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Review on the emerging white feces syndrome in shrimp industry

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Abstract
In the past few decades, shrimp industry is facing many uncertainties, especially by disease outbreaks, which are causing severe economic as well as production losses throughout the world. White Feces Syndrome (WFS), a type of bacterial disease, is a kind of disease where farmers do not have any curative measures to revive the infected animals. Similarly, another disease called EHP (*Enterocytozoon Hepatopenaei*), caused by a microsporidian parasite is often associated with WFS. In both cases, the sudden reduction in actual feed consumption, hepatopancreas discoloration, loose shell, and growth reduction are the commonly noticed symptoms. To date, there is no novel treatment has been found to fight this deadly WFS disease. This article briefly discusses the WFS disease outbreak conditions, and their preventive measures for farm level adoption by farmers to keep their animal away from WFS.

Keywords: *Vibrio* sp., *Penaeus vannamei,* shrimp disease, biofloc

Introduction
In the recent past, among the livestock producing sectors, aquaculture remains the fastest growing sector in India. In aquaculture, among the cultivable fish, shrimp is a highly demanded commodity both in the international and domestic market, as a source of nutrient rich food. In recent years, shrimp cultivators are facing a problem with disease control and management. White feces syndrome (WFS) is a major threat to the shrimp industry since 2010. WFS was first reported in Thailand during 2009 [1]. Presently, like EHP, WFS is posing a serious problem in the world's shrimp aquaculture industry. In India, Andhra Pradesh, Tamil Nadu, Gujarat, and West Bengal are the states, which are largely affected by WFS disease. It may lead to significant economic loss for both shrimp cultivators as well as to the national economy. Since, in the past few decades, frozen shrimp, the product produced through aquaculture, is the major exporting commodity which is contributing more to the national economy [2]. The present article briefly discusses the causes of WFS conditions responsible for WFS outbreak and its control or preventive measures which might be helpful for the farmers to combat the WFS.

Causative agent of White Feces Syndrome (WFS)
WFS is occurring due to the imbalance or disturbances in the intestinal microbial community of culture animals. Still today, the underlying reasons for the outbreak of WFS remain unclear. A decrease in intestinal microbial diversity and an increase in heterogeneity of intestinal microbiota cause WFS disease [3]. In India, from the WFS affected Penaeus sp. *Vibrio harveyi*, *V. alginolyticus* and *V. anguillarum* has been reported [4]. Similarly, from the feces of WFS affected shrimp *Vibrio* pathogens, such as *Vibrio parahaeolyticus*, *V. fluvialis*, *V. mimicus, V. alginolyticus* and *Vibrio* sp. been reported [5]. The major causative agent of White feces syndrome is *Vibrio alginolyticus* and *Vibrio fluvialis* [6]. According to the available knowledge, *Vibrio* load is very high in the fecal matters of WFS affected shrimp, which indicates the presence of *Vibrio* pathogen in culture water and intestine of shrimp.

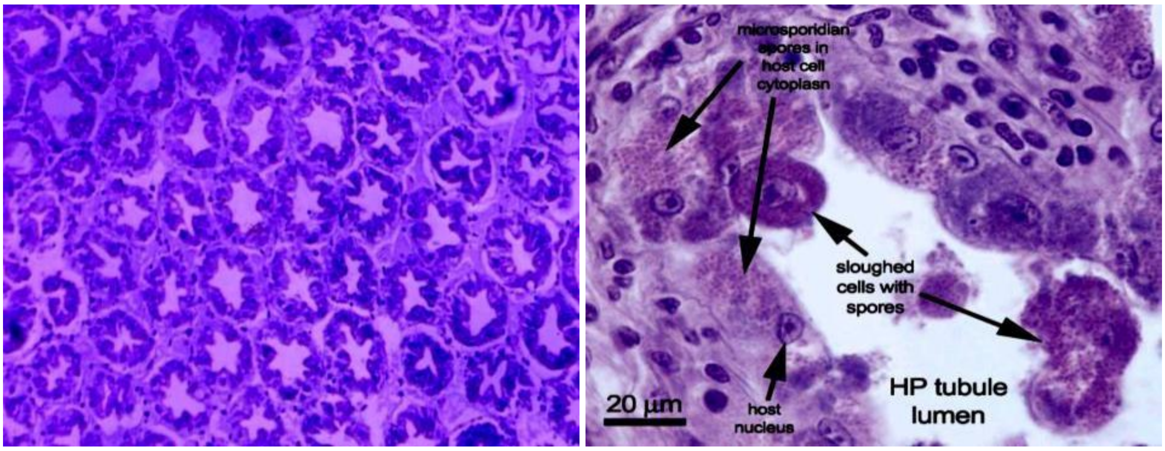
When compared intestinal microbiota of healthy and WFS affected shrimp, in WFS infected shrimp *Candidatus bacilloplasma* and *Phascolarctobacterium* levels were increased and *Paracoccus* and *Lactococcus* levels were decreased [3]. However, WFS affected shrimp intestine and hepatopancreas also contains protozoan Gregarines [1]. EHP (*Enterocytozoon hepatopenaei*) is more often associated with WFS disease [7]. Among the reports in different geographical areas, *V. parahaemolyticus* and *V. alginolyticus* are the two dominant *Vibrio* species in all WFS affected shrimp ponds.

Generally, it is known that intestinal microbial balance plays an important role in maintaining host health, nutritional absorption and immune response [8]. WFS causes 20-30% of mortality and total rejection of WFS affected shrimps was reported in Sri Lanka. In 2010, Thailand faced a crash in overall shrimp production (over 15-20%) due to the WFS outbreak [1].

EHP and white feces syndrome

Enterocytozoon hepatopenaei (EHP) is a microsporidian and its outbreak in tiger shrimp was first reported in Thailand during 2009. It is an obligate, intracellular microsporidian parasite. They form spores and remain viable for six months and can retain its infectivity to a maximum of one year in aqueous conditions [9]. EHP in shrimp is associated with growth retardation (Fig. 1) [10] (Size variation in the same aged group). The target organ for EHP is hepatopancreas and, it affects the function of digestion and nutrients absorption, which results in poor growth or retarded growth and poor immunity. EHP can be transmitted horizontally by cannibalism of shrimp. Horizontal transmission by water is also common. Artemia and *Grapsidae* family crabs are considered as a potential carrier of EHP [11]. EHP can be easily diagnosed by PCR and histology of hepatopancreas (Fig. 2) [10] and intestine of the affected animal.

Fig 1: Size variation in the same aged group



A. B.

Fig 2: A comparison of normal hepatopancreas and EHP affected hepatopancreas. A. Histology of normal shrimp hepatopancreas, B. Histology of EHP affected shrimp hepatopancreas (arrow mark shows, accumulation of spores on the tubules of hepatopancreas).

EHP (*Enterocytozoon hepatopenaei*) is more often associated with WFS disease [7]. The occurrence of EHP is more prevalent with WFS (96.4%) than without WFS (39.7%). In addition to *Vibrio* sp. fecal matters of WFS affected shrimp contains spores of microsporidian [12]. Microsporidian such as *Enterocytozoon hepatopenaei* is not a causative agent of white feces syndrome in white leg shrimp (*Penaeus vannamei*) [13]. No treatments and/or drugs have been developed to treat EHP affected shrimp ponds. Only one solution for EHP affected pond is partial harvesting and the only way to keep EHP away from our culture system is to follow better management practices (BMPs) and maintaining proper biosecurity throughout the culture period. However, Indian farmers are using garlic and bitter guard paste at the rate of 30-40 g/kg feed to treat EHP affected animals [10]. In Indonesia, probiotics, garlic paste (10-20 g/kg feed) and vitamin C (2 g/kg feed) were used to treat as well as to avoid the EHP and WFS outbreaks [12].

In EHP affected ponds, spores settle at the bottom and it can viable for six months. To remove the microsporidian spores, farmers need to apply lime @ 6 tons per hectare followed by ploughing of the pond bottom. It raises the pond bottom pH up 12 and maintains this pH for one week with adequate moisture content. This process disinfects the pond bottom and eliminates the parasitic spores [9]. Farmers should stock EHP free shrimp seeds and periodically analyse the fecal samples and stored feed to find out the presence of EHP spores.

How to identify WFS (White Feces Syndrome) affected shrimp ponds

The outbreak of WFS in shrimp is not clear, but the examination of intestinal microbiota and hepatopancreas gives an idea about the causative agent. One can easily identify WFS by observing the check tray and surface of the culture water (Fig. 3) [1]. Normally in WFS affected pond, white fecal strings float over the surface of the culture water and sudden reduction in feed consumption was noticed. In some cases, affected shrimp shows loose shell, dis-colouration in hepatopancreas, and white gut (Fig. 4) [6]. The outbreak of WFS is more prominent during the end of the second month of culture, probably 10-12g sized shrimp. WFS outbreak is more severe during the summer season (during high-temperature periods) than the rainy season. WFS causes growth retardation and severely affected shrimp pond shows high mortality.

White feces are not true feces, which are composed of non-assimilated, egested shrimp feed. Instead, the white fecal matter comprised of EHP spores, gut mucus, and necrotized epithelial cells [12]. Sometimes fecal strings are golden brown or yellow colour in the intestine of WFS affected shrimp (Fig. 6) [1].

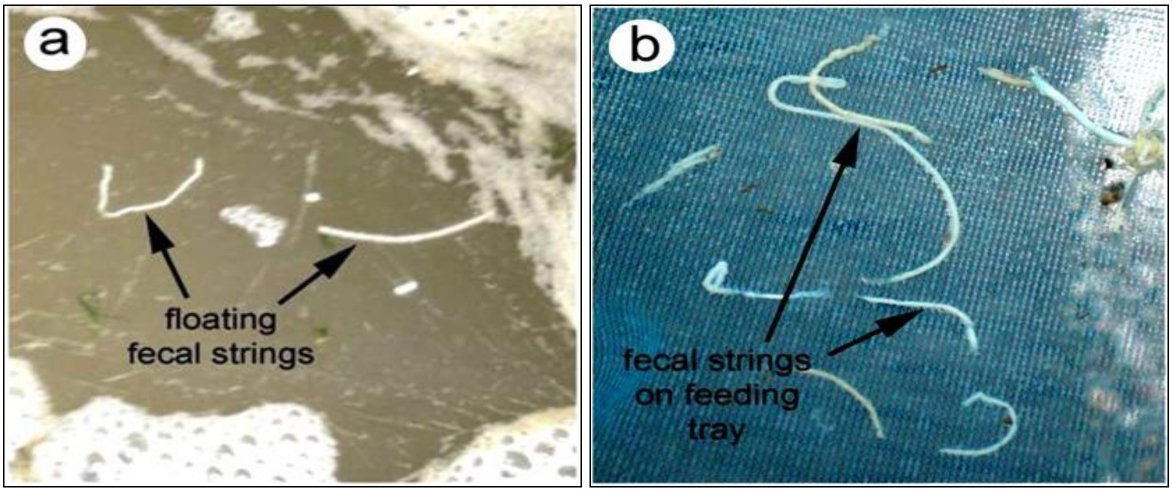


Fig 3: A. Floating fecal strings on the water surface, **B.** Fecal strings on check tray

Fig 4: A. WFS affected shrimp gut and hepatopancreas (white colour gut and discoloured hepatopancreas), **B.** unaffected shrimp gut and hepatopancreas (without white gut and discoloration in hepatopancreas)

Fig 5: Comparison of healthy animal feces with WFS affected animal feces. **b1**. Healthy shrimp fecal matter, **b2**. WFS affected shrimp fecal matter (white in colour) [3].

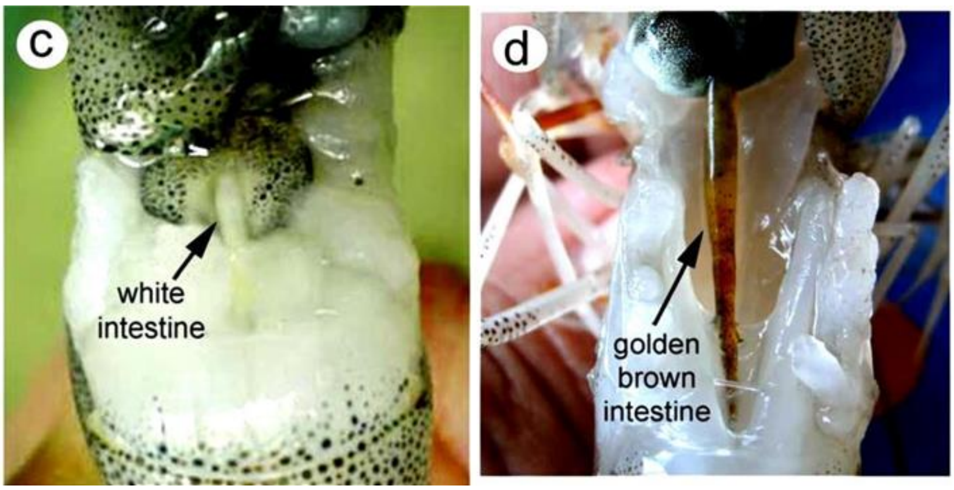


Fig 6: The intestine of affected shrimp. **C.** white intestine of an affected animal, **D.** Golden brown intestine of an affected animal.

Pathophysiological changes in WFS affected shrimp

Energy metabolism is completely/partially disturbed in WFS affected shrimp, results in poor or retarded growth [3]. The intestinal microbial balance is much essential for the enzymatic digestion of ingested food material, because, the beneficial microbial community in the gastrointestinal tract

(GIT) of culture animal produces digestive enzymes (amylase, lipase, protease, and cellulase), which enhances the enzymatic digestion and active absorption of available nutrient. The decrease of microbial diversity in the GIT of WFS affected shrimp shows reduced enzymatic digestion and active absorption process, which results in reduced feed intake and growth rate. WFS affected shrimp shows slow growth and increased food conversion ratio (2.5-3.0) as compared to healthy shrimp ponds FCR 1.4-1.7 [12]. WFS affects the functions of hepatopancreas (digestive and absorptive functions) and turns the colour of hepatopancreas into pale.

Conditions responsible for WFS outbreak

- Poor water quality management is the prime reason for the WFS outbreak. It increases the bacterial community especially *Vibrio* pathogen.
- High temperature during the summer season.
- Other conditions include -
- High stocking density,
- Algal crash in the culture system,
- Poor quality feed and
- Polluted environmental conditions.

Control measures of WFS

Maintaining biosecurity Good management practices (GMPs) and biosecurity are the two ways to prevent or/and control the emergence of WFS in the shrimp culture ponds. Maintaining good water quality, disinfection of materials, good hygiene of workers, proper chlorination and de-chlorination of culture water before and after use, selecting good quality feed and better storage of feed are the important GMPs. Critical better management practices (CBMPs) and critical bio-security measures (CBSMs) such as proper use of probiotics are needed to be followed to prevent the entry and spread of *Vibrio* pathogen in the culture system. Avoid overstocking during the summer season. Removal of white feces, floating over the surface of culture ponds, and periodic removal of sludge, accumulated at the bottom of culture ponds, reduces the chances of spreading of pathogens and bacterial load in the culture water.

Farmers should maintain a reservoir pond to treat the water before it is used for culture purposes, which is an important component in BMPs of shrimp farming. Though the farmers stock healthy shrimp seeds (disease-free seeds) in their culture ponds, the use of untreated water is contaminating the culture water with deadly pathogens [14, 15].

Use of feed additives

Feed additives can be used to reduce pathogens in the GIT of the cultured shrimp. Indonesia farmers are using garlic paste, in the form of freshly crushed garlic or processed powder (10-30 g/Kg feed), allicin and Vitamin C (2 g/kg feed) (stress reliefs) feed additives. Indian farmers are using garlic paste around 80 g/kg feed to control WFS and they got good results in large-scale farming. Initially, garlic paste (80 g/kg feed) is blended with 150ml of freshwater and 5-10 ppm of potassium permanganate ($KMnO_4$). The mixture is then sprayed over the feed and fed to the animal.

Other than this, supplementation of organic acids, probiotic (different strains of *Bacillus* sp.), β-glucans or oligosaccharides and garlic paste or garlic extract with feed by surface coating control the WFS disease. Use adequate water to mix these four ingredients and spray over the feed uniformly. Then use good surface binder (sodium alginate) to prevent the leaching of added ingredients. Finally, allow it to dry for a minimum of 30 min. In WFS affected pond use 10-15 g of this ingredient mix per kg of feed for two meals in a day.

Use of organic acids

Organic acids generally regarded as safe with antimicrobial. Organic acids serve as a growth promoter as well as prophylactic agents against bacterial pathogens. Organic acids such as citric acid, sorbic acid, acetic acid, propionic acid, and butyric acid reduce the pH of GIT of culture animals and fight against the *Vibrio* pathogen. Among the above-mentioned organic acids, formic acid shows the strongest inhibitory effect on *Vibrio* spp., Use of formic acid at lower concentrations (less than 0.039%) positively inhibits the growth of five *Vibrio* pathogens (*Vibrio alginolyticus*, *Vibrio cholerae*, *Vibrio harveyi*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*) [16]. 2% of organic acids in the shrimp diet decrease the *Vibrio* spp. counts in hepatopancreas of *Penaeus vannamei* which increased the phosphorous digestibility [17]. Normally in WFS affected shrimp, gut pH increase to alkaline condition, which enhances the colonization of pathogenic bacteria in the epithelial layer of the gastrointestinal tract. The addition of organic acids to the shrimp, through the formulated feed as a feed additive, reduces the gut pH, which consequently reduces the incidence of pathogenic colonization. At lower pH, minerals in the feed soluble in GIT of culture animal and available for absorption.

Use of organic acids (25% citric acid and 16.7% sorbic acid) and essential oils (1% vanillin and 1.7% thymol) in the feed enhance the non-specific immune response, which increased the disease resistance of shrimp against *V. parahaemolyticus* and maintain the intestinal microbial balance [18].

Control of WFS using probiotics

The use of probiotics (*Bacillus subtilis*) controls the *Vibrio* pathogen. The added *Bacillus subtilis* serves as a bio-remediator as well as a probiotic. It enhances immune performance, disease resistance, survival and growth rate. The multimodal strategy needs to be followed to avoid or control the disease outbreak which includes the proper implementation of BMPs with probiotic supplementation. Farmers in Indonesia are using *B. subtilis* to eliminate *Vibrio* spp. from the shrimp intestine and as well as from the culture environment [12].

Biofloc system and WFS disease

So far there are no known reports are available on the outbreak of WFS in the biofloc system. 'Biofloc' the word defined as "the macro aggregates of algae, bacteria, protozoans and other kinds of particulate organic matters, which includes uneaten feed and fecal matters". Biofloc system provides complete biosecurity throughout the culture period by maintaining good water quality, eliminating pathogenic bacterial community, optimizing FCR, minimizing water exchange and it enhances the immune level of culture animals. Biofloc system is a zero water exchange system, which is a basic better management practice (BMP) in shrimp farming. Biofloc itself serves as a basic feed for shrimp; hereby it reduces the feed usage. Biofloc is produced by applying organic carbon (molasses, spent wash, rice bran) as a carbon source. With available carbon level microorganisms utilize total ammonia nitrogen (TAN) and convert it into a microbial protein. The added carbon is used as an energy source by the microorganism to immobilize the TAN. Therefore, farmers can easily adapt to the biofloc system, it requires only proper maintaining of C: N ratio (15-20:1) and continuous aeration for the suspension of organic load and floc. This system eliminates *Vibrio* spp. from the culture environment and maintains the microbial balance in both the intestine of culture animal and culture water. Therefore, it reduces the risk of WFS outbreak in shrimp aquaculture ponds.

Conclusion

Shrimp is the most dominant and prevalent culture crustacean (>4.1 million tons per year, globally), being affected with newly emerging diseases. In recent days, white feces syndrome, a major threat to shrimp farming practices, which causes huge loss, globally, even its causal agents remain unknown. There is a need for control and preventive measures to address the WFS problems in shrimp farming industry.

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