# Gamma Ray Irradiation: A Valuable Tool for Fresh Feed Disinfection

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**Abstract:** Shrimps aquaculture remains as an important sector in the aquaculture industry in Malaysia as of 2015 to 2018 with the production of more than 187,000 MT and the value exceeded RM4, 238 million. However, most of the hatchery operators rely on specific pathogen-free (SPF) shrimp broodstock imported from broodstock multiplication centres (BMCs) outside Malaysia. These broodstocks are expensive ranging from USD 100-200 per piece. Hatchery operators spend more than RM 100,000 in a single shipment. Although it is the SPF broodstock, outbreak of diseases such as early mortality syndrome (EMS), white spot syndrome virus (WSSV) and *Enterocytozoon hepatopenaei* (EHP) infections still occur at farm level. It could be due to management issue at farms. Nevertheless, the hygiene of fresh feed at hatcheries is often overlooked. This fresh feed has been documented to be a potential source as host of pathogens particularly for marine polychaetes and squids. There is no procedure yet to disinfect prior to use at private hatcheries. At FRI Pulau Sayak, these fresh feeds are disinfected by gamma irradiation prior to feeding. Gamma ray irradiation on positive sample proved to be effective in disinfecting the potential pathogens. Gamma radiation destroys the pathogen's DNA directly or by the effect of water ionization that produces free radicals. The effective dose is between 5 – 10 kGy of gamma ray from cobalt ($^{60}Co$). Although gamma irradiation is a new method for food disinfection in Malaysia, the use is crucial for the local aquaculture industry to ensure hygienic feed for the shrimp and fish broodstocks.

**Keywords:** Gamma ray, fresh feed, broodstock

**Abstrak:** Akuakultur udang kekal sebagai sektor penting dalam industri akuakultur di Malaysia setakat 2015 hingga 2018 dengan pengeluaran lebih 187,000 MT dengan nilai melebihi RM4, 238 juta. Walau bagaimanapun, kebanyakan pengusaha hatceri bergantung kepada stok induk udang bebas pathogen (SPF) yang diimport dari pusat penggandaan induk (BMC) di luar Malaysia. Induk induk ini mahal dengan harga antara USD 100 - 200 seekor dan biasanya pengusaha akan membelanjakan lebih daripada RM 100,000 dalam setiap penghantaran. Walaupun ia adalah stok induk SPF, wabak penyakit seperti sindrom kematian awal (EMS), virus sindrom bintik putih (WSSV) dan jangkitan Enterocytozoon hepatopenaei (EHP) masih boleh berlaku di peringkat ladang yang mungkin disebabkan oleh isu pengurusan di ladang. Namun begitu, kebersihan makanan segar di hatceri sering diabaikan. Makanan segar telah dilaporkan sebagai sumber atau tuan rumah patogen terutamanya polikit marin dan sotong. Sehingga kini belum ada prosedur khas untuk membasmi kuman sebelum digunakan di hatceri. Di FRI Pulau Sayak, makanan segar dibasmi kuman dengan sinaran gamma sebelum diberi makan kepada udang. Sinaran gamma pada sampel positif terbukti berkesan dalam membasmi patogen. Sinaran gamma memusnahkan DNA patogen secara langsung melalui pengionan air yang menghasilkan radikal bebas. Dos berkesan adalah antara 5 – 10 kGy sinar gamma daripada kobalt (60Co). Walaupun sinaran gamma adalah kaedah baru untuk pembasmian kuman di Malaysia, penggunaannya adalah perlu bagi industri akuakultur tempatan bagi memastikan sumber makanan yang bersih untuk induk udang atau ikan.

## Introduction

Marine shrimp is an important aquaculture species for Malaysia with the production of more than 187,000 MT, and the value of more than RM4, 238 million between 2015 and 2018. According to the national Annual Fisheries Statistics by Department of Fisheries Malaysia, the production has increased tremendously from 2,339 MT in 1990 to 48,588 MT in 2020 as shown in Figure 1. The main cultured species are *Penaeus monodon, Penaeus merguiensis* and *Penaeus vannamei*. However, the shrimp aquaculture scenario is not without its problems. In the 90s, the industry was marred by the outbreak of white spot syndrome virus (WSSV) infection that caused huge economical lost with the estimation of US25 million annually (Dacho & Mustafa, 2007). Initially, *P. monodon* was the only cultured species. However, WSSV infection has forced farmers to look for other alternative shrimp species such as *P. mergueinsis* and *P. vannamei*. WSSV was first detected in Japan back in 1992. The disease was then spread to a few countries and subsequently to Southeast Asian countries in 1994 (Walker & Mohan, 2009). The prevalence of white spot disease could be caused by wild broostocks which are detected to be WSSV positive (Remany et al., 2012; Withyachumnarnkul et al., 2013; Orosco & Lluisma, 2017). Currently, *P. vannamei* becomes the most cultured species due to the availability of domesticated broodstock that is specific pathogen-free (SPF) (Lightner, 2011; Alday-Sanz et al., 2018), which boosted the shrimp production globally.

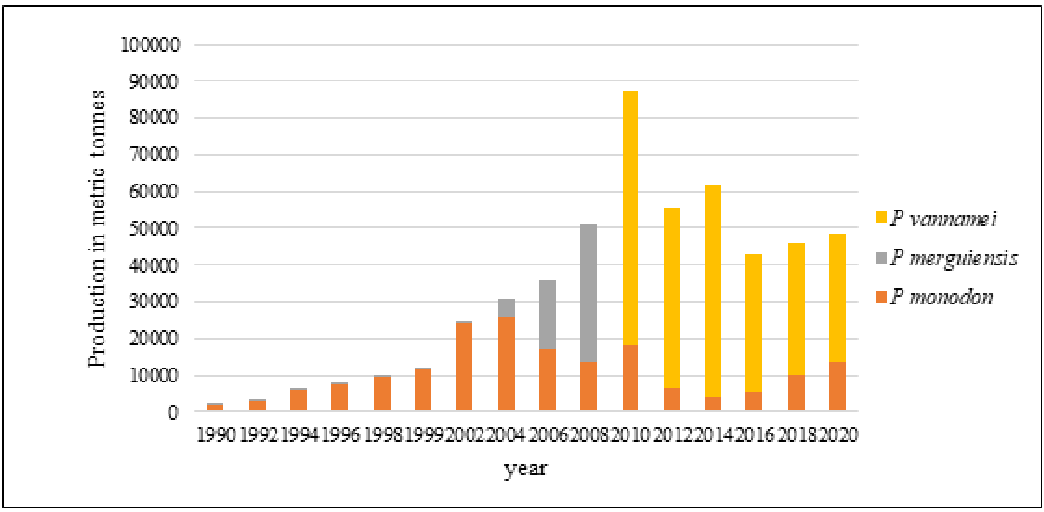


**Figure 1**: National production of penaeid shrimp culture in Malaysia. The main cultured species are *P. monodon*, *P. merguiensis* and *P. vannamei*. (source: Department of Fisheries Malaysia)

The prevalence of diseases did not end with the introduction of SPF domesticated broodstock. In 2009, early mortality syndrome (EMS) which was renamed as acute hepatopancreatic necrosis disease (AHPND) was detected in *P. vannamei* in China. The disease eventually has spread to Southeast Asian countries and subsequently to South America, Mexico and USA. The AHPND is caused by specific strain of bacteria such as *V. parahaemolyticus*, *V. punensis*, *V. harveyi*, *V. owensii*, *V. campbelli* and *Shewanella* sp. that contain pVA1 plasmid (63–70 kb) encoding the binary $PirA^{VP}$ and $PirB^{VP}$ toxins. The disease has caused the loss of USD 43 billion to the shrimp industry (Kumar et al., 2020, 2021).

Another pathogen that causes economic loss in the shrimp industry is *Enterocytozoon* *hepatopenaei* (EHP). It is estimated to have cause the loss of USD 567.62 M annually in India (Patil et al., 2021). The presence of such microsporidium was first described in *P. monodon* in Malaysia in 1984. However, the presence of such microsporidium in *P. japonicus* was first detected in Australia in 2001 and later, in 2004, it was detected in *P. monodon* in Thailand. It was then formerly described as EHP in 2009 (Chaijarasphong et al., 2021).

One of the most widely used tool to detect WSSV (Flagel, 2002), AHPND and EHP is the PCR test. PCR test screens the presence of pathogens. When the screening test on WSSV comes out negative, consequently the shrimp production will increase due to absence of the virus (Vaseeharan et al., 2003; Seok et al., 2007). Similarly, PCR test is also developed for the detections of APHND (Nunan et al., 2014; Sirikharin et al., 2015) and EHP (Flegel & Sritunyaluucksana, 2018; Hou et al., 2021). Therefore, PCR test has proved that it has subsequently helped to contain the spread of deadly pathogen from shrimp to shrimp and lessen the economic loss.

Many studies have shown that pathogen infections are not limited to penaeid shrimps. It could be found in other aquatic animal as well. Polychaetes have been reported as passive carrier for EHP and WSSV (Desrina et al., 2013; Haryadi et al., 2015; Desrina et al., 2018; Desrina 2020; Krishnan et al., 2021). Apparently, *V. paraheamoliticus* has also been reported in blood clams and squids (Tan et al., 2020). Polychaetes and squids are used as maturation diet for shrimps in hatcheries and blood clamps are being fed occasionally as well. The fresh feeds therefore post a threat to the shrimp industry.

Although hatchery operators can reject the infected broodstock after PCR test by selecting pathogen-free shrimps, when it comes to maturation diet, fresh feeds still pose a threat via vertical transmission. However, by solely relying on formulated maturation diet, it may increase the operational cost. Thus, one of the possible solutions that will enable fresh feed to be used is by gamma ray irradiation treatment. Irradiation of food using ions of beta or gamma rays can inactivate or destroy the food spoilage pests, microorganisms and their toxins (Munir & Federighi, 2020). Besides that, gamma irradiation offers high penetrating power which makes it possible for bulk treatment (Arapcheska et al., 2020).

## Materials & Methods

### PCR Test

PCR test was conducted at FRI Pulau Sayak facility using Biorad model CFX96 Touch Real-time PCR System. Polychaetes samples were collected from the cultured polychaetes which was purchased from local supplier. Screening was done before and after gamma ray irradiation. Each sample was being tested for AHPND, WSSV and EHP.

### Gamma irradiation

The fresh feeds, the squids and the polychaetes were packed inside microwavable food container and frozen prior to packing. Gamma ray irradiation services were done at Malaysian Nuclear Agency, in Bangi, Selangor. For gamma ray irradiation service, the fresh feeds were arranged in a styrofoam box. A block of dry ice was packed together with the frozen fresh feeds. The box weighted 25 kg each. These fresh feeds were then irradiated with 10 kGy gamma ray radiation.

## Result & discussion

PCR test result of the fresh feeds showed that polychaetes were positive of EHP while squids were positive of WSSV as in Figure 1 and Figure 3, respectively. After the gamma ray irradiation, WSSV and EHP were not detected in both polychaetes and squids as in Figure 2 and Figure 4, respectively.

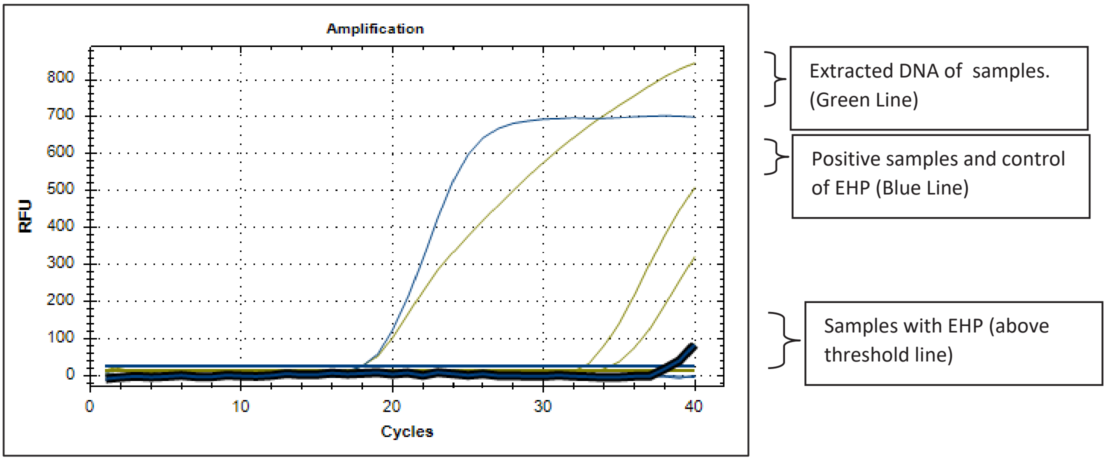


**Figure 1:** Sample of fresh polychaetes, tested positive for EHP before gamma ray irradiation.

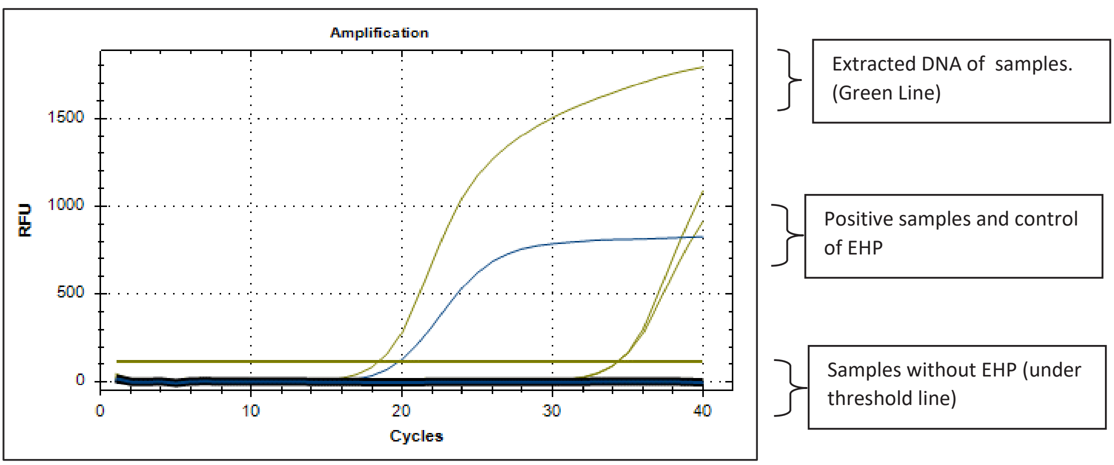


**Figure 2:** Sample of fresh polychaetes, tested negative for EHP after gamma ray irradiation.

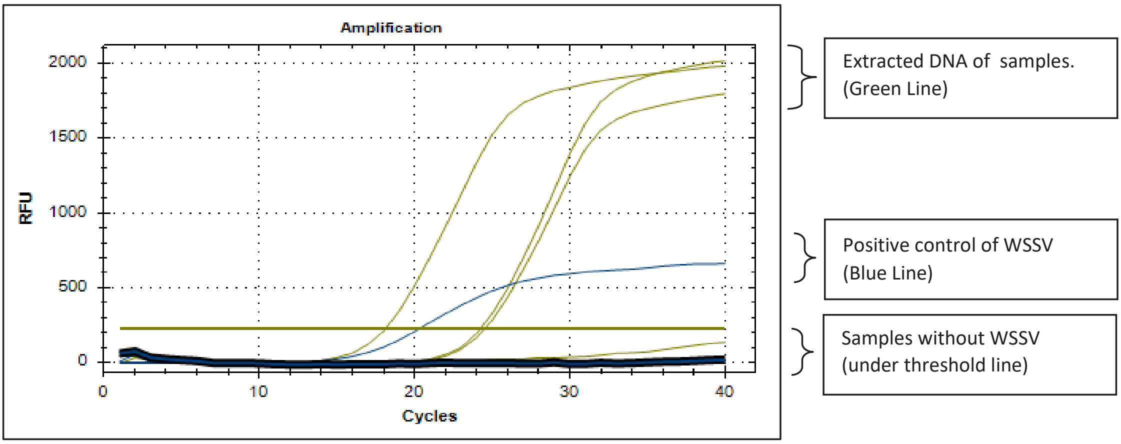


**Figure 3:** Fresh squids that tested positive for WSSV before gamma ray irradiation

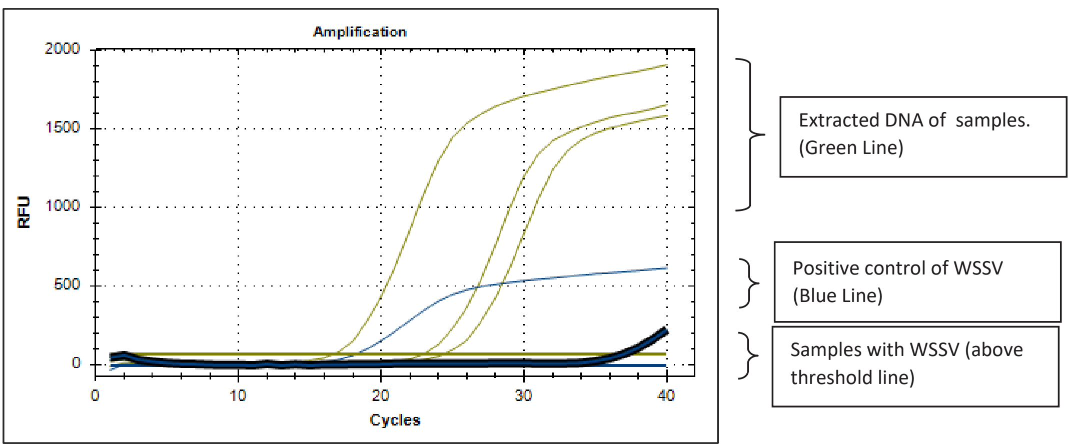


**Figure 4**: Fresh squids that tested negative for WSSV after gamma ray irradiation.

There is limited study on gamma ray irradiation are limited to reduce storage losses, to extend shelf life, and also to improve microbiological and parasitological safety of foods (Arapcheska et al., 2020). Earlier study on gamma ray irradiation performed on clam was meant for food safety. The study showed that coliform and faecal coliform were 90% eliminated at 1.32 kGy and 1.39 kGy (Harewood et al., 1994). Another study by Chen et al. (2000) found that at 12 kGy, bacteria were not detected in animal feed after irradiation. In the present study, at 10 kGy, EHP and WSSV were not detected after gamma irradiation. The results indicated that gamma ray is capable of disinfecting fresh feeds for the use of shrimp maturation. Thus, gamma ray irradiation will enable locally available polychaetes and squids for the use as maturation diet that are pathogen-free. Consequently, the production cost of having to import fresh feed from other countries can be lowered.

## Conclusion

Gamma irradiation has been proven to be a useful tool for disinfecting pathogens in fresh feed especially in the aquaculture maturation diet. The current study was done on the maturation diet for shrimps. Thus, perhaps it should be extended to the maturation diet for fishes and feeds for ornamental fish as this technique could reduce food-borne disease to the cultured animal.

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