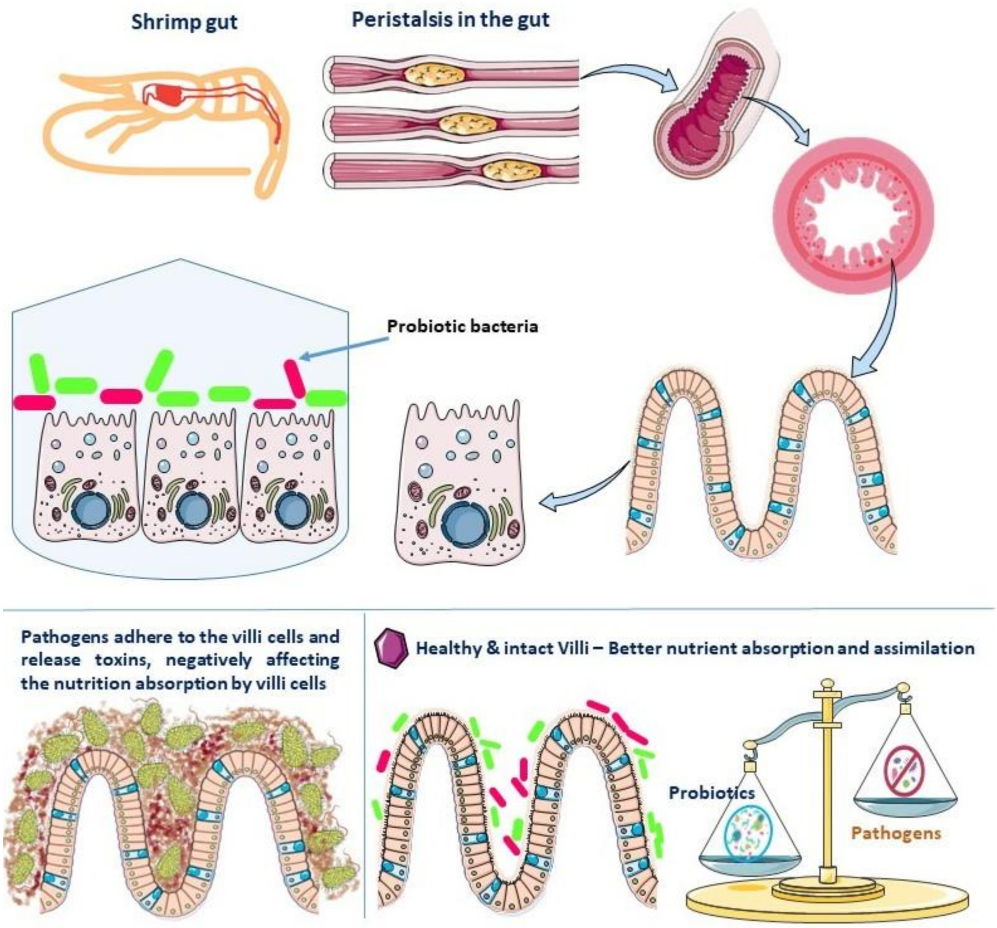

Figure 1. Schematic representation of gut probiotics acting on villi (Parts of the figure are created/adapted from SMART – Servier Medical Art, Servier: https://smart.servier.com).

Microbial strains used in shrimp aquaculture have been well reviewed (Farzanfar, 2006; Van Hai & Fotedar, 2010; Jamal et al., 2019; Amenyogbe et al., 2020; Ringo & Song, 2016; Knipe et al., 2021). Various criteria in selecting probiotic strains for shrimp aquaculture have been studied and reviewed (Gomez-Gil & Roque, 1998; Farzanfar, 2006; Van Hai & Fotedar, 2010; Lakshmi et al., 2013; Butt et al., 2021). Clostridium butyricum a butyric acid and lactic acid producing bacterium is proven to exhibit antagonistic effect against *Vibrio harveyi*, to provide protection against it in *Macrobrachium rosenbergii* and improve the growth of the animal (Sumon et al., 2018). *Enterococcus faecalis* is a proven probiotic for aquaculture. *E. faecalis* is known to exhibit antagonist activity against pathogenic *V. harveyi*. Supplementing *E. faecalis* in diet improves enzymatic activity, immunity and growth performance of *Macrobrachium rosenbergii* (Khushi et al., 2022). Lactic acid bacteria of *Streptococcus* spp. are qualified probiotics for aquaculture (Gatesoupe, 1999; Swain et al., 2009). *S. faecium* is reported to enhance weight gain, feed conversion ratio (FCR) and specific growth rate (SGR) in carp

(Bogut et al., 1998). Dietary supplementation of *Bacillus* sp. and yeast is found to positively impact the growth and survival in the *L. vannamei* (Nimrat et al., 2021). *Saccharomyces cerevisiae* when given along with feed enhanced the immunity and exhibited antioxidant activity in *Penaeus vannamei* promoting the health of the animal. *Saccharomyces cerevisiae* along with *B. subtilis* are proposed as potential probiotics combination for shrimp culture (Sundaram et al., 2017).

Prebiotics are the nourishment to the indigenous gut bacteria or beneficiary bacteria (Amenyogbe et al., 2020). Prebiotics ideally enable the establishment of desired microbiota and should have a synergistic effect with the probiotic species. Prebiotics are predominantly cost effective plant oligosaccharides, fibres that are not digested by the host but by the indigenous gut bacteria or by the supplied probiotics (Charalampopoulos & Rastall, 2009; Wee et al., 2022).

Probiotics in combination with prebiotic nourishment can be referred to as synbiotics. Synbiotics are gaining more importance in shrimp farming as they promote feed utilization, nutrient assimilation and growth of the animal. Shrimp farming being a disease prone industry synbiotics approach is one of the methods as alternative to antibiotics (Ringø & Song, 2016; Pérez-Sánchez et al., 2018). Providing prebiotic nourishment along with the probiotics enable the probiotics to compete better with r – strategist bacteria. The benefits of the synbiotics on shrimp health and feed utilization might be attributed to the host animal receiving more energy and nutrients, as well as improved absorption or digestion of certain nutrients and enzymatic activity (Ringo & Song, 2016). Mechanism of action of synbiotics are illustrated in Figure 2. Ecoforce as a synbiotic contains six probiotic bacterial strains, yeast, enzymes, dried yeast and nutrients (Table 1).

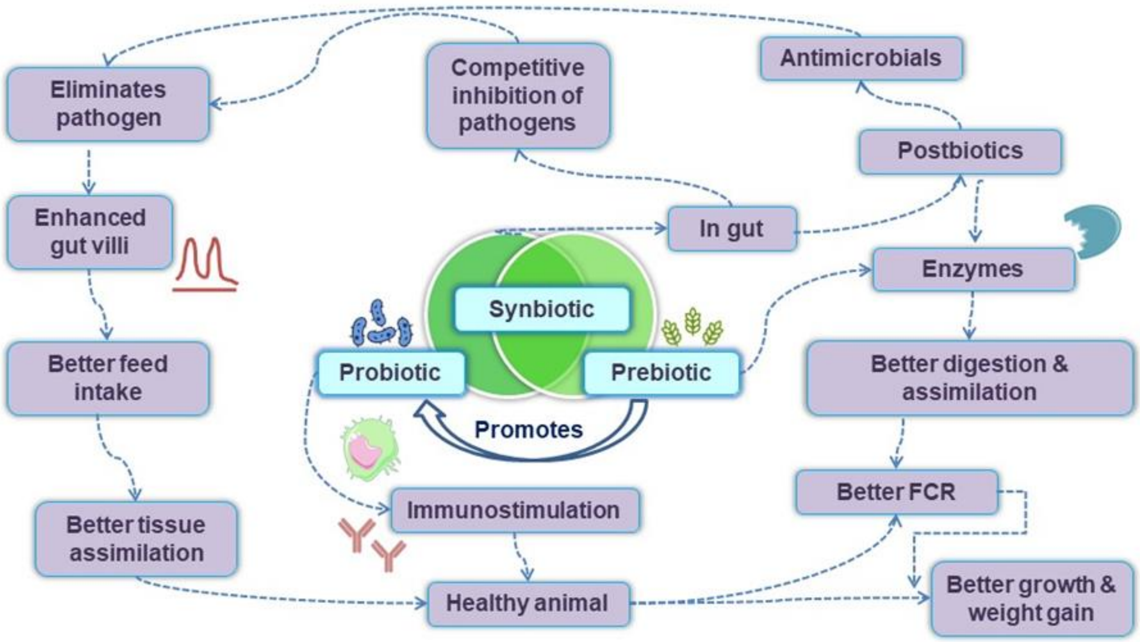

Figure 2. Mode of actions of a symbiotic (Parts of the figure are created/adapted from SMART – Servier Medical Art, Servier: https://smart.servier.com).

Several factors influence shrimp farming. The productivity in shrimp farming can be defined by the survival rate, growth rate, average body weight (ABW) and feed conversion ratio (FCR) attained in a particular crop. While there are best management practices to achieve a disease-free shrimp farming, using probiotics for a productive crop for boosting growth rate, better FCR, improving the immunity, is a viable and sustainable choice. Probiotics have grown to become an integral part of shrimp farming.

Survival rate

Survival rate in shrimp farming can be defined as the count of shrimp alive or harvested as a percentage of the number stocked in the pond. Survival rate depend on several factors such as seed quality, water quality, and stocking density. In intensive and semi-intensive shrimp farming gut probiotics enable better survival of the shrimps through their biological activity in the gut. Behera and Nayak (2011) reported 60-75% survival in five ponds of P. monodon culture at a stocking density of 96,000/ha with the use of Ecoforce as a gut probiotic along with water probiotics in all the five ponds. Mohan et al. (2011) in their farm level study in three ponds with one control pond and two Ecoforce used ponds, reported the survival rate of P. monodon for two crops. In the summer crop, the survival rate of *P. monodon* was recorded as 73%, 85% and 82% for the treated ponds. Similarly, in the winter crop the survival rate of *P. monodon* was recorded as 72%, 80% and 84%. In both crops the survival rate was higher in Ecoforce treated ponds (Mohan et al., 2011). Elumalai et al. (2013) in a farm level study, observed the effect of Ecoforce to be significantly better on the survival of *Penaeus monodon* with 76% survival rate in Ecoforce treated pond as against the 59% in the control pond. In another farm level study on *P. monodon* the survival rate significantly increased with the application of the Ecoforce. A survival rate of 86.8% was observed in the Ecoforce treated pond whereas only 76.6% was attained in the control pond. With an increased dosage of the gut probiotic slightly higher survival rate of 88.1% was attained (Rajinikanth et al., 2010). Ecoforce reduces the mortality rate effectively as observed by George et al. (2019) in a farm level study on *Litopenaeus vannamei* with 13 to 15 dead shrimps per day in the control pond and 0 to 1 dead shrimps in the Ecoforce treated pond (Table 2).

Table 1. Composition of the synbiotic, Ecoforce per 500 g

Ingredients	Quantity
Streptococcus faecium ABPL153	5 g
Streptococcus faecalis (T-110)	5 g
Bacillus mesentericus TO-A	3.33 g
Bacillus subtilis ABPL 151	3.33 g
Bacillus sp ABPL 152	3.33 g
Clostridium butyricum TO-A	10 g
Saccharomyces cerevisiae	5 g
Alkaline protease	5 g (500,000 Units)
Lipase	5 g (20,000 Units)
Dried yeast	205 g
Triticum aestivum	239.00 g
Excipient	q.s.
Calories	812.60 cal
Protein	28.00 g
Fat	5.26 g
Carbohydrate	172.00 g
Fibre	4.78 g
Ash	4.06 g
Calcium	95.60 mg
Phosphorous	901.03 mg
Iron	8.37 mg
Potassium	956.00 mg
Thiamine	1.31 mg
Riboflavin	0.26 mg
Niacin	11.47 mg

Growth rate and weight gain

Gaining a marketable weight in an appropriate period of culture makes shrimp farming profitable. This can be achieved only with a good growth rate. Gut probiotics encourage growth rate and weight gain in juvenile and adult shrimps. Swain et al. (2009) in a laboratory level study of assessing the potential of probiotic bacteria against Vibriosis in *Penaeus monodon* post larvae studied the effect of Ecoforce (Table 2). The specific growth rate (SGR) was good and 100% more than the control with an SGR of 0.552 mg/day in the control and 1.07 mg /day in the Ecoforce treated. The post larvae treated with Ecoforce attained a 53 mg weight which was high in comparison with 28 mg in the control (Swain et al., 2009). In a farm level study by Rajinikanth et al. (2010) in *P. monodon* the ABW at 130 days of culture (DOC) was 30.2 g in Ecoforce treated pond which was high compared with the 23.1 g in the control pond. In that study, it was also observed that the ABW increased significantly to 32.0 g with increase in the dosage of Ecoforce. Mohan et al. (2011) reported a growth of 29.92±0.37 grams in 120 DOC with use of Ecoforce as feed supplement in *P. monodon*, whereas the control pond was harvested at 21.7 grams in 105 DOC due to white spot. Behera and Nayak (2011) in their study in five ponds of *P. monodon* culture reported ABW of 30, 30, 32, 32 and 33 grams with use of Ecoforce in all the five ponds. A higher ABW of 29.2g in Ecoforce treated pond against 22.6 g in the control pond was observed in *P. monodon* at the end of the culture in a farm level study (Elumalai et al., 2013). In a study on L. vannamei from DOC 55 to 65 an ABW of 11.9g was observed in Ecoforce treated pond as against 11.1g in the control pond. Moreover, the influence of Ecoforce in weight gain in 9 days in the mentioned study was remarkable with 2.2g in Ecoforce treated pond as against 1.5g in the control pond (George et al. 2019).

Table 2. Studies that discussed the efficacy of Ecoforce

Author & year	**Study type**	**Objective of the study**
Swain et al. (2009)	Laboratory level study	To study the efficacy of selected probioitcs against *Vibrio* infection in *P. monodon* PL
Rajinikanth et al. (2010)	Farm level study	To assess the efficacy of probiotics and growth promoters in *P. monodon*
Behera & Nayak (2011)	Farm level study	To assess the role of probiotics in shrimp farming
Mohan et al. (2011)	Farm level study	To assess the effect of Ecoforce on growth and survival of the *P. monodon*
Elumalai et al. (2013)	Farm level study	To assess the effect of Ecoforce on growth and survival of the *P. monodon*
Pattukumar et al. (2013)	Laboratory level study	To study the immunomodulatory effect of selected probiotics in *P. monodon* PL
George et al. (2019)	Farm level study	To assess the potency of Ecoforce against Vibriosis in *L. vannamei*

Feed conversion ratio (FCR)

FCR in shrimp farming can be termed as the ratio of the quantity of feed given over weight gain in shrimp in a known period of time. Lower FCR indicates highly cost effective feeding and less polluting pond bottom, which also translates to better water quality in the pond. Gut probiotics play a crucial role by enabling better digestion and nutrient assimilation which ultimately helps in getting good FCR. The farm level observation by Rajinikanth et al. (2010) up to DOC 115 establish the efficacy of Ecoforce in getting low FCR with 1:1.5 as FCR in Ecoforce treated pond in comparision with 1:2.1 in the control pond. In the same study an even lower FCR of 1:1.25 was observed in the pond administered with high dosage of Ecoforce (Rajinikanth et al., 2010). Behera and Nayak (2011) in their study in five ponds of *P. monodon* culture in Balasore, Orissa, reported FCR ranging between 1.51 to 1.59 using Ecoforce as a feed supplement. Elumalai et al. (2013) in their farm level study on *P. monodon* assessed the potential of Ecoforce in impacting the FCR which revealed an FCR of 1:1.80 in the control pond and 1:1.47 in the Ecoforce treated pond. The above study in line with Rajinikanth et al. (2010) establishes the efficacy of Ecoforce in attaining a highly efficient FCR in shrimp culture.

Effectiveness against Vibriosis

Vibrio infection remains as one of the major threats in shrimp farming. *Vibrio*, provided with their rapid multiplying ability and as r-strategist species take predominance in the aquaculture system when the organic nutrient load is high. This increases the chance of shrimp getting infected. Apart from using probiotics as biocontrol agents to competitively inhibit the Vibrio in the pond water to prevent infection, it is also essential to treat the shrimp with probiotics to control Vibriosis when required.

In depth studies have been done to understand the effectiveness of Ecoforce against Vibriosis. Swain et al. (2009) studied the efficacy of probiotics against *Vibrio harveyi* in *P. monodon* post larvae in which the mortality was significantly reduced from 40% (control) to 32% (Ecoforce supplemented). In a laboratory level study by Pattukumar et al. (2013) the efficacy of probiotics in Vibrio parahaemolyticus challenge study was assessed in which the mortality rate in juvenile and adult *P. monodon* was controlled significantly in Ecoforce supplemented animals. The accumulated mortality rate in the control was 65% and 68% in juvenile and adults, respectively which was reduced to 25% and 31% in Ecoforce supplemented group. A farm level study in *L. vannamei* by George et al. (2019) examined the potential of Ecoforce in treating shrimp ponds affected by Vibriosis in which the study was for 9 days from DOC 55. The percentage of the infected shrimps came down drastically from 53% (on DOC 55) to 3% on DOC 65 with administration of Ecoforce whereas the infection was observed to increase from 41% to 60% in the same period without Ecoforce supplementation. In the same study, the daily mortality of shrimps daily decreased from 63 to 3 in Ecoforce supplemented pond whereas in the control pond it increased from 42 to 95 (George et al., 2019).

Immunomodulatory effect

Probiotics offer beneficiary immunomodulatory effects to the host. Prebiotic dried yeast acts as a rich source of proteins contributing to the nutrition of the shrimp and with β-glucans offering excellent immunomodulatory effect to the shrimp. Pattukumar et al. (2013) studied the immunomodulatory effects of probiotics in shrimp in detail and observed a significantly higher total haemocyte count, phenol oxidase activity, phagocytic activity and nitroblue tetrazolium (NBT) respiratory burst in juvenile and adult *P. monodon* supplemented with Ecoforce in comparision with the control. In a farm level study supplementation of Ecoforce increased the total haemocycte count and the time required for clotting of haemolymph reduced significantly compared with the control pond devoid of Ecoforce (George et al., 2019).

Multispecies probiotics are more effective in offering benefits to the host in comparison with a single strain probiotic and to a lesser extent in case of multistrain probiotics (Timmerman et al., 2004; Kwoji et al., 2021; Puvanasundram et al., 2021). With different metabolites offered by different species a synergistic probiotic effect is established in the gut. Seo et al. (1989) reported that the mixed cultures of *Streptococcus faecalis* T-110 and *Clostridium butyricum* TO-A inhibited the growth of enteropathogens (enterotoxigenic *Escherichia coli*, *Salmonella typhimurium*, *Vibrio parahaemolyticus*, *Clostridium difficile* and *Clostridium botulinum*). These probiotic strains achieve this by symbiosis between them. These selected probiotic strains are known to produce organic acids such as butyric acid, lactic acid and bring down the pH creating unfavourable environment for the pathogenic bacteria (Seo et al., 1989). The probiotic strains in Ecoforce act symbiotically among themselves facilitating the proliferation of each other (Seo et al., 1989; Huang et al., 2013). Tripathi (2020) in his survey observed the predominant use of Ecoforce as feed supplement in the shrimp farms in Gujarat, India.

Conclusion

Ecoforce has emerged not only as an effective synbiotic product in shrimp health management but also as a sustainable tool in preventing the use of antibiotics in shrimp farming. Synbiotics are the alternatives for antibiotics and chemotherapeutic agents in aquaculture disease management. This review emphasizes the importance, functions and administration of synbiotics in shrimp aquaculture. Though there are other possible biological approaches such as post biotic, quorum sensing interference and phage therapy they are neither cost effective nor sustainable in shrimp health management. In case of phage therapy there are possibilities of resistance development and transfer of virulence. Whereas probiotic do not enable development of resistance and do not form residue in the meat produced. Probiotics and synbiotics are the way forward for the sustainable aquacultured shrimp health management.

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Ethical approval

The author declares that this study complies with research and publication ethics.

Informed consent

Not available.

Conflicts of interest

There is no conflict of interests for publishing of this study.

Data availability statement

The data used to support the findings of this study are included in the article.

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Author contribution

Jeganathan Arun: Conceptualization, Literature search, Writing original draft, Illustrations, Revisions.

Godfred Ponraj Jeyaraj: Conceptualization, Review, Editing.

Seerengaraj Vijayaram: Critical Review, Editing.

All authors contributed to the final manuscript.

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