Aquaculture Research, 2001, **32** (Suppl. 1), 383–387

Effect of dietary shrimp head meal contaminated with white spot syndrome virus (WSSV) on detection of WSSV in black tiger shrimp (*Penaeus monodon* Fabricius)

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Abstract

The effects of supplementary shrimp head meal contaminated with white spot syndrome virus (WSSV-SHM) in the diet on detection of WSSV in *Penaeus monodon* Fabricius were investigated. In Experiment I, 15 shrimp with a mean body weight of 18.2 g were fed to apparent satiation with each of four diets for 8 weeks. Diet 1 was the control diet containing no WSSV-SHM; Diets 2–4 contained wet-cooked WSSV-SHM (autoclaved at 115°C for 15 min), dry-cooked WSSV-SHM (oven-dried at 90°C for 1 h) and commercial SHM at a level of 10% in the diets, respectively. In Experiment II, five diets were used: Diet 1 as the control diet without WSSV-SHM, Diets 2–5 containing steamed WSSV-SHM (100°C for 15 min), oven-dried WSSV-SHM (60°C for 8 h), raw fresh WSSV-SHM and freeze-dried WSSV-SHM at 10% in each diet, respectively. Shrimp, weighing 10.8 g, were fed each diet for 6 weeks to satiation. In both Experiments I and II, the pooled hemolymph samples from five shrimps were taken with 2-week feeding interval and determined in triplicate for WSSV detection using polymerase chain reaction (PCR) assay. In both Experiments I and II, PCR products from hemolymph samples showed the negative results for all dietary treatments. These results suggested that using commercial SHM and WSSV-SHM in diets had no adverse effects on WSSV infection in *P. monodon*.

Keywords: penaeid shrimp, white spot syndrome, shrimp head meal.

Introduction

As shrimp culture in Thailand has rapidly expanded throughout the coastal areas, shrimp culturists have faced serious disease problems in the industry. The disease problems seem to be more serious at present in many areas where intensive shrimp culture is practised. Viral diseases have caused high mortalities and no treatment has been reported. Among the viral diseases, red disease with white patches has caused severe damage to the shrimp culture industry; and the white spot baculovirus (WSBV) is reported as the causative agent for this disease (Kasornchandra, Boonyarapalin & Itami 1998). In addition, the horizontal transmission of this disease has been suspected via the water and carriers such as wild shrimp or other crustaceans (e.g. krill, crabs), and via infected shrimp carcasses (ASCC 1996). Shrimp culturists have claimed that shrimp heads used to produce the meals (SHM) might be contaminated with WSSV; and consequently they have postulated that WSSV-contaminated shrimp head meal (WSSV-SHM) used in commercial shrimp feeds has been the carrier of this disease. Therefore, this study

was conducted to clarify the supplemental effect of WSSV-SHM and commercial SHM in diets on WSSV infections in *Penaeus monodon* Fabricius.

Materials and methods

Two feeding experiments (Experiments I and II) were designed using diets containing WSSV-SHM with different processing conditions as shown in Figs 1 and 2, respectively.

Preparation of WSSV-SHM

Viruses extracted from shrimp with clinical signs of white spot syndrome were processed using the method of Boonyaratpalin, Supamattaya, Kasornchandra, Direkbusaracom, Aekpanithanpong & Chantanachooklin (1993). Shrimp with a body weight of about 20 g were injected intramuscularly with WSSV and kept in a 250-L tank until they showed the symptoms of red disease with white patches. The head part (cephalothoraces) of the diseased shrimp were collected and stored at –80 °C until used. The frozen shrimp heads contaminated with WSSV were prepared into the meal with different treatments as shown in Figs 1 and 2. The WSSV-SHM was supplemented in each diet at a level of 10% in Experiments I and II. The WSSV-SHM and the experimental diets used in both Experiments I and II were confirmed positive for WSSV by the PCR as described by Kasornchandra *et al.* (1998).

Experimental shrimps

Shrimp (*P. monodon*) from private shrimp farms were transferred to the National Institute of Coastal Aquaculture, Songkla, Thailand and maintained in 150 (W) × 400 (L) × 100 (H) cm tanks. They were acclimated and fed with commercial shrimp feed for 1 week prior to the experiment. The hemolymph samples were collected from each shrimp initially and individually screened for WSSV infection using PCR assay before use in Experiments I and II.

Experiment I

Four experimental diets were used: Diet 1 as the control diet containing no WSSV-SHM and Diets 2–4 containing wet-cooked WSSV-SHM (autoclaved at 115°C for 15 min), dry-cooked WSSV-SHM (oven-dried at 90°C for 1 h) and commercial SHM at a level of 10% in the diet, respectively. All the diets with about 30% moisture were processed using a meat grinder and the spaghetti-like feed was broken into pellets. This process was followed by 5 h drying in an air-flow oven at 55 °C until the moisture content was below 10%. The dry pellets were held at 3–5 °C until use. The composition of the experimental diets is given in Table 1. The nutrient

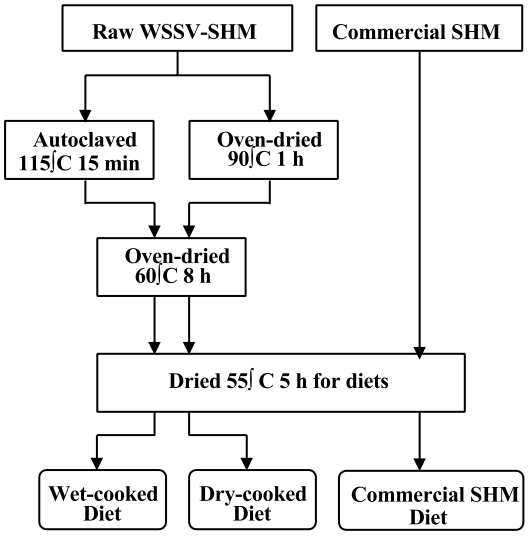

Figure 1 Processing condition of WSSV-SHM and the diets used in Experiment I.

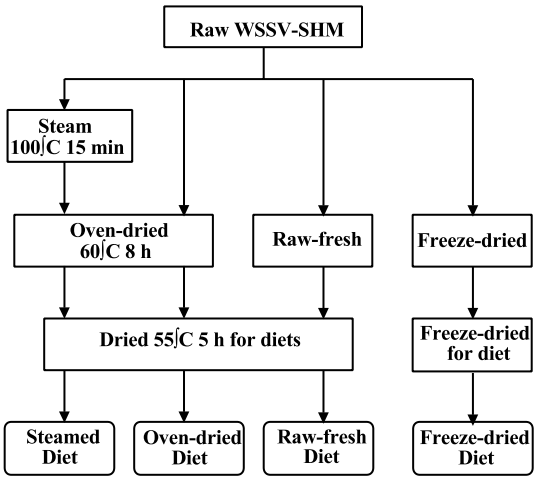

Figure 2 Processing condition of WSSV-SHM and the diets used in Experiment II.

contents of the diets were analyzed using the methods of AOAC (1980) as shown in Table 2.

Fifteen shrimp with an initial mean body weight of 18.2 g were selected and distributed into each of four glass aquaria [47 (W) × 80 (L) × 30 (H) cm]. In each aquarium, 15 chambers of 15 (W) × 15 (L) × 30 (H) cm were separated and shrimp were stocked individually in order to prevent cannibalism during the molting period. They were fed each experimental diet to apparent satiation five times a day for an 8-week period. The experimental aquaria were supplied with a flow-though sea water system at a flow rate of 0.5 L min^{-1} and with individual aeration. The water temperature averaged 29 ± 1 °C and the salinity ranged between 28 and 37 ppt. At each 2-week feeding interval, dietary groups of shrimp were screened in triplicate for WSSV infection using a PCR assay. Hemolymph samples from each shrimp were taken and pooled samples of five shrimp were used for each PCR determination.

Table 1 Composition of the experimental diets used in Experiments I and II

Ingredients (g 100 g^{-1} diet)	Control	Test diets in Experiments I[1] and II[2]
WSSV-SHM	–	10.00
Fish meal	28.00	28.00
Squid meal	9.00	5.00
Soybean meal	10.00	10.00
Wheat gluten	6.00	6.00
Wheat flour	26.00	20.00
Rice flour	10.15	10.15
Fish oil	2.00	2.00
Lecithin	2.00	2.00
Cholesterol	0.50	0.50
Vitamin mixture[3]	0.83	0.83
Mineral mixture[3]	4.00	4.00
BHT	0.02	0.02
Zeolite	1.50	1.50

[1]Diets 2–4 contained wet-cooked WSSV-SHM, dry-cooked WSSV-SHM and commercial shrimp head meal, respectively.
[2]Diets 2–5 contained steamed WSSV-SHM, oven-dried WSSV-SHM, raw-fresh WSSV-SHM and freeze-dried WSSV-SHM, respectively. The moisture content of raw-fresh WSSV-SHM was 79%, therefore 48 g of this ingredient was used per 100 g diet.
[3]Boonyaratpalin & Pongmaneerat (1995).

Experiment II

Five diets containing different treatments of WSSV-SHM were used: Diet 1 as the control diet without WSSV-SHM and Diets 2–5 containing steamed WSSV-SHM (100 °C for 15 min), oven-dried WSSV-SHM (60 °C for 8 h), raw-fresh WSSV-SHM and freeze-dried WSSV-SHM at 10% in each diet, respectively. Diets 1–4 were processed using the same method described in Experiment I. Diet 5, which contained freeze-dried WSSV-SHM, was similarly processed into pellets but was freeze-dried until the moisture content was below 10%. The dry pellets were also kept in a refrigerator until used. The composition and nutrient contents of the

Table 2 The nutrient contents of the experimental diets used in Experiments I and II

Experimental diets	Nutrient contents (% dry basis)			
	Crude protein	Crude lipid	Ash	Moisture
Experiment I:				
Control diet	43.09	9.40	9.61	7.11
Wet-cooked WSSV-SHM diet	43.77	9.74	11.46	7.38
Dry-cooked WSSV-SHM diet	43.99	9.27	11.46	8.77
Commercial SHM diet	43.17	9.88	12.11	7.77
Experiment II:				
Control diet	43.76	9.31	9.87	6.22
Steamed WSSV-SHM diet	44.53	9.94	11.57	5.28
Oven-dried WSSV-SHM diet	44.55	10.55	11.10	7.07
Raw-fresh WSSV-SHM diet	44.64	10.33	10.97	8.64
Freeze-dried WSSV-SHM diet	44.59	9.89	11.72	7.39

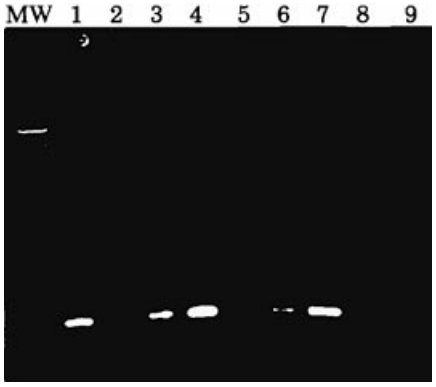

Figure 3 PCR products of WSSV-DNA from WSSV-SHM and diets used for *P. monodon* in Experiment I. Lanes 1, 8, positive control of WSSV; lane 2, one-step PCR of commercial SHM; lane 3, wet-cooked WSSV-SHM; lane 4, dry-cooked WSSV-SHM; lane 5, control diet without WSSV-SHM; lane 6, diet containing wet-cooked WSSV-SHM; lane 7, diet containing dry-cooked WSSV-SHM; lane 9, two-step PCR of commercial SHM.

experimental diets are given in Tables 1 and 2, respectively.

Shrimp, weighing about 10.8 g, were selected and distributed into 20 glass aquaria [25 (W) × 50 (L) × 30 (H) cm] with five shrimps each. They were fed each diet in four replicates, five times a day, to satiation for 6 weeks. The water temperature averaged 28 ± 1 °C. The experimental aquaria were supplied with a flow-though sea water system in the same way as in Experiment I. The transmission of WSSV in the shrimps via the dietary WSSV-SHM was diagnosed with 2-week feeding interval by the PCR assay that was performed in four replicates in each dietary group. The pooled hemolymph samples of five shrimps were used for each PCR determination as previously described.

Results and discussion

Experiment I

The results of PCR products of the WSSV-SHM, commercial SHM and the experimental diets used are given in Fig. 3. The single band of PCR product of 520 bp after analysing in agarose gel electrophoresis in lane 1 was the positive control of WSSV (Fig. 3). The single band of wet-cooked WSSV-SHM (lane 3) and dry-cooked WSSV-SHM (lane 4) were also detected. Likewise, the diets containing both wet-cooked WSSV-SHM and dry-cooked WSSV-SHM exhibited the single bands of WSSV (lanes 6 and 7). These results confirmed that WSSV-DNA still remained in those supplemental wet-cooked and dry-cooked WSSV-SHM and the diets. The control diet did not show any positive band (lane 5), suggesting that this diet was not contaminated with WSSV. In addition, the PCR assay on the commercial SHM normally used in commercial shrimp feed was also negative obtained by one-step PCR (lane 2). But the WSSV-DNA was detected after two-step PCR was performed (lane 9). It was pointed out that the commercial SHM tested was contaminated with a very low concentration of WSSV. The diet containing commercial SHM was not subjected to analysis by PCR.

After 2, 4, 6 and 8 weeks of feeding, PCR assays from hemolymph samples of shrimp fed the control diet did not show positive bands. Similarly, PCR assay on shrimp fed diets containing wet-cooked WSSV-SHM, dry-cooked WSSV-SHM and commer-

Table 3 Effect of dietary WSSV-SHM on WSSV infection in *P. monodon* (Experiment I)

	Result of PCR assay at week				
Dietary groups	**0**	**2**	**4**	**6**	**8**
Control	–	–	–	–	–
Wet-cooked WSSV-SHM	–	–	–	–	–
Dry-cooked WSSV-SHM	–	–	–	–	–
Commercial SHM	–	–	–	–	–

–, Negative PCR test.

Table 4 Effect of dietary WSSV-SHM on WSSV infection in *P. monodon* (Experiment II)

	Results of PCR assay at week			
Dietary groups	**0**	**2**	**4**	**6**
Control	–	–	–	–
Steamed WSSV-SHM	–	–	–	–
Oven-dried WSSV-SHM	–	–	–	–
Raw-fresh WSSV-SHM	–	–	–	–
Freeze-dried WSSV-SHM	–	–	–	–

–, Negative PCR test.

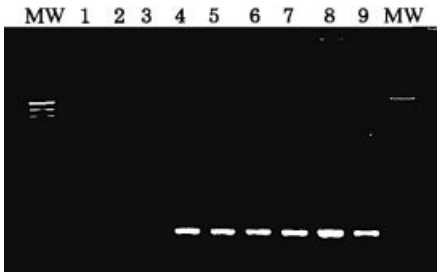

Figure 4 PCR products of WSSV-DNA from WSSV-SHM and diets used for *P. monodon* in Experiment II. Lane 1, control diet without WSSV-SHM; lane 2, diet containing steamed WSSV-SHM; lane 3, diet containing oven-dried WSSV-SHM; lane 4, diet containing raw-fresh WSSV-SHM; lane 5, diet containing freeze-dried WSSV-SHM; lane 6, steamed WSSV-SHM; lane 7, oven-dried WSSV-SHM; lane 8, freeze-dried WSSV-SHM; lane 9, positive control of WSSV.

cial SHM did not produce the WSSV-DNA-specific bands (Table 3). These results suggested that using commercial SHM and WSSV-SHM treated under those processing conditions in the diets for *P. monodon* did not transmit WSSV infections. However, the SHM used in this experiment was treated with high temperature processing that might destroy all the live virus particles in the diets. Consequently, Experiment II was carried out to determine the effect of dietary raw-fresh WSSV-SHM and freeze-dried WSSV-SHM on WSSV infection in the shrimp.

Experiment II

The results of PCR products of the WSSV-SHM and the experimental diets used are shown in Fig. 4. The WSSV-SHM used in this experiment, including steamed, oven-dried and freeze-dried WSSV-SHM, exhibited the single band of WSSV-DNA as shown in lanes 6–8, respectively. The raw-fresh WSSV-SHM was not subjected to analysis by PCR assay. The experimental diets containing those of WSSV-SHM also showed the specific band of WSSV-DNA (lanes 2–5), except for the control diet (lane 1) that contained no WSSV-SHM. Thus, the PCR assay of WSSV-SHM and the diets produced concordant results.

The PCR assay of WSSV infection in the shrimp after receiving diets containing WSSV-SHM are presented in Table 4. After 2, 4 and 6 weeks of feeding, none of the experimental shrimp had detectable WSSV-DNA-specific bands by PCR assay. This finding indicated that supplementation of WSSV-SHM, including steamed, oven-dried, freeze-dried and raw-fresh SEM-SHM in the diets, had no correlation with the occurrence of white spot syndrome baculovirus.

Conclusion

Using the cooked WSSV-SHM (steamed WSSV-SHM and oven-dried WSSV-SHM), the uncooked WSSV-SHM (freeze-dried WSSV-SHM and raw-fresh WSSV-SHM) and the commercial SHM in the diets did not induce WSSV infection in the shrimp. Thus, it was demonstrated that horizontal transmission of white spot syndrome baculovirus occurring in *P. monodon* does not involve dietary SHM, under the experimental conditions used in this study, including WSSV-SHM and commercial SHM.

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