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The Effects of Microsporidian (*Thelohania*) Infection on the Growth and Histopathological Changes in Pond-reared Pacific White Shrimp (*Litopenaeus vannamei*)

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ABSTRACT

The effects of microsporidian (*Thelohania*) infection on the growth and histopathological changes in intensive pond-reared Pacific white shrimp (*Litopenaeus vannamei*) were studied. Postlarvae 10 (PL10) were stocked at 125 PL/m^2 in three earthen ponds of approximately 5 rai (8,000 m^2) in an area with a salinity of 30-35 ppt. Shrimp were fed with commercial pelleted feed throughout the 120 days of the culture period. Shrimp were first observed to be infected with microsporidian parasites between 20-25 days after stocking. The highest prevalence of infection between 25-28% was found at day 60 in all growout ponds. Subsequently, the prevalence of infection decreased until the shrimp were harvested at day 120 when only 3-5% of shrimp remained diseased from the three rearing ponds. Shrimp infected in both hepatopancreas and abdominal muscle had the lowest weight, which was significantly different ($P<0.05$) from shrimp infected in only abdominal muscle and the uninfected group from first sampling at day 45 throughout the 120 days of the culture period. Histopathological changes in diseased shrimp from the hepatopancreas-and-muscle-infected group revealed that white masses of microsporidian parasites had partially infested the hepatopancreas and other organs, including the stomach, lymphoid organ and abdominal striated muscle, while in the muscle-infected group only the striated muscles were partially infested by microsporidians. These results clearly indicated that the severity of microsporidian infection negatively affected the growth and survival of white shrimp.

Key words: microsporidian, Pacific white shrimp, *Litopenaeus vannamei,* histopathological change, growth

INTRODUCTION

Microsporidiosis caused by microsporidian parasites has been reported in several genera of penaeid shrimp (Sprague and Couch, 1971; Newman *et al*., 1976). Diseased shrimp are recognized by the white opacity or milky discoloration associated with the musculature. Such microsporidian-infected shrimp are known as "cotton shrimp" or "milk shrimp" in English (Lightner, 1988) and "white back" in Thai (Donyadol *et al*., 1985; Limsuwan, 1991; Flegel *et al.,* 1992). In Thailand, microsporidian from infected black tiger shrimp, *Penaeus monodon,* as well as banana shrimp, *P. merguiensis,* has been initially identified as *Agmasoma* (*Thelohania*) *penaei* (Hazard and Oldacre, 1975; Flegel *et al*., 1992). These microsporidian-infected shrimp cause a significant loss to the industry because they are discarded in processing plants (Overstreet,

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1973; Lightner *et al*., 1975; Sindermann, 1990). The Pacific white shrimp, *Litopenaeus vannamei,* was introduced into Thailand for commercial culture in 2002 and later, during the culture period, *Thelohania* infection was reported from intensive pond-reared shrimp located in Nakornsrithammarat province, southern Thailand (Limsuwan *et al*., 2008). The highest percentage of *Thelohania* infection was 25% in cultured ponds that did not use calcium hypochlorite for water treatment before stocking postlarvae (PL), which caused heavy losses due to the lower survival rate and a marketing problem resulting from milky discoloration in the abdominal muscles.

Currently, the global economic crisis in major importing countries, such as the United States of America (USA), Japan and the European Union (EU) has seriously affected shrimp prices and caused declining consumption, whilst shrimp production has increased tremendously from both Asia and Latin America. In order to maintain competitiveness in this business, most small-scale shrimp farmers reduce the cost of production by stopping the use of any chemicals for water treatment to eliminate potential intermediate hosts or carriers of most diseases prior to stocking PL into the growout ponds. Thus, shrimp may be prone to microsporidian infection, particularly in areas where microsporidian outbreaks have previously occurred. The objectives of this study were to evaluate the prevalence of microsporidan infection in the genus *Thelohania* (*Agmasoma*) and its effects on the growth and histopathological changes of pond-reared *L. vannamei*.

MATERIALS AND METHODS

This study was conducted in a shrimp farm located in Prachaup Khiri Khan province, central Thailand. Microsporidian infection had been observed in this farm for the last two growout cycles. Three earthen ponds of approximately 5 rai (8000 m^2) in area with a salinity of 30-35 ppt during the culture period were used.

Water preparation and culture techniques

After the shrimp were harvested, routine pond preparation was carried out by removing the sludge from the middle of the pond. Seawater was pumped into three ponds to a depth of 1.5 m through a bag filter of approximately 26 mesh/$inch^2$. One week later, specific pathogen free (SPF) PL10 determined to be free from white spot syndrome virus (WSSV), Taura syndrome virus (TSV) yellow head virus (YHV) and microsporidian (*Thelohania* sp.) by polymerase chain reaction (PCR) and reverse transcriptase polymerase chain reaction (RT-PCR) assays, were stocked into each pond at 125 PL/m^2. Commercial pelleted feed was provided throughout the culture period. No water was exchanged during the first 40-50 days. Thereafter, more water exchange was required to maintain optimal water quality, particularly during the month before harvesting the shrimp.

Severity of microsporidian infection during the culture period

Shrimp were observed for clinical signs of microsporidian infection from the feeding trays daily during the first 40 days after stocking the PL. At day 45, shrimp were first sampled by cast net and then were sampled every 15 days until harvested. At each sampling event, at least 600 shrimp were randomly sampled from three different areas in the pond to determine the prevalence of microsporidian infection. Shrimp specimens were classified into three groups: (a) microsporidian infection in the hepatopancreas and abdominal striated muscles, (b) muscle infection only and (c) uninfected healthy shrimp (Figure 1). Shrimp from the three groups were weighed and recorded. After shrimp were harvested at day 120, data of shrimp weights from the three groups at each sampling time were statistically compared using Duncan's multiple range test (Steel and Torrie, 1980).

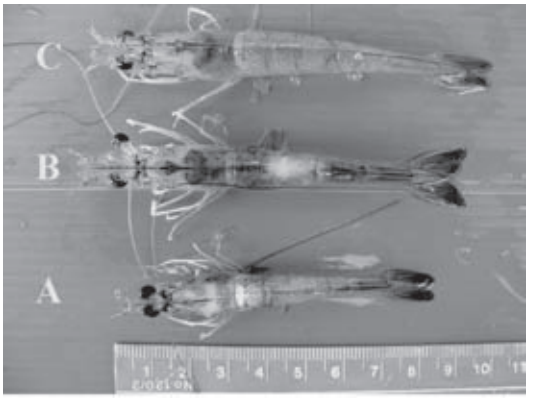

Figure 1 Microsporidian infection in: (a) the hepatopancreas and abdominal striated muscles; (b) muscle infection only; and (c) an uninfected healthy shrimp.

Histopathological study of microsporidian infections

At each sampling time, 10 shrimp from each infected group were fixed with Davidson's fixative and processed for routine histology using hematoxylin and eosin (H&E) staining as described by Bell and Lightner (1988). Histopathological changes of infected shrimp of different severity were examined and compared.

RESULTS AND DISCUSSION

Clinical signs

Shrimp were first observed to be infected with microsporidian parasites between 20-25 days after stocking the PL into the growout ponds. Affected shrimp had a whitish or milky appearance on various parts of the body. As the shrimp grew larger, this clinical sign was more easily observed especially dorsally from the hepatopancreas to the middle of the body. However, some infected shrimp had a whitish appearance near the last segment of the abdominal muscle (Figure 2). Internal gross signs of infected shrimp revealed that white masses of microsporidian parasites had infested the striated muscles as well as other organs, including the hepatopancreas, stomach and lymphoid organ (Figure 3). These external signs resembled the microsporidian infection previously reported in pond-reared *L. vannamei* (Limsuwan *et al*., 2008) and in *P. monodon* (Limsuwan, 1991; Flegel *et al.,* 1992).

Prevalence of the disease

The percentage of microsporidian infection in Pacific white shrimp in three cultured

Figure 2 White shrimp infected with microsporidian with a whitish or milky appearance in various parts of the body at day 45.

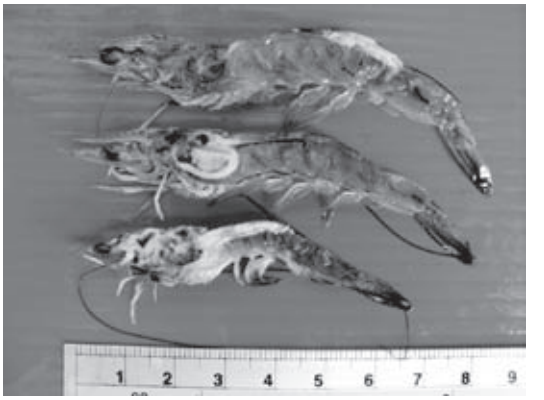

Figure 3 White masses of microsporidian in partially infested striated muscle and other tissues at day 45.

ponds is shown in Table 1. The highest prevalence of infection between 25-28% was found at day 60 in all three experimental growout ponds. Subsequently, the prevalence of infection decreased slightly until day 90 and then sharply declined until day 105. After the shrimp were harvested at day 120, only 3-5% of diseased shrimp were observed from the three rearing ponds. This result was similar to microsporidian infection reported in pond-reared *L. vannamei* that did not use calcium hypochlorite for water treatment before stocking PL (Limsuwan *et al*., 2008). In this study, the prevalence of infection reached 28.5% in pond 3 after 60 days post-stocking. Later during the culture period, despite infected shrimp being eaten by healthy shrimp, the prevalence of infection did not increase. This suggested that transmission was unsuccessful when healthy shrimp ate infected shrimp or healthy shrimp were exposed to waterborne spores as reported by Iversen *et al*. (1987). However, microsproridiosis was successfully transmitted experimentally in pink shrimp, *P. duorarum* PL, by feeding them on the feces of spotted sea trout (*Cynoscion nebulosus*) that had been fed infected shrimp (Iversen and Kelly, 1976).

Table 1 Percentage of microsporidian infection of Pacific white shrimp (*Litopenaeus vannamei*) during the culture period of 120 days.

Pond	Culture period																	
	45 days			60 days			75 days			90 days			105 days			120 days		
	A	B	Total	A	B	Total	A	B	Total	A	B	Total	A	B	Total	A	B	Total
1	19.1	1.4	20.5	25.5	2.2	27.7	23.0	1.9	24.9	16.9	1.6	18.5	4.5	1.3	5.8	2.1	0.9	3
2	16.5	2.1	18.6	23.2	2.1	25.3	23.6	1.5	24.1	19.8	1.7	21.5	6.7	1.3	8.0	3.4	0.8	4.2
3	20.1	2.5	22.6	26.3	2.2	28.5	22.0	1.8	23.8	18.2	1.3	19.5	6.3	0.9	7.2	2.8	0.5	3.3

A = microsporidian infection in the hepatopancreas and abdominal muscle.
B = microsporidian infection in the muscle only.

Effect of the disease on growth

Average weights of white shrimp with different severities of microsporidian infection during the 120 days of the culture period are shown in Tables 2, 3 and 4. Shrimp infected with microsporidian in both the hepatopancreas and abdominal muscle had the lowest weight, which was significantly different ($P<0.05$) from shrimp infected in only the abdominal muscle and the uninfected group from first sampling at day 45 throughout the 120 days of the culture period. Shrimp infected with microsporidian in the muscle only had an average body weight significantly lower than the uninfected group but higher than the groups in which both the hepatopancreas and muscle were infected. Shrimp heavily infected with microsporidian, particularly in the

Table 2 Average weight (g) of Pacific white shrimp (*Litopenaeus vannamei*) in pond 1 with different severities of infection.

Severity of infection	Culture period					
	45 days	60 days	75 days	90 days	105 days	120 days
Normal shrimp	4.4±0.4[a]	5.9±0.5[a]	7.9±0.4[a]	10.1±0.6[a]	12.4±0.5[a]	15.2±0.8[a]
Muscle only	3.8±0.5[b]	5.6±0.7[b]	7.1±0.8[b]	9.1±0.9[b]	10.9±0.8[b]	14.1±1.1[b]
Hepatopancreas and muscle	2.9±0.6[c]	4.2±0.3[c]	4.8±0.7[c]	6.6±0.5[c]	10.4±0.6[b]	11.5±1.1[c]

Values in the same column followed by different letters were significantly different (P<0.05).

Table 3 Average weight (g) of Pacific white shrimp (*Litopenaeus vannamei*) in pond 2 with different severities of infection.

Severity of infection	Culture period					
	45 days	60 days	75 days	90 days	105 days	120 days
Normal shrimp	4.4±0.3[a]	6.0±0.5[a]	8.0±0.4[a]	10.1±0.6[a]	12.3±0.4[a]	16.2±0.3[a]
Muscle only	3.7±0.5[b]	5.0±0.7[b]	7.1±0.8[b]	9.0±0.9[a]	10.5±0.4[b]	13.8±1.5[b]
Hepatopancreas and muscle	3.0±0.5[c]	4.1±0.3[c]	4.8±0.7[c]	6.8±0.4[b]	10.4±0.6[c]	11.3±1.3[c]

Values in the same column followed by different letters were significantly different (P<0.05).

Table 4 Average weight (g) of Pacific white shrimp (*Litopenaeus vannamei*) in pond 3 with different severities of infection.

Severity of infection	Culture period					
	45 days	60 days	75 days	90 days	105 days	120 days
Normal shrimp	4.4±0.4[a]	5.9±0.5[a]	7.9±0.4[a]	10.1±0.6[a]	12.4±0.5[a]	15.9±0.4[a]
Muscle only	3.8±0.5[b]	5.1±0.7[b]	7.1±0.8[b]	9.1±0.8[a]	10.9±0.8[b]	14.6±0.4[b]
Hepatopancreas and muscle	2.9±0.6[c]	4.2±0.3[c]	4.8±0.7[c]	6.6±0.5[b]	10.4±0.6[b]	12.1±1.6[c]

Values in the same column followed by different letters were significantly different (P<0.05).

hepatopancreas and abdominal muscle, had loose shells and grew slower than shrimp in other groups. In fact, the most severely infected shrimp were very weak due to microsporidians having infested most of the hepatopancreas and other vital organs. Therefore, damage of the hepatopancreas and other vital organs could have resulted in decreased feeding, metabolism and growth of the shrimp. When the shrimp were 75-105 days old, the prevalence of infected shrimp from cast-net sampling decreased because they gradually had died off or been consumed by the healthy shrimp. After shrimp were harvested at day 120, the production and survival rate were related to the percentage of microsporidian infection during the early culture period. During this period, pond 2 had the highest production and survival rate (Table 5), which was due to a lower percentage of microsporidian infection at 45-60 days post stocking. This was similar to the results reported by Limsuwan *et al*. (2008) that indicated microsporidian infection occurred after the infective spore stages were eaten by the PL. Environmental conditions during PL stocking were more suitable for microsporidian infection of the PL compared to the water quality conditions after 50 days because larger shrimp were more resistant to this parasite.

Table 5 Production of shrimp at harvest.

Pond	Total yield (kg)	Yield (kg/rai)	Survival rate (%)
1	10 536	2107	69.5
2	11 140	2228	71.6
3	10 086	2017	67.2

Histopatological changes in diseased shrimp

Histopathological changes in diseased shrimp from the hepatopancreas-and- muscle-infected group at day 30 revealed that white masses of microsporidian parasites had partially infested the hepatopancreas and other organs, including the stomach (Figure 4) and abdominal striated muscle, while in the muscle of the infected group, only the striated muscles were partially infested by microsporidia (Figure 5). At day 45, histological changes revealed a greater severity of infection in that microsporidia had infested most of the hepatopancreas. Hepatopancreatic tubules of the heavily infected shrimp were dilated and necrotic (Figure 6). The number of hepatopancreatic R-cells was lower when compared with uninfected shrimp. Other vital organs, including the stomach, lymphoid organ (Figure 7), pericardial chamber, gills and striated abdominal muscle were most infested by microsporidia. It was interesting to note that the tegmental glands appeared normal and microsporidia had only infested the surrounding tissues (Figure 8) as well as nervous tissues (Figure 9) and hemopoietic tissues (Figure 10). A light inflammatory response was observed in the striated muscle (Figure 11) and hepatopancreas of some diseased shrimp. In contrast, Lightner (1996) found that infection of microsporidian in striated muscle did not cause a host inflammatory response.

From days 60, 75, 90, 105 and 120, the histopathological changes in microsporidian-infected shrimp were similar to those observed at day 45. This finding indicated that heavily infected shrimp after 60 days post-stocking PL gradually died off due to damage to various organs, including the hepatopancreas, lymphoid organ, stomach and abdominal muscle resulting in decreased normal physiological function and finally consumption by the healthy shrimp. The histopathological changes in this study resembled microsporidian infection in pond-cultured *P. monodon* in Malaysia (Anderson *et al*., 1989). Microsporidian *A. penaei* was reported to infect the blood vessels, heart, gonads, gills, hepatopancreas, gut and connective tissues, as well as muscle of white shrimp, *P. setiferus* (Rigdon *et al*., 1975; Kelly, 1987).

CONCLUSIONS

Pacific white shrimp were first observed to be infected with the microsporidian between 20-25 days after stocking PL into the ponds. The highest infection prevalence of between 25-28% was found at day 60. Shrimp infected with microsporidian in both the hepatopancreas and abdominal muscle had the lowest body weights, which were significantly different from either the shrimp infected in only the muscle or the uninfected shrimp. The histopathological changes in heavily infected shrimp revealed that most of the vital organs, including the hepatopancrease, lymphoid organ, stomach and other organs were infested by microsporidian parasites. This study clearly indicated that the severity of microsporidian infection negatively affected the growth and survival of white shrimp.

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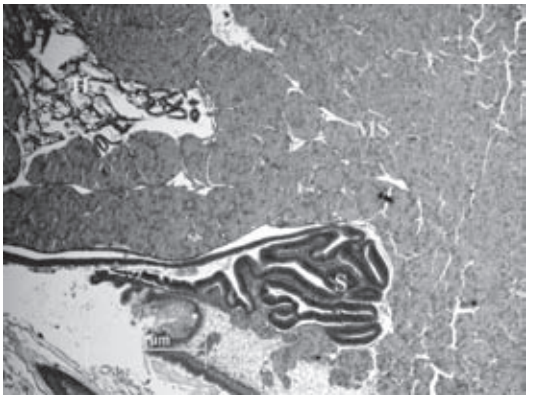

Figure 4 Microsporidian parasites (MS); partially infested hepatopancreas (H); and stomach (S). (H&E staining).

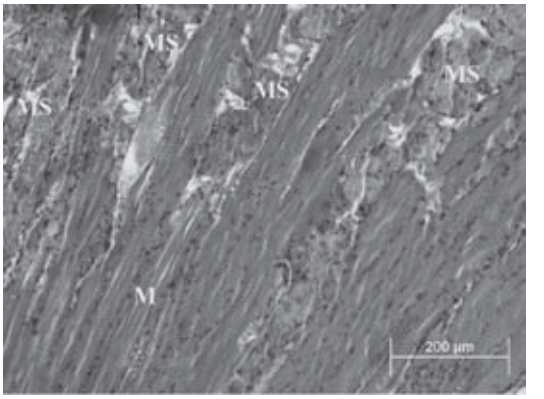

Figure 5 Microsporidian parasites (MS); partially infested striated muscle (M). (H&E staining).

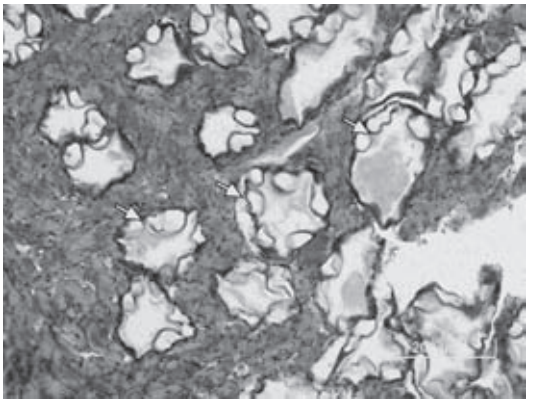

Figure 6 Hepatopancreatic tubule epithelium of heavily microsporidian-infected shrimp were dilated and necrotic (arrows). (H&E staining).

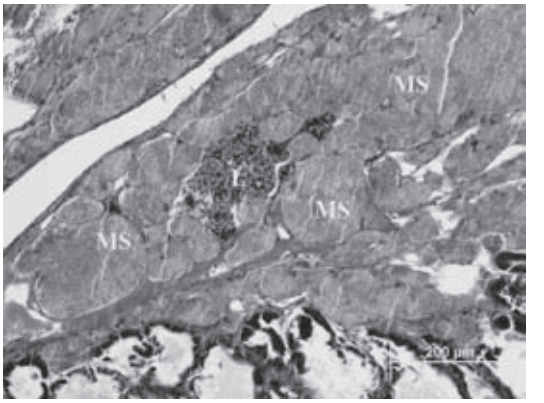

Figure 7 Lymphoid organ (L) infested by microsporidian parasites (MS). (H&E staining).

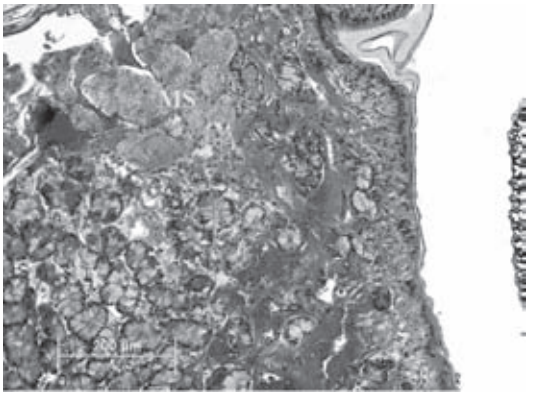

Figure 8 Microsporidian parasites (MS) infested surrounding tissues but tegmental glands (T) appeared normal. (H&E staining).

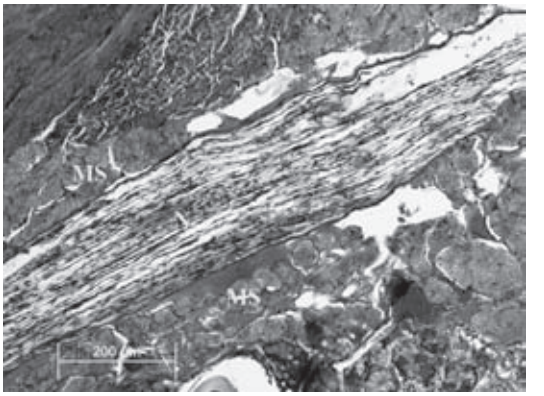

Figure 9 Microsporidian parasites (MS) infested surrounding tissues but nervous tissue (N) appeared normal. (H&E staining).

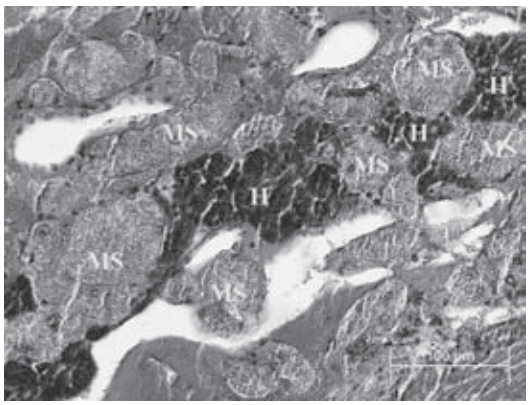

Figure 10 Microsporidian parasites (MS) infested surrounding tissues, while hemopoietic tissue (H) appeared normal. (H&E staining).

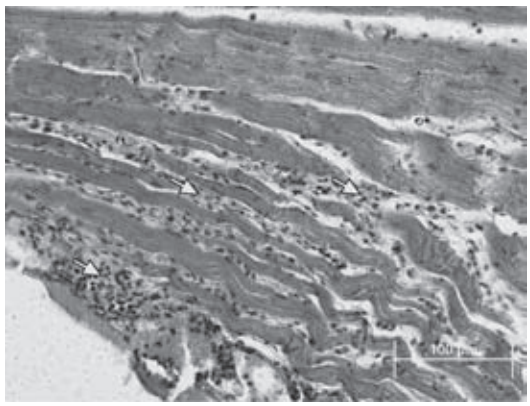

Figure 11 Striated muscles of infected shrimp showing light inflammatory response (arrows). (H&E staining).

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