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Incidence of white feces syndrome (WFS) and its management with herbal supplements (*Allium sativum* and *Curcuma longa*) by improving the growth and gut health of shrimp *Litopenaeus vannamei* (Boone-1931)

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Abstract:
White Gut Disease, also known as White Feces Syndrome (WFS), is a prevalent and economically significant condition affecting the Pacific white shrimp (*Litopenaeus vannamei*) aquaculture industry in worldwide. This condition is distinguished by the presence of pale or whitish excrement in shrimp that are affected, along with gastrointestinal problems. The exact etiology of white gut disease in *L.vannamei* remains unclear, although various factors have been implicated. The present study suggests that the disease may be multifactorial, involving a combination of infectious agents, dietary imbalances, environmental stressors, and improper management practices. White gut disease has significant negative impacts on shrimp health and productivity. Infected shrimp may exhibit reduced feed intake, growth retardation, and increased mortality rates. The WFS disease also has economic implications, leading to financial losses for shrimp farmers due to decreased production and increased treatment costs. The present study reveals that, the role of herbal supplements *Allium sativum* and *Curcuma longa* on improving growth and gut health of the shrimp *L.vannamei* in the culture pond environment.

Keywords: White Feces Syndrome, herbal supplements, growth, *L. vannamei*, shrimp farming.

1. Introduction:

The White Feces Syndrome (WFS) is a gastrointestinal illness that affects cultured penaeid prawns all over the world. The presence of white fecal strings floating on the water surface of culture ponds characterizes the illness. Infected shrimps typically have pale to yellowish midguts, slow growth, significant size variation, decreased average daily growth, higher feed conversion ratios (FCR), loose exoskeletons, and occasionally mortality (Aranguren Caro *et al.*, 2021 and Munkongwongsiri *et al.*, 2022). The intestine histology indicated a thin intestinal wall, detachment of intestinal epithelial cells, and a decrease or removal of microvilli (Huang *et al.*, 2020). WFS often occurs between 50 to 60 days of culture (DoC) after stocking the seed in the culture ponds. It weakens the shrimp and causes chronic mortalities, reducing output yield by up to 60% (Durai *et al.*, 2015 and Huang *et al.*, 2020).

At the farm level, the illness has been linked to a number of issues, including shrimp growth retardation, size discrepancies, poor feeding, and chronic mortalities. Several articles have linked WFS to various aetiologies, such as *Vibrio cholera*, gregarine-like organisms, *Bacilloplasma sp*., and *Phascolaractobacterium sp*. Siriporn Sriurairatana *et al*., (2014) has been reported that white feces syndrome (WFS) in shrimp occurs due to the conversion, shedding, and clustering of hepatopancreatic microvilli into worm-like structures, which bear a resemblance to protozoan gregarines.

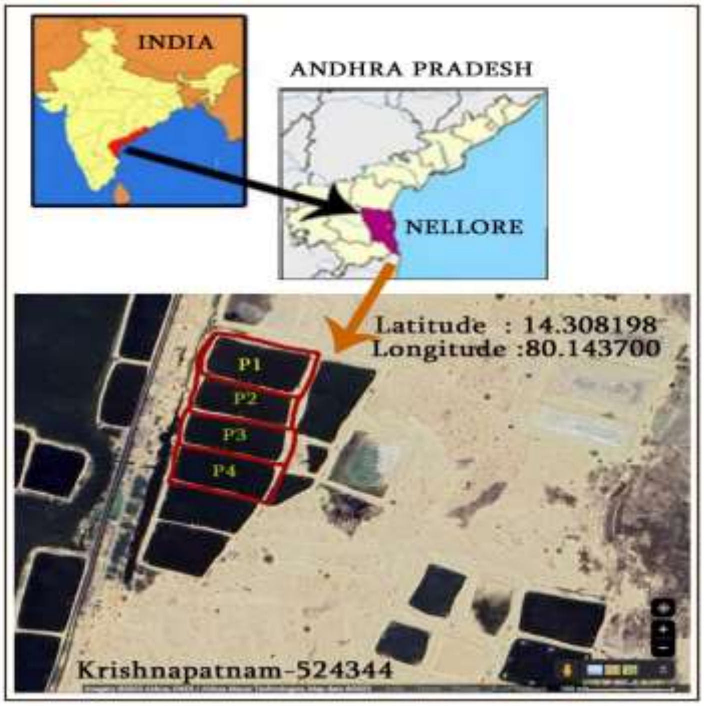
White fecal syndrome (WFS) in *L.vannamei* is correlated with several conditions, including elevated stocking numbers, subpar water quality, deteriorated pond bottoms, excessive plankton blooms, inadequate feed management, and heightened pollutants in pond water. (S.A. Masthan, 2015). The White Feces Syndrome (WFS) has emerged as a significant issue in the global shrimp aquaculture industry, particularly in the cultivation of *L.vannamei*, in recent years. (Satish Kumar *et al.,* 2022). The large-scale production of shrimp, farmers have begun to include antibiotic growth promoters in feed to address the issue of waterborne fungal infections in the culture ponds. However, because of its adverse effects such as the buildup of residues in fish tissue and the rise of antibiotic-resistant microorganisms, natural substances are more readily accepted by the public. The herbal immunostimulants that have been documented to improve the efficiency of feed utilization and promote animal productivity (Levic *et al*., 2008). Herbal plants offer a wide range of qualities, including antioxidants, antimicrobials, anticarcinogens, analgesics, insecticides, anti-parasitic, anticoccidials, growth promoters, appetite stimulants, bile secretion stimulants, and digestive enzyme activity stimulants. laxatives and antidiarrhea, hepatoprotection (Coutteau *et al*., 2011).

Garlic (*Allium sativum*) and turmeric (*Curcuma longa)* It has been utilized in the treatment of numerous ailments and has various advantageous effects for both humans and animals. These advantages include antibacterial, antioxidant, and antihypertensive qualities (Sivam, GP 2001). Garlic possesses the ability to effectively control infections, including those caused by bacteria and fungi, hence enhancing animal well-being. (Corzo, 2007). Allicin and curcumin, which are the active constituents, exhibit a broad spectrum of activity against fungus, bacteria, and helminths (Ali Hussein *et al.,* 2021). Hence, the primary objective of this study was to assess the effectiveness of garlic and turmeric in promoting the growth and maintaining the gut health of shrimp *L.vannamei* impacted by WFS in a culture pond setting.

2. Materials and methods:

The current study was conducted at a privately-owned shrimp farm, located at Krishnapatnam coastal village (latitude / longitude: 14.24° / 80.14°) of Muthukur mandal in Nellore district of Andhra Pradesh, India. The four semi-intensive earthen ponds 0.5 ha (P1, P2, P3 and P4) of shrimp *L.vannamei* infected with WFS (P3 & P4) were adopted for this study (Fig.1).

Fig 1: Map showing study area of shrimp experimental ponds at Krishnapatnam village, Nellore coast of Andhra Pradesh.



At the time of experiment with an average wet weight of shrimp juveniles having 3-5 grams of 30 to 40 days of culture (DoC) was selected in this study. The experimental culture ponds maintaining the salinity of 17-19ppt with ground water, and ideal pH ranges 7.6-8.3 maintained in the entire the experimental duration. The culture ponds selected for this study are uniformly prepared, adhering to standard management procedures such as drying, leveling, plowing, liming and the pond water level was maintained up to 1.2 to 1.3 m depth (Table:1). This was followed by usual pre- stocking management practices of manuring with fermented juice for fertilization of all ponds to grow the useful plankton bloom in pond water. There was no exchange of water over the entire duration of the culture phase. Nevertheless, water replenishment was carried out periodically from the reservoir to compensate for water loss caused by evaporation or soil seepage. The water quality variables were carefully maintained and controlled to ensure ideal conditions throughout pond culture.

Table 1: Details of Experimental culture pond.

Culture Ponds	Water Spread Area (Ha)	Water Depth (m)	Stocking Density (SD)
P1	0.5	1.2 to 1.5	35/m^2
P2	0.5	1.2 to 1.5	35/m^2
P3	0.5	1.2 to 1.5	40/m^2
P4	0.5	1.2 to 1.5	40/m^2

2.1.Experimental feed preparation:

During the experiment, cultured shrimp were fed with CP shrimp feed with 35% protein content (CP Aquaculture India Pvt. Ltd., Chennai, India) for the entire culture duration in different feeding rations and schedule suggested in CP shrimp feed chart and adjusted according to the proper daily check tray observation. Shrimp feed was mixed with 1:2 ratio 5gr of garlic (*Allium sativum*) paste and 10gr of turmeric (*Curcuma longa*) powder per kg of shrimp feed in the form of paste by adding with 48 hours fermented curd with feed mixing and dry in shade in 10-15 min after that, evenly broadcasted in the culture ponds in one meal every day at morning.

2.2.Physical observation and growth:

The shrimp *L. vannamei* was examined by check tray observation regularly in terms of movement, activity of the animal, appearances, body, gut and hepatopancreas color, appendages, feeding gut, size variation, floating of fecal strings etc. The shrimp growth was examined through weekly sample nettings in the culture pond at every 15 days of intervals. The average body weight (ABW) of the shrimp was calculated as described by Mustafa and Ridzwan (2000).

2.3.Water quality analysis:

The water samples were obtained from the selected culture ponds by submerging 500ml clean polythene bottles at a depth of 1-2 feet between 6 to 7 am. The samples were then transported to the laboratory for analysis of different chemical parameters such as salinity, pH, total alkalinity, total hardness, calcium, magnesium, total ammonia, nitrate, and dissolved oxygen. The analysis was conducted using established standard methods. APHA.,1998.

2.4. Statistical analysis:

All data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using the statistical software SPSS version 22.0

3. Results & Discussion:

Mean values of water quality variables were recorded in shrimp culture ponds (P1, P2, P3 and P4) are presented in table-2. It is evident from the results that there was no significant difference in the water quality parameters between the culture ponds. Water quality plays a critical role in shrimp farming and has a direct impact on the health, growth, and overall performance of shrimp populations.

Table 2: Water quality parameters (Mean ±SD) in experimental shrimp ponds (P1, P2, P3 and P4).

S. No	Parameter	Units	P1	P2	P3	P4
1	Temperature	°C	28.62±2.34	28.18±2.12	28.47±1.54	28.97±1.96
2	Salinity	ppt	18.54±2.45	17.63±2.33	18.46±2.27	18.52±2.65

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3	pH	0-14	7.86±1.2	7.86±1.3	7.75±1.7	7.92±1.6
4	Total Alkalinity	ppm	127±21.41	136±26.35	129±23.48	132±26.62
5	Total Hardness	ppm	4000.61± 256.29	5128.82± 348.25	3997.48± 464.34	4109.39± 433.88
6	Calcium	ppm	626.51±111.4	716±126.7	634±122.4	727±126.8
7	Magnesium	ppm	1012±156	1024±146	996±123	1014±167
8	Total Ammonia	ppm	0.9±0.04	0.8±0.06	0.9±0.07	1.0±0.09
9	Nitrite	ppm	0.14±0.04	0.16±0.06	0.18±0.03	0.19±0.08
10	Dissolved Oxygen	ppm	6.37±1.06	6.44±1.04	6.88±1.15	5.96±2.35

Table 3: Feeding and ABW of shrimp *L.vannamei* in culture ponds.

S. No	Days of Culture DOC	Feed % Given	Check tray monitor time (hrs)	ABW of the shrimp in the culture ponds (grams)			
				P1	**P2**	**P3***	**P4***
1	01-05	Full	NA	0.3	0.3	0.3	0.3
2	06-10	Full	NA	0.8	0.9	0.8	0.8
3	11-15	Full	NA	1.3	1.5	1.2	1.3
4	16-20	Full	NA	2.1	2.3	2.0	2.1
5	21-25	Full	NA	2.6	2.8	2.5	2.6
6	26-30*	9	2.5	3.2	3.4	**3***	3.5
7	31-35*	8	2.5	3.9	4.3	**3.3***	**4.1***
8	36-40*	7	2.5	4.3	5.6	**3.7***	**4.7***
9	41-45*	6	2	4.8	6.3	**4***	**5.3***
10	46-50*	5.5	2	5.6	7.1	**4.2***	**5.8***
11	51-55	4	2	7.0	8.5	6.0	7.4
12	56-60	4	2	9.0	9.0	7.2	8.8
13	61-65	3.5	2	10.3	10.6	8.8	10.2
14	66-70	3	2	11.6	11.8	10.0	11.5
15	71-75	2.7	2	12.4	12.7	11.4	13.1
16	76-80	2.5	2	13.9	13.4	12.9	14.6
17	81-85	2.5	2	15.5	14.8	14.5	16.1
18	86-90	2.5	1.5	17.1	16.0	16.2	17.8
19	91-95	2.5	1.5	18.7	17.4	17.6	19.4
20	96-100	2	1.5	20.3	19.0	19.3	20.5
*WFS infected ponds DOC & Slow growth of shrimp were observed in P3, P4 ponds.							

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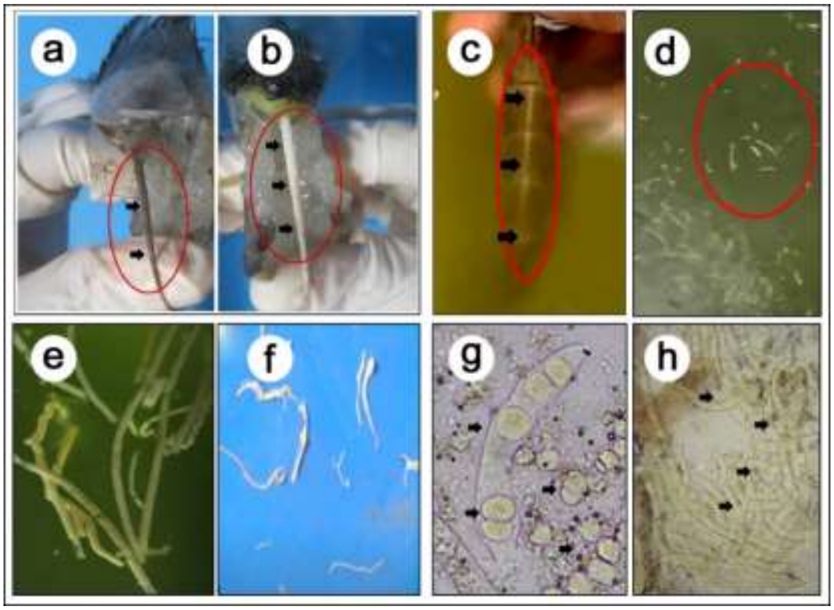
The significant seasonal variation in the water temperature has been noticed among all the culture ponds from January to April-2023 due to the onset of summer. It is observed that, the lowest temperature of 28.18±2.12°C in P3 and the highest temperature of 28.97±1.96 °C was observed in P4, indicating the influence of high temperature on the shrimp ponds. It is also one of the most important factors controlling the growth of marine shrimp Wyban JA. *et al.*, 1987. The pH levels ranged from a minimum of 7.75±1.7 to 7.92±1.6. The salinity of the culture pond water ranged from 17.63±2.33 to 18.54±2.45 ppt. The dissolved oxygen levels in all of the culture ponds in this study ranged from 5.9 to 6.8 ppm. The total alkalinity ranged from 127±21.41 to 136±26.35ppm, consistently maintained within the optimal levels with little fluctuations. The hardness levels in all the culture ponds ranged from 3997.48±464.34 to 5128.82±348.25. Further, it is also observed that the calcium hardness ranged from 626.51±111.4 to 727±126.8 and Magnesium hardness level ranged between 996±123 to 1024±146. The ammonia levels were noticed from 0.8±0.06 to 1.0±0.09 ppm. Nitrites varied from 0.14±0.04 ppm to 0.19±0.08 ppm as shown in Table 2.

Besides the specific parameters mentioned above, other water quality parameters such as turbidity, total suspended solids (TSS), alkalinity, and hardness also influence shrimp farming. Monitoring of these parameters and maintaining them within the ranges are important for ensuring optimal conditions for shrimp growth and minimizing stress. The maintaining good water quality requires regular monitoring, appropriate management practices and efficient waste management. It has been observed that raising the pH level along with ammonia can enhance the toxicity of ammonia to shrimp in culture ponds, as documented by Boyd, C. E., & Tucker, C. S. (1992) and Browdy, C. L., & Brune, D. E. (2009). Proper filtration systems, aeration, and regular water exchange can help improve and maintain water quality in shrimp ponds. Maintaining good water quality is essential for healthy shrimp growth. Effective water management practices include monitoring water parameters (e.g., temperature, dissolved oxygen, pH), regular water exchange, aeration, and controlling nutrient levels to prevent pollution and disease outbreaks Briones-Moreno *et al*., 2017 and Phillips *et al*., 2018.

Fig 2: WFS observations of shrimp *L.vannamei* (a). Normal shrimp gut (b) WFS infected shrimp gut (c). WFS infected shrimp gut visible from pond water (d,e) fecal strings floating in pond water. (f) Collected fecal strings of shrimp (g) Microscopic observation showing bubble-like structures later found to be sloughed lipid droplets (h) Under a light microscope, WFS affected shrimp display an accumulation of aggregated, altered microvilli.



3.1. Disease management with garlic and turmeric:

Disease outbreaks pose a significant threat to shrimp farms worldwide. The use of antibiotics and chemicals for disease control raises concerns about environmental pollution and antibiotic resistance. Garlic and turmeric offer potential alternatives for disease management in shrimp farming. For instance, garlic extracts have demonstrated efficacy against various shrimp pathogens, including Vibrio species (Sahu et al., 2020; Ramasamy *et al*., 2021). Turmeric, on the other hand, has shown promise in controlling pathogens such as *Vibrio harveyi* and *Vibrio parahaemolyticus* (Dang *et al*., 2020; Sharifi *et al*., 2018). The garlic has been recognized for its numerous health benefits and bioactive compounds. Several studies have investigated the potential use of traditional herbs in shrimp farming Huang Y, *et al*.,2018. Turmeric (*Curcuma longa*) is another natural compound that has gained attention in shrimp farming. Curcumin, the active ingredient in turmeric, exhibits antimicrobial, anti-inflammatory, and antioxidant properties. Studies have shown that turmeric extracts can enhance shrimp immune responses, improve growth performance, and alleviate the negative effects of stressors in shrimp farming (Vaseeharan B, *et al*., (2011), Citarasu T, *et al*., (2015), Mandal A, *et al*., (2016), (Sharifi *et al*., (2018); Di *et al*., (2021).

The bioactive compounds present in turmeric contribute to their beneficial effects in shrimp farming. Garlic and turmeric compounds possess antimicrobial properties and can modulate immune responses in shrimp (Rattanachuay *et al*., 2019; Sahu *et al*., 2020). Turmeric's curcumin acts as an immunostimulant, antioxidant, and anti-inflammatory agent, which can improve shrimp health and disease resistance (Sharifi *et al*., 2018; Di *et al*., 2021). The use of turmeric in shrimp farming can be applied through various methods, such as dietary supplementation, immersion baths, and incorporation into feed formulations. These approaches have demonstrated positive outcomes in terms of shrimp health, growth performance, and disease resistance (Rattanachuay *et al*., 2019; Dang *et al*., 2020). Turmeric offer potential as natural alternatives for enhancing shrimp health and disease management in shrimp farming. Their bioactive compounds exhibit antimicrobial, antioxidant, and immunostimulant properties, which can improve shrimp immunity, growth performance, and disease resistance.

The majority of the faeces produced by feed digestion in a healthy shrimp consists of leftover vacuoles from hepatopancreas cells, which entail a key mechanism for packaging and eliminating waste materials from the digestive tract. When WFS-infected prawns are released from the tubule into the GI tract, the combination of lipids, digested/undigested meal, WFS, and the bacterial mass contributes to the production of a whitish faecal string (Fig:2 a,b,c). Because of the presence of lipids in the faeces, the faecal mass may become lighter than faeces from a healthy prawn and float on the water's top instead of sinking to the pond bottom (Fig:2 d,e). Changes in the gastrointestinal tract are an indication of the disease. Modifications affecting the gastrointestinal tract, characterized by the presence of pale or white coloured faeces in *L. vannamei* shrimp (Fig:2 f,g,h), serve as an indication of the disease.

Our study clearly shows that the pathogenic bacteria due to poor pond management may be induce WFS in shrimp and decisively shows that herbal supplements plays major role in the development of gut health and also show betterment of immunity, antibacterial activity and also resistance with result as good growth and body weight of the shrimp by maintaining the healthy gut during the culture period followed by the better management practices like pond preparation and water quality management, feed management and animal health management.

3.2. Growth of *L. vannamei* fed with garlic and turmeric:

L. vannamei is a popular species of shrimp that is widely cultivated in aquaculture. While shrimp farmers typically feed them with commercial shrimp feeds, incorporating natural ingredients like garlic and turmeric into their diet has been explored for their potential benefits. Garlic (*Allium sativum*) and turmeric (*Curcuma longa*) are both known for their potential health benefits due to their bioactive compounds. Here are some potential effects they may have on the growth of *L. vannamei* shrimp. Garlic and turmeric possess antimicrobial properties that may help reduce the risk of bacterial and fungal infections in shrimp. By including these herbal supplements in feed, it is observed that improved health of the shrimp, potentially leading to improved growth (Table.3).

The garlic and turmeric can enhance the taste and palatability of shrimp feed, encouraging better consumption and utilization of the nutrients in the diet. This improved feed intake can positively affect shrimp growth. While these potential benefits suggest that incorporating garlic and turmeric into the diet of *L. vannamei* shrimp may have positive effects on growth, it's essential to conduct further research and trials to determine the optimal inclusion levels and long-term effects.

Additionally, it's important to consult with shrimp farming experts or aquaculture specialists who can provide more up-to-date and species-specific recommendations regarding the use of these natural ingredients in shrimp feed. In the present study we observed that the WFS infected pond shrimp (P3 & P4) shown poor growth from DOC 25 to 50, later increased health and growth. The ABW were observed in *L. vannamei* daily fed with the garlic and turmeric supplemented diets were demonstrated better health and growth performance throughout experimental period (Table.3).

4. Conclusion:

White faeces syndrome (WFS) disease has been a significant concern in shrimp aquaculture, particularly affecting the gut health and overall performance of shrimp, including the popular species *Litopenaeus vannamei*. WFS is characterized by the presence of white or pale faeces in affected shrimp, indicating underlying gastrointestinal issues and potential impacts on growth and immune function. In recent years, the use of traditional herbal supplements, such as Allium sativum (garlic) and Curcuma longa (turmeric), has gained attention as a potential management strategy to improve gut health and mitigate the incidence of WFS in shrimp farming. Both garlic and turmeric are known for their antimicrobial, antioxidant, and immunostimulant properties, which can contribute to enhancing the overall health and disease resistance of shrimp. This study shown promising results regarding the use of garlic and turmeric in improving the growth and gut health of shrimp, *L. vannamei* and reducing the incidence of WFS. These herbal supplements have demonstrated the potential to enhance the balance of gut microbiota, improve nutrient absorption, boost the immune system, and mitigate the negative impacts of pathogenic microorganisms. However, further research and field trials are necessary to optimize dosage, application methods, and long-term effects, as well as to ensure the compatibility of these herbal supplements with other management practices in shrimp aquaculture.

5. Future scope:

The use of traditional herbs in shrimp aquaculture is promising. Herbal remedies have the potential to prevent diseases, reduce stress, improve water quality, enhance feed supplementation, and meet the growing demand for sustainable seafood. By harnessing the antimicrobial and immunostimulant properties of herbs, shrimp farmers can reduce reliance on antibiotics and chemical treatments, while promoting natural growth and disease resistance. The use of herbal extracts in aquaculture systems can lead to more environmentally friendly practices and cater to consumer preferences for eco-friendly and healthy products. However, further research, collaboration, and validation are needed to fully realize the benefits of herbal efficiency in shrimp aquaculture.

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7. **Conflict of Interest**: None

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