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การตรวจสอบจุลินทรีย์บ่งชี้สุขลักษณะในบ่อเลี้ยงกุ้งขาวแวนาไม จังหวัดตรัง เพื่อพัฒนาสู่ระบบการเลี้ยงปลอดภัย

Inspection of Sanitary Indicator Bacteria in The Pacific White Shrimp (*Litopeneaus vanamei*) Pond Culture, Trang Province for Developing a Safety Culture System

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Abstract

The sanitary indicator bacteria from bottom soil, water and pacific white shrimp (*Litopeneaus vanamei*) in shrimp pond was determined from May to August 2015 on 8 shrimp ponds. All samples were inspected for total coliforms (TC), fecal coliforms (FC), fecal streptococci (FS) and *Salmonella*. Most of samples were found that TC, FC and FS from soil and shrimp were less than 3 MPN/g. Pond-water samples were found 0-10 MPN/100 ml. Faecal Streptococci group was examined : *Enterococcus faecium, E. faecalis* and *E. casseliflavus* from bottom soil and pond-water; *E. faecalis* and *E. casselitflavus* form shrimp. *Salmonella* was examined: *Salmonella javianaand Salmonella brunei*, but *Salmonella* was not detected from shrimp samples.

Keyword : bacteria, soil, water, pacific white shrimp, coliforms

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1. Introduction

In Thailand, there are a number of shrimp farms growing every year, especially on the coast. The data contained from 1998-2004 shows that a number of shrimp farms were likely to increase. This affects the increase of production and cost. The quality of water is not suitable for growing shrimps, and it causes shrimp disease. The value of the shrimps is also going down. The shrimp farms have expanded to the water area that has less saltiness. Having too many shrimps in the pond, and trying to manage the quality of the production, need to be considered because they affect the farming systems. Shrimp farming farmers need to consider in safety, cleanness of the farms, and the production for consumers. Nowadays, many countries that order shrimp from Thailand consider consumer safety. In 2004 Thailand had a campaign of Food Safety Year; therefore, shrimp farming in Thailand needed to be inspected, and think about the ways to prevent the bacteria to contaminate the shrimp. It is for the food safety.

The management of the factors occurring in the pacific white shrimp farm is important in order to get good quality production. The ways to keep as many shrimps as possible alive and to keep the quality of the sanitary bacteria are the indicators for the safety and the cleanness in the fishery production system. The good bacteria are called coliforms. They are used to evaluate the quality of water. This kind of bacteria is important for the hygiene of food and water. If the bacteria is found in the pond being inspected, it means that food and water in the pond is contaminated with human or warm-blooded animal's feces. It is because this kind of bacteria is found in the large intestine of humans and some kinds of animals. Coliforms are the most suitable bacteria to indicate the safety and the hygiene of food because analyzing coliforms is easy and obvious. There are four types of coliforms; *Escherichia*, *Citrobacter*, *Klebsiella*, and *Enterobacter*. However, coliform called enteric *Escherichia coli* causes gastrointestinal disease. It is not found in food. Fecal coliform is a kind of bacteria that gives gas and acid when growing in EC broth at the temperature of 44-46 °C. (Wilkes University, 2006) [1] Most countries that import shrimp don't accept shrimp that is contaminated with this kind of bacteria. (Dalsgaard, 1995) [2] It is because there are many types of *Salmonella*. Each type of *Salmonella* has different ecological characteristics which causes differences in symptoms of diseases. The diseases caused by *Salmonella* are gastroenteritis, septicemia, and typhoid fever. 10^8-10^9 cells of Salmonella can cause salmonellosis. In some cases an amount of *Salmonella* cells less than 10^9 cells can also cause this disease. *Salmonella* is easily destroyed by heating to the temperature of 60 °C for 4-5 hours or at the temperature of 100 °C for 1 minute .

This study aims to investigate the amount of coliforms (TC), fecal coliforms (FC), and fecal streptococci (FS) and to check the amount of *Salmonella* in water, soil, and shrimp in

shrimp ponds in Trang in order to anticipate the problems and improve the hygiene of the farms to reach the standard criteria of COC and GAP. This study also focuses on investigating the important factors occurring in the pacific white shrimp farms.

2. Research Methodology

2.1 Study area

This study was conducted within eight pacific white shrimp ponds. The study area is located in costal of Andaman sea, Trang province, south of Thailand, May to August 2015.

2.2 Sample collection

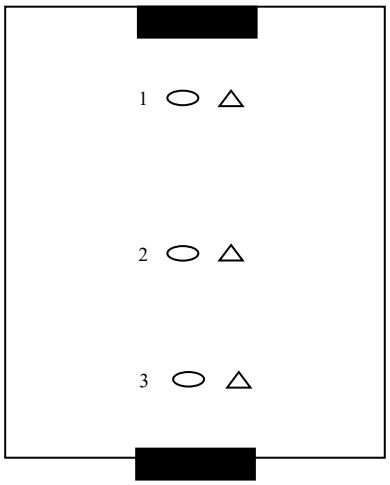
Water sample, bottom soil sample and pacific white shrimp were collected to be the samples of the study

1. Bottom soil samples were collected before releasing water into the pond and after harvesting shrimps in three positions. (Figure 1)

2.Water samples were collected continuously for 4 months, at the water exit door and opposite the water exit door, and at the stocked water pond in the farm, and at the canal.

3. Pacific white shrimp meat were collected from shrimp pond while harvesting at the end of period culture for 4 months.

All the samples for bacteria inspection were packed in sterilized bags and transferred to laboratory. Which were transported to the laboratory at low temperature (4-7°C) and protected from light, to be processed immediately for bacteria analysis.



Water Inflow

Water Outflow

○ = Collecting soil samples

△ = Collecting water samples

Station 1 Specify the position at water outflow

Station 2 Specify the position in the middle of the pond

Station 3 Specify the position at water inflow

Fig.1 Specify the positions for collecting samples in the pacific white shrimp *(Litopeneaus vanamei)* ponds.

2.3 Sample analysis

The sample were analyzed of the amount and types of sanitary bacteria from soil, water, and shrimp as follow:

1. Total coliforms and fecal coliforms were analyzed by multi-tube fermentation (APHA, 1989)[3]. The amount of bacteria is calculatedby the value of Most Probable Number- MPN from MPN Table.

2. Fecal streptococci using the criteria of APHA (1989)[3]. It was analyzed by analysis the amount of bacteria from reading the value of MPN (Most Probable Number- MPN) from the MPN

Table and sent them to classify the types at the Department of Science Service.

3. *Salmonella,* using the criteria of ISO 6597:1993(E) and sent it to the department of Science Service to classify the types of serovar. The results of classification were serovar of *Salmonella* such as *Salmonella javiana, Salmonella brunei.*

3. Results and Discussions

From investigating coliform in soil, water, and shrimp in the 8 pacific white shrimp ponds in Trang during May to August 2015 using multiple tube fermentation technique found that total coliforms (TC), fecal coliforms (FC), fecal streptococci (FS) and *Salmonella* found in soil for 0-3 MPN/g., in water for 0-10 MPN/100 ml. *Enterococcus faecium*, *E. faecalis* and *E. casseliflavus* were found in water samples and soil. *E. faecalis*and, *E. casseliflavus* were found in shrimp. *Salmonella javiana* and *Salmonella brunei* were found in water and soil but not shrimp.

The amount of 3 groups of sanitary bacteria; total coliform, fecal coliform and fecal streptococci found in soil, water, and shrimp in 8 pacific white shrimp ponds in Trang showed that these three groups of bacteria is found very little in soil, water, and shrimp. Therefore, the standard of almost all kinds of fishery production doesn't identify the amount of bacteria contamination. However, in Thailand, total coliforms 1,000MPN/100 ml, natural fecal coliforms, and *Salmonella* are not identified Golas *et al.* (2004) [4] investigated total coliforms, fecal coliforms, and fecal streptococci in water and fish food in the pond and found fecal streptococci in water for 1-300, 1-65 CFU/100 ml respectively. It suggested that bacteria were found in fish ponds at the same time as found in shrimp ponds, and the amount of fecal coliform and fecal streptococci were less than the value of total coliforms. Bouchriti *et al.* (1992b) [5] reported the investigation of Fecal Streptococci, the bacteria found in sea water and *Crassostrea gigas* showed that *Streptococcus faecails*, *S. faecium*, and *S. bovis* were found in the area that has C*rassostrea gigas* at Oualidia Lagoon in Morocco. Later, *Streptococcus* was called *Enterococcus* which is found in Thailand.

The results of investigating where the contamination is from, found that the pacific white shrimp in the pond got the contamination from human excrement. It is because this didn't showed the value of FC/FS ratio more than 4. It indicated the sources of contamination. The contamination of waste in the pacific white shrimp originated from warm-blooded animals, human, and the community (Wilkes University, 2006)[1]. Bouchriti *et al.* (1992a)[6]stated that if the value of FC/FS ratio less than 0.7, it means that the beginning of the excrement contamination is from warm-blooded animals. If the value of FC/FS ratio more than 0.7, the contamination is from human. And if the value of FC/FS ratio more than 4.0 means the contamination is from the community. Alabama (2006) [7] and Jones (2006) [8] reported that the ratio of FC/FS less than 1 means it is

contamination from animals. If the value is more than 4.0, it means that the contamination is from human. If the value of FC/FS is between 1-2 , it means the contamination is from both human and animals. Ostrolenk *et al*, 1947 [9] was the first person who compare enterococci with coliforms. In general, human's excrement has less *Enterococci* than *E. coli* which show the ratio of fecal coliform to *Enterococci* at 4:1 or higher. It indicates that the contamination is from human's excrement.

Salmonella contamination was found in soil, water, and shrimp at the pond samples, natural canal and the water flows into clarifier sample, so keeping the water in the clarifier sample before releasing it to the shrimp pond is so important for raising shrimp. Koonse *et al*. (2005) [10] stated that *Salmonella* found in shrimp had the relationship with the amount of fecal bacteria in shrimp pond. However, this finding indicated that salmonella wasn't found in shrimp which means that the shrimp still in the pond before catching is contaminated by very little bacteria. Therefore, the bacteria contamination didn't occur during the nursery step, but it might occur during or after catching shrimp. As a result, the investigation of the sanitary bacteria after catching shrimp needs to be discovered. Kruczynski (1999) [11] stated that in the United States, there is a development of the standard bacteria from the river and fecal coliform bacteria will be found when the animals live in sea water. However, fecal coliform is sometimes found in tropical environments.

4. Conclusion

The amount of the three kinds of bacteria; total coliform, fecal coliform, and fecal streptococci found in water, soil, and shrimp samples in Trang was very little which was in the standard criteria. The results of seeking where the sanitary bacteria contamination was from, found that the shrimp in the farm weren't contaminated with bacteria from human's excrement. It is because all samples didn't have the value of FC/FS ratio more than 4. The bacteria called *Salmonella* wasn't found in shrimp. However, it was found in soil and water from outside the pond (from the natural canal flowing into the pond).

5. Acknowledgment

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