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Effectiveness of Ozonization on Total Vibrio Bacteria and Viruses in Sea Worms *(Nereis sp.)* as Natural Feed of Vannamei Shrimp *(Litopenaeuse vannamei)*

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Abstract

The total bacteria and viruses in sea worms as vannamei shrimp mother feed are greatly influenced by the ozonization treatment in worms. The purpose of this study is to determine the effect of ozonation on the number of vibrio bacteria and viruses in sea worms. This research was carried out at the CV Laboratory. Source Bangka Hatchery, Mapur Village, Riau Silip District, Bangka Regency, Bangka Belitung Province. The research method used is mixed methods with experimental testing procedures designed with a Complete Acal Design (RAL) with 4 treatments with 3 replicas each which aims to determine the effect of ozonation on the number of vibrio bacteria and viruses in sea worms. The results showed that ozonization treatment had a significant influence on the microbiological quality of sea worms, especially in terms of total Vibrio bacteria and the level of viral contamination. The ozonization treatment has been proven to be able to reduce the total number of Vibrio bacteria present in the body of sea worms. Vibrio bacteria are a group of microorganisms that are often associated with the risk of disease in humans and aquatic animals. This decrease in the number of bacteria shows that ozone can be an effective antimicrobial agent in suppressing the growth and spread of pathogenic bacteria. In addition, ozonization also affects the level of viral contamination in sea worms. Ozone is known as a powerful oxidizer that can damage the structure of proteins and nucleic acids in viruses, thereby reducing the viability and infectivity level of viruses contained in sea worm tissues. The results showed a marked difference between the control group and the ozonized treatment group, where the treatment group showed significantly lower levels of virus contamination.

Keywords: ozonation; sea worm; vannamei shrimp; Vibrio sp. virus

Abstrak

Total bakteri dan virus pada cacing laut sebagai pakan induk udang vaname sangat dipengaruhi treatmen ozonisasi pada cacing. Tujuan dari penelitian ini adalah mengetahui pengaruh ozonisasi terhadap jumlah bakteri vibrio dan virus pada cacing laut. Penelitian ini dilaksanakan di Laboratorium CV. Sumber Hatchery Bangka, Desa Mapur, Kecamatan Riau Silip, Kabupaten Bangka, Provinsi Bangka Belitung. Metode penelitian yang digunakan adalah mixed methods dengan prosedur pengujian melalui eksperimental yang didesain dengan Rancangan Acal Lengkap (RAL) dengan 4 perlakuan dengan masing-masing 3 ulangan yang bertujuan untuk mengetahui pengaruh ozonisasi terhadap jumlah bakteri vibrio dan virus pada cacing laut. Hasil penelitian menunjukkan bahwa perlakuan ozonisasi memiliki pengaruh signifikan terhadap kualitas mikrobiologis cacing laut, khususnya dalam hal total bakteri Vibrio dan tingkat kontaminasi virus. Perlakuan ozonisasi terbukti mampu menurunkan jumlah total bakteri Vibrio yang terdapat dalam tubuh cacing laut. Bakteri Vibrio merupakan kelompok mikroorganisme yang sering dikaitkan dengan risiko penyakit pada manusia maupun hewan akuatik. Penurunan jumlah bakteri ini menunjukkan bahwa ozon memiliki kemampuan sebagai agen antimikroba yang efektif dalam menekan pertumbuhan dan penyebaran bakteri patogen. Selain itu, ozonisasi juga berpengaruh terhadap tingkat kontaminasi virus pada cacing laut. Ozon dikenal sebagai oksidator kuat yang dapat merusak struktur protein dan asam nukleat pada virus, sehingga dapat menurunkan tingkat viabilitas dan infektivitas virus yang terdapat dalam jaringan cacing laut. Hasil penelitian menunjukkan adanya perbedaan nyata antara kelompok kontrol dan kelompok yang diberi perlakuan ozonisasi, di mana kelompok perlakuan menunjukkan tingkat kontaminasi virus yang lebih rendah secara signifikan.

***Kata Kunci**: cacing laut; ozonisasi; udang vaname; vibrio sp. virus*

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INTRODUCTION

Vannamei shrimp *(Litopenaeus vannamei)* cultivation is one of the leading sectors in the aquaculture industry in Indonesia. High market demand both nationally and internationally drives intensive production increase, (Amelia et al., 2021; Mustafa et al., 2023). (Liang et al., 2022) explained that one of the key factors in the success of shrimp production is the quality of the broodstock used in the hatchery process. A healthy and quality broodstock will produce superior naupli and have resistance to disease, . Good broodstock management must be supported by natural feeding that is in accordance with the physiological needs of shrimp. In this context, sea worms of the *Polychaeta* type, specifically Nereis sp., have proven to be a very beneficial natural feed due to their high nutrient content. These worms contain 56.29% protein and 11.32% fat, making them an excellent source of nutrients to support the shrimp reproductive process, (Rajuansah et al., 2021). However, the use of sea worms as natural feed is not free from challenges, especially related to the health risks of shrimp mothers, (Mustafa et al., 2023; Palupi et al., 2018). Most of the seaworm needs in the hatchery industry are still met through capture directly from nature, which has the potential to carry various types of pathogens such as viruses, bacteria, fungi, and parasites. Disease transmission can occur either vertically, from mother to offspring, or horizontally through the surrounding environment, (Purnomo et al., 2022). Diseases transmitted through natural feed such as Nereis sp. can have a major impact on the success of shrimp hatcheries, given that shrimp's immune system is relatively sensitive to environmental changes and pathogenic microbial contamination, (Palupi et al., 2018).

One of the methods that is starting to receive attention in the field of aquaculture to overcome microbial contamination in natural feed materials is ozonization, (Binh et al., 2025; Pumkaew et al., 2021). Next, (Jhunkeaw et al., 2021) menjelaskan bahwa Ozon (O3) It is a compound that has high oxidative power and has been shown to be effective in inactivating various pathogenic microorganisms including bacteria and viruses. The use of ozonization in water and food treatment has long been applied in various industries due to its safety and does not leave harmful residues. In the context of shrimp hatchery, ozonation has the potential to be an efficient alternative method to lower Total Vibrio Count (TVC) and viral infection rates in sea worms before being used as mother feed, (Binh et al., 2025). Nonetheless, the utilization of ozone in biological substrates such as *the Nereis sp*. worm still needs further research to ascertain its effectiveness and impact on feed nutrient quality. Bacteria from the genus Vibrio are one of the main causes of disease in shrimp, especially in the larval phase. Some species such as Vibrio harveyi and Vibrio alginolyticus are known to cause up to 100% death in shrimp larvae if not handled properly, (Pumkaew et al., 2021). Vibrio infection not only interferes with the survival of the fry, but also inhibits growth and decreases hatchery productivity. In addition to bacteria, viruses are also a serious threat in shrimp farming, although information on the type and mechanism of transmission is still limited.

Previous research has shown that ozonation is effective in lowering the population of pathogenic bacteria in the water and on the surface of various organic matter. In the context of vannamei shrimp hatchery, it is necessary to conduct a specific study on the effectiveness of ozonization on Nereis sp. sea worms used as mother feed. This effectiveness is not only seen from the decrease in the total number of Vibrio bacteria, but also from the possibility of decreasing the presence of viruses that are potentially contagious to the mother shrimp. In addition, it is important to evaluate whether the ozonization process affects the nutritional quality of the worms, so as not to reduce their benefits as reproductive feed. This study is expected to be a reference in developing a safer and more sustainable natural feed management system. Based on this description, this study aims to determine the effectiveness of ozonation on total Vibrio bacteria and the presence of viruses in sea worms *(Nereis sp.)* as natural feed for vannamei shrimp *(Litopenaeus vannamei)*. This research was carried out in the Laboratory of CV's Vannamei Shrimp Hatchery Unit. The source of the Bangka Hatchery is in Mapur Village, Riau Silip District, Bangka Regency, Bangka Belitung Province. It is hoped that the results of this research can make a real contribution to improving the biosecurity system in shrimp hatcheries and support the development of natural feed technology that is safe, efficient, and environmentally friendly. This effort is a strategic step in supporting the target of sustainable and competitive national shrimp production in the global market.

METHOD

The research methods used are mixed methods (Creswell & Clark, 2017; Samsu, 2021) with the research procedure, namely Complete Random Design (RAL) (Brown & Melamed, 1990; Rahmawati & Erina, 2020) with experimental labolatory testing, (Arziyah et al., 2022; Brown & Melamed, 1990) With 4 treatments with 3 replicates each which aims to determine the effect of ozonation on the number of vibrio bacteria and viruses in sea worms. The location of the research was carried out at CV. Source Bangka Hatchery Mapur Village, Riau Silip District, Bangka Regency, Bangka Belitung Province. The tools and materials used in this study are 30 liter ozone volume as an ozonization device, a 30-liter volume basin as a research container, an aerator machine to supply the oxygen needs of test animals, a 5 mm diameter aeration hose to supply oxygen, an SS-A1000 Sonic electric scale weighing 0.1 - 1000 grams to weigh the weight of test animals, a 50-liter volume basin as an adaptation medium, 20 cm size seser for worm rinsing, memmert oven 1 dry sterilization unit, 50 L wet sterilization autoclave, 1 set of surgical equipment for sampling, erlenmeyer 500 ml TCBS media making, magnetic strirer SH-3 1 unit heating media, 90-100 mm pertri cup media making, refrigerator 1 media storage unit, incubator 1 bacterial incubation unit, laminar LAF bacterial culture, autoclave 50 l wet sterilization, marker counting tool and bacteria counting counter, PCR MIC PCR Real-Time PCR samples. Meanwhile, the materials in this study are sea worms as test animals, seawater sea worm media, aquades media making materials, TCBS Himedia media as vibrio bacteria growth materials, NaCl 0.85% bacterial culture materials, Real-Time PCR PCR reagents Pcr. Sampling namely worms for control or pre-treatment and post-treatment is carried out randomly on each strefoam then we separate worm samples with different containers and given a sample code so that later they do not exchange. The data collection technique was carried out by observation, interview, documentation, and providing questionnaires in the form of questions to respondents, (Aramo-Immonen, 2013). Meanwhile, the data analysis technique, namely the measurement of parameters at the end of the study, was tested with Fingerprint Analysis to see the differences between treatments. Measurement results of each parameter Complete Random Design (RAL) (Brown & Melamed, 1990; Rahmawati & Erina, 2020) to see the difference between pre- and post-ozonation treatments.

RESULT AND DISCUSSION

The use of ozone in water and food treatment systems has long proven effective, including in the aquaculture industry. Previous research has shown that ozone treatment is able to significantly lower the concentration of *Vibrio* in water, mud, and other substrates in hatcheries. In addition, ozone nanobubbles have also been studied to have high efficacy in improving water quality and reducing the prevalence of diseases in vannamei shrimp. However, the application of ozone to biological substrates such as sea worms is still relatively limited, so more in-depth studies are needed to assess its effectiveness in reducing the total *Vibrio* and viruses without damaging the nutrient content of feed. The results of the study on the effectiveness of ozonation on total vibrio bacteria and viruses in sea worms *(Nereis sp.)* as a natural feed of the mother of vannamei shrimp *(Litopenaeuse vannamei)* as follows:

Total Bacteria Vibrio

Vibrio bacteria are a group of gram-negative bacteria that are predominantly found in waters, including seas, lakes, and rivers, with some species being pathogenic and can cause diseases in humans and animals, such as cholera and vibriosis. The results of the study showed a significant decrease in worm samples after ozone treatment, which can be seen in the table below:

Table 1.
Total Bacteria Vibrio

Sampling Date	Sample	Area	TCBS (cfu/ml)			Remark
			Yellow	Green	TCBS-TLV	
01/03/2025	Pre-Treatment Worms	Fresh Feed	3.800	2.520	0	Body
01/03/2025	Post-Treatment Worms	Fresh Feed	3.480	1.720	0	Body
03/03/2025	Pre-Treatment Worms	Fresh Feed	1.880	2.400	0	Body
03/03/2025	Post-Treatment Worms	Fresh Feed	1.440	1.640	0	Body
04/03/2025	Pre-Treatment Worms	Fresh Feed	4.320	160	0	Body
04/03/2025	Post-Treatment Worms	Fresh Feed	2.880	80	0	Body
05/03/2025	Pre-Treatment Worms	Fresh Feed	6.080	0	0	Body
05/03/2025	Post-Treatment Worms	Fresh Feed	3.520	0	0	Body
06/03/2025	Pre-Treatment Worms	Fresh Feed	6.960	0	0	Body
06/03/2025	Post-Treatment Worms	Fresh Feed	4.120	0	0	Body
07/03/2025	Pre-Treatment Worms	Fresh Feed	1.400	120	0	Body
07/03/2025	Post-Treatment Worms	Fresh Feed	3.600	0	0	Body
09/03/2025	Pre-Treatment Worms	Fresh Feed	2.920	2.160	0	Body
09/03/2025	Post-Treatment Worms	Fresh Feed	3.120	960	0	Body

Source: research data processing results

Based on the results of the research that has been carried out, each treatment given can reduce the number of vibrio bacteria in worms, according to the statement that ozone is not only used to kill microbes, but also can neutralize excessive/toxic organic mineral substances in the water, (Binh et al., 2025; Renaldo et al., 2021). The cause of the decrease in the number of vibrio bacteria in worms is because Ozone can extend the shelf life of fruits, vegetables, and other food products by reducing the growth of destructive microorganisms. This is in accordance with the statement that the use of ozone will increase the concentration of oxygen in the water so that it is fresher and healthier, (Andhini, 2019; Jhunkeaw et al., 2021; Pumkaew et al., 2021). So that ozone is used in drinking water treatment to remove organic and bacterial contaminants, as well as improve water quality to maintain the storage process of food products.

The worm ozonization process is carried out using pure oxygen that is fed into an ozone machine and aerated for 30 minutes at a pressure of 250 Volts and an O3 level of up to 15 ppm. In the use of ozone for worms we must pay attention and learn more about the need for concentration, dosage and proper ozone timing so as not to cause a lot of deaths in worms. The residual ozone concentration value is used to determine the ozone activity in the sample, where the higher the residual ozone concentration value in the sample, the more active the ozone, (Cinthya et al., 2019), and does not reduce the nutrient content in sea worms and does not decompose in worms. In accordance with the statement that the more ozone concentrations contained in the water, it can cause health disturbances in the human body, especially it can cause poisoning and cancer, (Meunpol et al., 2003; Renaldo et al., 2021). Because ozone is a toxic gas if inhaled in high concentrations, the use of ozone must be done with caution and in accordance with the recommended dosage. So that the use of ozone must be in accordance with the standards set by the government, (Renaldo et al., 2021). There are several important factors that affect the disinfection process, namely the level of disinfectant used and the time of contact. The percent of bacterial degradation will increase as the contact time increases and the level of disinfectant used increases until it reaches optimal levels. This is because with the increase in ozone levels, the free radicals produced by ozone to degrade microorganisms are also increasing. Ozone attacks guanine and thymine residues in bacteria. The process of disinfection using ozone will cause the conversion of closed circular plasmid DNA into circular open DNA plasmids so that bacterial cells that encounter ozone will undergo lysis, (Jannah et al., 2021).

Viruses

Viruses are a type of microscopic disease with super-small organisms that can attack humans, plants, animals and sometimes very deadly. The presence of viruses in worms as a mother feed for vannamei shrimp will have a big impact on shrimp farming production in hatcheries and ponds. So that worm treatment is carried out with the ozonization method, which can be seen in the table below:

Table 2.
Viruses in Sea Worms

		Virus Parameters												
		AHPND/EMS					WSSV				EHP			
Date	Sample	Pir	Pir	C	CT	Result	WSSV	IC***	CT2	Result	EHP	IC***	CT6	Result
2 Feb 2025	Cacing Pesaren Pre-Ozon	+	-	-	37,15	Positive	-	-	-	Negative	-	-	-	Negative
	Pasca-Ozon	-	-	-	-	Negative								
12 Feb 2025	Cacing Bali Pre-Ozon	-	-	-	-	Negative	+	-	34,46	Positive	-	-	-	Negative
	Pasca-Ozon						+	-	37,68	Positive				
16 Feb 2024	Cacing Bali Pre-Ozon	+	-	-	36,95	Positive	-	-	-	Negative	-	-	-	Negative
	Pasca-Ozon	-	-	-	-	Negative								
28 Feb 2024	Cacing Lokal P. Pongok Pre-Ozon	-	+	-	36,73	Positive	-	-	-	Negative	-	-	-	Negative
	Pasca-Ozon	-	-	-	-	Negative								
5 Mar 2024	Cacing Bali Pre-Ozon	-	-	-	-	Negative	-	-	-	Negative	+	-	36,7	Positive
	Pasca-Ozon										-	-	-	Negative
7 Mar 2024	Cacing Pesaren Pre-Ozon	+	+	-	36,73/35,70	Positive	-	-	-	Negative	-	-	-	Negative
	Pasca-Ozon	-	+	-	38,12	Positive								
12 Mar 2025	Cacing Pesaren Pre-Ozon	-	-	-	-	Negative	-	-	-	Negative	+	-	36,22	Positive
	Pasca-Ozon										-	-	-	Negative

: Negative (CT >40) :Medium Positive (CT 35-28)

: Light Positive (CT 40-35) : High Positive (CT < 28)

Source: research data processing results

Based on the results of the study, ozone can disappear viruses in sea worms with very low viral contamination values and in some conditions moderately contaminated worms cannot be eliminated but there is a decrease in contamination value according to the opinion (Jafari-Oori et al., 2022) For mild-moderate COVID-19 patients, ozone adjuvant therapy can accelerate the patient's PCR results to be negative and alleviate the patient's symptoms such as cough and shortness, however, there is no difference in the results of the patient's chest X-ray between the groups given ozone and not. The worm ozonization process to eliminate the virus is carried out for 60 minutes according to the statement (Shin & Sobsey, 2003) that the virus disinfection process is carried out after the water in the reactor is conditioned with ozone for 60 minutes. In general, the results obtained in the study show that ozone administration is beneficial, because in some diseases it also utilizes ozone therapy which can reduce mortality, shorten the length of treatment in the hospital, and improve patients' clinical symptoms. Ozone therapy techniques, according to (Li & Pu, 2024; Zeng & Lu, 2018) Ozone is a very powerful molecule that can identify bacteria, so it is good for the treatment of chronic infections. In general, the results of research on ozonization technology have been widely used as anti-microbial and anti-viral in various products and treatments because they do not leave harmful residues and are environmentally friendly. This is in line with the statement that the unstable and volatile nature of ozone makes ozone leave no residue on the media in direct contact with the surrounding environment, (Panebianco et al., 2022; Zhang et al., 2025). (Anisha et al., 2024) stated that the advantage of ozone technology is "*green technology*", because it does not leave residues and ozone is easily decomposed into oxygen.

CONCLUSION

Based on the results of research that has been carried out while at CV. Source: Bangka Hatchery Mapur Village, Riau Silip District, Bangka Regency, Bangka Islands Province, It can be concluded that ozonization treatment has a significant influence on the microbiological quality of sea worms, especially in terms of total *Vibrio bacteria* and the level of viral contamination. The ozonization treatment has been proven to be able to reduce the total number of *Vibrio bacteria* present in the body of sea worms. Vibrio bacteria are a group of microorganisms that are often associated with the risk of disease in humans and aquatic animals. This decrease in the number of bacteria shows that

ozone could be an effective antimicrobial agent in suppressing the growth and spread of pathogenic bacteria. In addition, ozonization also affects the level of viral contamination in sea worms. Ozone is known as a powerful oxidizer that can damage the structure of proteins and nucleic acids in viruses, thereby reducing the viability and infectivity level of viruses contained in sea worm tissues. The results showed a marked difference between the control group and the ozonized treatment group, where the treatment group showed significantly lower levels of virus contamination.

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