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Effect of astaxanthin and yeast (*Saccharomyces cerevisiae*) supplemented in diets on growth performance and resistant against virulence *Vibrio parahaemolyticus* (*Vp* $_{AHPND}$) in sand worms (*Perinereis nuntia*)

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Abstract

Sand worm (*Perinereis nuntia*) are commonly used to feed shrimp broodstocks in Thailand. The wild sandworm is in serious trouble, because of this the production from culture in a biosecurity system hatchery is necessary. The objective of this research was to study the effect of feeding supplementation with astaxanthin and yeast (*Saccharomyces cerevisiae*.) on growth, chemical composition and inhibitory effect against the growth of *Vibrio parahaemolyticus* (Acute Hepatopancreatic Necrosis Disease; AHPND) of farmed sand worms (*Perinereis nuntia*). A completely randomized design (CRD) was carried out using 4 treatments with 3 replications. The sand worms (average weight of 0.05 g) were reared in black plastic tanks and fed for 90 days with 4 different types of diet as follows: T1, commercial Pacific white shrimp diet (control); T2, commercial Pacific white shrimp diet supplementation with astaxanthin (AS); T3, commercial Pacific white shrimp diet supplementation with live cell yeast (LY); and T4, commercial Pacific white shrimp diet supplementation with autolyzed yeast (AY). For the results, the sand worms fed with AS, LY and AY diets (T2,T3,T4) had weight gain, average daily gain, specific growth rate and survival rate significantly higher than the control group ($p<0.05$). Moreover, feed intake and feed conversion ratio of sand worms in T2, T3 and T4 were significantly lower than the control group ($p<0.05$). Protein and lipid contents of the sand worms meat in T2, T3 and T4 were significantly higher than the control group ($p<0.05$). The results indicated that feed supplementation with astaxanthin and yeast (both live cell and autolyzed yeast) increased growth, protein and lipids contents of farmed sand worms. The coelomic fluid of sandworms in T2, T3 and T4 showed significantly higher inhibitory effect against the growth of *Vp* $_{AHPND}$ than the control group. The results indicated that feed supplementation with astaxanthin and yeast increased growth, protein, lipids contents and inhibitory effect against the growth of *Vp AHPND*. These findings could significantly contribute to the value of farmed sand worm in further studies.

Keywords: *astaxanthin, Perinereis nuntia, sand worm, yeast, growth performance, Vibrio parahaemolyticus*(*Vp* $_{AHPND}$)

1. Introduction

Sand worm, *Perinereis nuntia* (Annelida:Polychaeta), are an invertebrate marine sand worm. It is a food source for shrimp broodstocks because of it containing high amounts of protein, lipid and polyunsaturated fatty acids, which are important for egg maturation in female breeder of aquatic animals (Techaprempreecha et al., 2011). In addition, sand worm contains carotenoid pigments (Bauernfeind, 1981; Goodwin, 1980), carotenoid also called tetraterpenoids, are yellow, orange, and red organic pigments that are produced by plants and algae, as well as several bacteria and fungi. The sand worms used in hatcheries are wild-caught. At present, wild sand worms are declining and not suffice for demand, especially those used as live feed of shrimp broodstocks. Therefore, they have been cultured; however cultured sandworms had growth and nutritional values lower than wild sand worms (Techaprempreecha et al., 2011). The natural sandworm is in risk of serious contamination of shrimp pathogens. With the increased sandworm culture in the biosecurity hatcheries, it is necessary to increase the amount of sand worm production to sufficient supply needs as well as helping to reduce the risk of severe pathogen infections of marine shrimp broodstocks. However, the increased nutritional value of sand worm feed with yeast supplementation to increase protein intake stimulates the growth of the sand worms (Rumsey et al., 1990). At the same time, yeast also boosts immunity, because yeast has a component that has a direct effect on disease resistance and enhances the immune system of aquatic animals, such as beta glucan, mannan and

chitin (Lipke & Ovalle, 1998). Astaxanthin has a role which stimulates the immune system and increases stress resistance in aquatic animals, which is a powerful antioxidant, and plays a role in increasing the function of the immune system. Therefore, astaxanthin and yeast-mixing with instant shrimp feed could be used for raising production and value of sand worms, which is an easy process for farmers in growing and improving the immune system (Rajabi, et al., 2012; Xiong et al., 2018). The aim of this study was to investigate the effect of dietary supplementation with astaxanthin and yeast on the growth of sand worms (*P. nuntia*) and the ability of the coelomic fluid to inhibit virulent strain of *V. parahaemolyticus* which causes early mortality syndrome/acute hepatopancreatic necrosis disease.

2. Objectives

1.-To study the effects of dietary supplementation with astaxanthin and yeast on growth survival rate chemical composition and the amino acids of sand worms (*P. nuntia*) among different supplementary diets.

2.-To study the ability of coelomic fluid of sand worms to inhibit Vp_{AHPND} among different supplementary diets.

3. Materials and methods

3.1 Experiment 1: Dietary supplementation with astaxanthin and yeast on the growth and nutritional values of sand worms (*P. nuntia*)

3.1.1 Animals preparation

Sand worms (*P. nuntia*) were obtained from Coastal Aquaculture Research and Development Regional Center 2 (Samutsakhon), Thailand. The sand worms were reared in plastic containers and fed with commercial Pacific white shrimp diet until 2 months old. The sand worms with average weight of 0.052±0.004 g were selected for further experiments.

3.1.2 Water preparation

Sea water was filled through a protein skimmer and then treated with 65% sodium hypochlorite at the concentration of 20 ppm. Water salinity was maintained at 30 ppt and alkalinity was adjusted between 110-120 mg L^{-1} by the addition of dolomite and hydrated lime.

3.1.3 Preparation of experimental tank

Twelve plastic tanks (oval shape, black color, size 0.30 x 0.90 x 0.50 m, Figure 1) were placed with cleaned fresh water sand (2 mm diameter) of 15 cm height and filled up with a water level of 20 cm. The water of each tank can be drained out by a PVC pipe (1-inch diameter) and intubated into the tank wall. A small mesh of a plastic nylon net was placed inside the tank between the sand and pipe to prevent sand from flowing out during water drainage.

3.1.4 Experimental design and feeding trials

A completely randomized design (CRD) was carried out using 4 treatments with 3 replications (2,000 sand worms per replicate). The sand worms were fed with 4 different types of diet as follows: control (T1), commercial Pacific white shrimp diet (Con); T2, commercial Pacific white shrimp diet supplementation with astaxanthin at 1,000 ppm (AS); T3, commercial Pacific white shrimp diet supplementation with live cell yeast at 4.95 x 10^3 cell/g (LY); and T4, commercial Pacific white shrimp diet supplementation with autolyzed yeast at 35% protein (AY). The commercial Pacific white shrimp were bought from Thai Union Feed Mill Co., Ltd., Thailand. All of the 4 diet types were coated with squid oil, air dried, then packed in vacuum plastic boxes and stored in the dark until used. The diets were prepared before each feeding time. All the experimental diets were estimated for chemical composition (AOAC, 2016) and presented in Table 1.

Two-months old sand worms were transferred to experimental tanks (2,000 sand worms per tank) and fed three times daily at 09.00 a.m., 14.00 p.m., and 19.00 p.m. Animals were fed with 2g/tank/time from day 0 to 14, followed by 5g/tank/time from day 15 to end of experiment. At each feeding time, cleaned sea water (as described above) was filled to a height above sand in the tanks and then drained out after feeding time 2 hr.

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Table 1. Chemical composition and carotenoids of experimental diets

Experimental diets	Chemical composition (%)					Carotenoid
	Protein	Lipid	Fiber	Moisture	Ash	
Con (T1)	38.24	8.67	3.87	10.31	11.04	0.07
AS (T2)	38.61	8.63	3.98	10.77	11.57	0.55
LY (T3)	38.43	7.96	3.02	9.23	10.71	0.11
AY (T4)	38.41	7.52	3.05	11.11	11.75	0.11

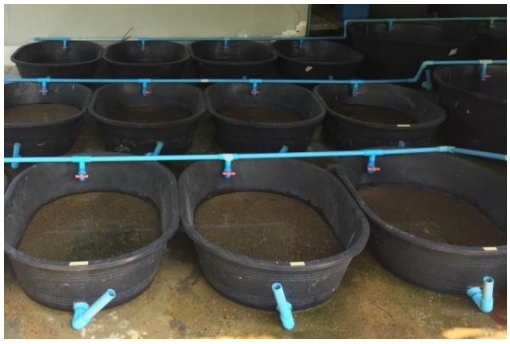

Figure 1 Plastic tanks (oval shape, black color, size 0.30 x 0.90 x 0.50 m) were placed with cleaned freshwater sand (2 mm diameter) of 15 cm height and fill the water 20 cm level.

3.1.5 Growth performance

Fifty sand worms of each replicate were randomly sampled. The samples were weighed twice at day 30 and 60. At day 90 (end of experiment), all of the sand worms were collected for analysis of weight, weight gain (WG), average daily gain (ADG), specific growth rate (SGR), feed intake (FI), survival rate (SR) and feed convention ratio (FCR) using the following equations:

WG (%/g/sand worm) = (FW – IW) / IW x 100
ADG (g/sand worm/day) = AFW – AIW / ND
SGR (% / day) = [ln (FW) – ln (IW)] x 100
FI (g/sand worm) = TFW/NS
SR (%) = (FNS / INS) x 100
FCR = Feed given (DW)/ body weight gain (WW)

Where FW = final weight,
IW = initial weight, AFW = average final weight, AIW = average initial weight, ND = number of culture days, ln = natural log, TFW = total of feed weight, NS = number of sand worms, FNS = final number of survived sand worms, INS = initial number of sand worms, DW= dry weight and WW = wet weight

3.1.6 Chemical composition and total carotenoid analyses

At the end of the experiment, the sand worms of each treatment were dried and then analyzed for protein, lipid, moisture and ash according to the AOAC (2016) official method of 990.03, 2000.05, 930.15 and 942.05, respectively. Fiber was determined by In-house method base on ISO 6865:2000 AOAC (2016). Total carotenoid was determined spectrophotometrically at 470 nm according to the method of Foss et al. (1984).

3.1.7 Water quality analyses

Water quality of all the experimental tank were measured once a week, *viz*. temperature, pH, salinity and dissolved oxygen (DO) using a mercury thermometer, digital pH meter, salinity refractometer and oxygen meter, respectively. While, alkalinity and total ammonia were analyzed according to potentiometric titration and phenol hypochlorite methods (Department of Fisheries, 2008).

3.1.8 Statistical Analysis

For all analyses, the mean and standard deviation were calculated and reported. The data were subjected to an analysis of variance (ANOVA). Where there was a significant difference ($p \leq 0.05$), the mean values were further separated by Duncan's multiple range test for WG, ADG, SGR, FI, SR, FCR, chemical composition and carotenoid contents. Statistical analysis was performed using the R program.

3.2 Experiment 2: Disease resistance against shrimp virulence pathogen

The ability test of coelomic fluid of sand worms to inhibit the growth of *Vp* $_{AHPND}$ by using agar well diffusion assay.

3.2.1 Bacteria preparation

V. parahaemolyticus was isolated from moribund AHPND-infected Pacific white shrimp and cultured in nutrient agar (NA) with 2% NaCl at 30 ^{o}C for 24 hours and transferred to the culture to Mueller Hinton Broth (MHB) for 24 hours. After that, the tissue was removed from supernatant by centrifuge (HERMLE Model Z 300k) speed 2,500 rpm at a temperature 4 ^{o}C, for 10 minutes and washed with sterile 2% NaCl solution. After that, the cultures were adjusted to contain the amount of 0.5 McFarland (10^8 CFU/ml).

3.2.2 Mueller Hinton Agar preparation

Prepare Mueller Hinton Agar (MHA, Difco, USA) by pouring 20 ml into a petri dish. Adjust the suspension of *V. parahaemolyticus* turbidity equal to McFarland No. 0.5. The Mueller Hinton Agar (MHA) plate surface was inoculated by spreading with a sterile swab moistened with the Vp_{AHPND} suspension 10^8 CFU/ml, inoculum over the entire agar surface. Then, a hole with a diameter of 3 mm is punched aseptically with a sterile cork borer (diameter 3 mm).

3.2.3 Animal preparation

One hundred sandworms per each experiment were washed and disinfected with sterile sea water. Then water was absorbed with sterile blotting paper.

3.2.4 Experimental design

This experiment was conducted by utilizing (Completely randomized design; CRD) for 4 treatments and 3 replications followed by sand worms were fed with 4 different types of diet as follows: control (T1), commercial Pacific white shrimp diet, T2, commercial Pacific white shrimp diet supplementation with astaxanthin at 100 ppm (AS); T3, commercial Pacific white shrimp diet supplementation with live cell yeast at 4.95 x 10^3 cell/g (LY); and T4, commercial Pacific white shrimp diet supplementation with autolyzed yeast at 35% protein (AY).

3.2.5 Experiment procedures and data collecting

Sand worms in each treatment were washed with sterile water and absorbed with sterile blotting paper. After which, the sandworms were cut into small pieces to allow coelomic fluid to come out and then used a 5 ml syringe to suction the liquid and removed tissue from supernatant by centrifuge 2,500 rpm at 4 ^{o}C for 5 minutes.

Coelomic fluid was filtered through a 0.45 µm syringe filter to remove bacteria, and filled with 100 µl of coelomic fluid of treatment 1 (2% NaCl), 2, 3, and 4. Then, agar plates were incubated at 30 ^{o}C for 24 hours.

Then, the researcher measured the size of the clear annular using a vernier caliper. The researcher measured 4 vertical lines from the coelomic fluid colonies that created an inhibitor to the clear border where bacteria could grow. Then, the researchers found the mean units used in millimeters (Bendich, 2019; Balouiri, et al., 2016).

3.2 6 Statistical Analysis

For all analyses, the mean and standard deviation were calculated and reported. The data were subjected to an analysis of variance (ANOVA). Where there was a significant difference ($p \leq 0.05$), the mean values were further separated by Duncan's multiple range test. Statistical analysis was performed using the R program.

4. Results

4.1 Growth performance

The values of growth performance parameters and statistical analysis of sand worms fed with four different diets are listed in Table 2. At day 30 and day 60, the highest weight of the sand worms were also observed in AS treatment (0.204 ± 0.003 and 0.488 ± 0.031 g/sand worm, respectively), but they were not significantly different among four treatments ($p>0.05$), and ranged from 0.199 ± 0.004 to 0.204 ± 0.003 g/sand worm and 0.436 ± 0.035 to 0.488 ± 0.031 g/sand worm, respectively (Table 2). At day 90, the weight of the sand worm were significantly lower in the control group (0.568±0.081 g/sand worm) than in the other treatments ($p<0.05$). Whereas, the weight of the sand worms AS, LY and AY treatments were not significantly different ($p>0.05$), and ranged from 0.626 ± 0.075 to 0.694 ± 0.072 g/sand worm (Table 2).

Weight gain (WG) was significantly higher in the LY and AY treatments (133.577 ± 1.015 and 128.467 ± 1.877 %/g/sand worm) than in the other treatments ($p<0.05$), while the lowest WG (100.750 ± 8.255 %/g/sand worm) was observed in the control group. The average daily gain (ADG) was significantly higher in LY

($p<0.05$), while this parameter was not significantly different between the AS and AY treatments ($p> 0.05$).

Specific growth rate (SGR) was significantly lowest in the control group (5.134 ± 0.089 %/day) ($p<0.05$), whereas SGR of sand worms fed with LY diets was significantly higher (5.477 ± 0.008 %/day) ($p<0.05$) (Table 2). The highest feed intake (0.464 ± 0.018 g/sand worm) was observed in the control group and they were not significant among AS, LY and AY treatments (0.436 ± 0.003 , 0.428 ± 0.003 and 0.432 ± 0.007 g/ sand worm, respectively).

Survival rate (SR) of sand worms fed with AS, LY and AY (95.133 ± 0.752, 96.983 ± 0.690 and 95.583 ± 1.531%) were significantly higher than the control group (92.001 ± 1.994%) ($p<0.05$). In contrast, feed conversion rate (FCR) was significantly highest in the control group (0.827 ± 0.066) ($p<0.05$). While, sand worms fed with AS, LY and AY diets were not significantly different ($p>0.05$).

4.2 Chemical composition and total carotenoid

As shown in Table 3, protein and lipid contents of sand worms (57.72 ± 0.25% and 17.87 ± 0.13%) were significantly lower in the control group ($p<0.05$). The sand worms fed with LY and AY diets had protein contents (66.37 ± 0.30% and 66.34 ± 0.04%) significantly higher than the control group and AS treatment ($p<0.05$). Lipid contents were not significantly different among AS, LY and AY treatments ($p>0.05$) but higher than the control group ($p<0.05$). Fiber and ash contents were not significantly different among the four treatments ($p<0.05$), and ranged from 0.32 ± 0.10 to 0.42 ± 0.22% of fiber, and 9.36 ± 0.71 to 9.94 ± 0.17 of ash (moisture 0 %).

Total carotenoid content was significantly higher in the sand worms fed with AS diet (0.31±0.02 µg/g) ($p<0.05$). Whereas, total carotenoid contents of the other treatments were not significantly different ($p>0.05$), and ranged from 0.049 ± 0.001 to 0.114 ± 0.114 µg/g. This result indicated that feed supplemented with astaxanthin strongly affected the accumulation of carotenoids in the sand worms.

4.3 Water quality

For 90 day of sand worm cultures, the physico-chemical properties of water of all treatments ranged as follows: water temperature 27.17 ± 0.58 - 27.83 ± 0.30 ^{O}C, salinity 29.13 ± 1.73 – 30.06 ± 1.73 ppt, pH 7.14 ± 0.23 – 7.70 ± 0.60, alkalinity 110.65 ± 0.26 – 124.33 ± 20.03 mg/L, DO 6.61 ± 0.76 – 7.07 ± 0.32 mg/L and total ammonia 0.30 ± 0.23 – 0.55 ± 0.48 mg/L. Statistical analysis showed that all parameters were not significantly different among the treatments ($p>0.05$) (Table 4).

Table 2 Growth performance of sand worms fed with four different diets

Parameters/Treatments	T1(Control)	T2(AS)	T3(LY)	T4(AY)
Weight (g/worm)				
At day 30	0.201 ± 0.004^{ns}	0.204 ± 0.003^{ns}	0.199 ± 0.004^{ns}	0.203 ± 0.006^{ns}
At day 60	0.436 ± 0.035^{ns}	0.488 ± 0.031^{ns}	0.479 ± 0.027^{ns}	0.476 ± 0.032^{ns}
At day 90	0.568 ± 0.081^{a}	0.626 ± 0.075^{b}	0.694 ± 0.072^{b}	0.674 ± 0.091^{b}
Weight gain (%/g/sand worm)	100.750 ± 8.255^{a}	118.135 ± 1.540^{b}	133.577 ± 1.015^{c}	128.467 ± 1.877^{c}
Average daily gain (g/sand worm/day)	0.006 ± 0.000^{a}	0.007 ± 0.000^{b}	0.008 ± 0.000^{c}	0.007 ± 0.000^{b}
Feed intake (g/sand worm)	0.464 ± 0.018^{b}	0.436 ± 0.003^{a}	0.428 ± 0.003^{a}	0.432 ± 0.007^{a}
Specific growth rate (%/day)	5.134 ± 0.089^{a}	5.311 ± 0.014^{b}	5.477 ± 0.008^{c}	5.404 ± 0.016^{bc}
Feed convention ratio	0.827 ± 0.066^{b}	0.703 ± 0.009^{a}	0.621 ± 0.004^{a}	0.646 ± 0.010^{a}
Survival rate (%)	92.001 ± 1.994^{a}	95.133 ± 0.752^{b}	96.983 ± 0.690^{b}	95.583 ± 1.531^{b}

Values are the means ± SE of triplicate. Data in the same row followed by different superscripts are significantly different ($p<0.05$), ns = non-significant

Table 3 Chemical composition and total carotenoids of sand worms fed with four different diets. (moisture 0 %)

Parameters/Treatments	T1(Control)	T2(AS)	T3(LY)	T4(AY)
Protein (%)	57.72 ± 0.25[a]	62.05 ± 0.50[b]	66.37 ± 0.30[c]	66.34 ± 0.40[c]
Lipid (%)	17.87 ± 0.13[a]	19.22 ± 0.47[b]	20.37 ± 0.74[b]	20.08 ± 0.48[b]
Ash (%)	9.36 ± 0.71[ns]	9.58 ± 0.50[ns]	9.78 ± 0.39[ns]	9.94 ± 0.17[ns]
Fiber (%)	0.42± 0.22[ns]	0.32 ± 0.10[ns]	0.34 ± 0.06[ns]	0.41 ± 0.04[ns]
Total carotenoids (µg/g)	0.049 ± 0.001[a]	0.313 ± 0.019[b]	0.109 ± 0.019[a]	0.114 ± 0.114[a]

Values are the means ± SE of triplicate. Data in the same row followed by different superscripts are significantly different ($p<0.05$), ns = non-significant

Table 4 Water quality of sand worm culture by feeding four different diets

Parameters/Treatments	T1(Control)	T2(AS)	T3(LY)	T4(AY)
Water temperature (^{O}C)	27.83 ± 0.29	27.17 ± 0.58	27.83 ± 0.76	27.67 ± 0.29
Salinity (ppt)	29.68 ± 1.53	29.13 ± 1.73	30.06 ± 1.73	29.34 ± 1.53
pH	7.23 ± 0.31	7.70 ± 0.60	7.14 ± 0.23	7.31 ± 0.79
Alkalinity (mg/L)	117.37 ± 21.55	110.65 ± 10.26	124.33 ± 20.03	110.67 ± 17.90
DO (mg/L)	6.80 ± 0.78	7.07 ± 0.32	6.61 ± 0.76	6.71 ± 0.53
Total ammonia (mg/L)	0.34 ± 0.14	0.30 ± 0.23	0.53 ± 0.24	0.55 ± 0.48

Values are the means ± SE of triplicate. Data of all parameters were not significantly different among the treatments ($P>0.05$)

Table 5 The clear zone (annular zone) of coelomic fluid of sand worm to inhibit *V. parahaemolyticus* (EMS/AHPND) were fed by each treatment.

Treatment	annular zone (mm)
T1(Control)	0.167 ± 0.052[a]
T2(AS)	0.367 ± 0.052[b]
T3(LY)	0.517 ± 0.041[c]
T4(AY)	0.417 ± 0.041[b]

Values are the means ± SE of triplicate. Data in the same row followed by different superscripts are significantly different ($p<0.05$)

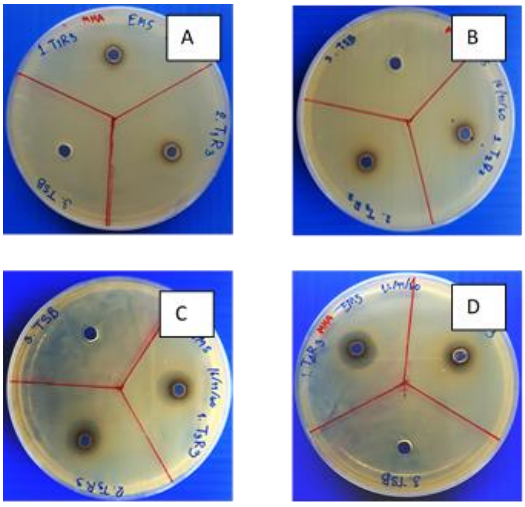

Figure 2 The result of the study showed the property of coelomic fluid from sand worms**.** The sand worms were fed with 4 different types of diet as follows: A; control (T1), commercial Pacific white shrimp diet (Con); B; T2, commercial Pacific white shrimp diet supplementation with astaxanthin at 1,000 ppm (AS); C; T3, commercial Pacific white shrimp diet supplementation with live cell yeast at 4.95 x 10^3 cell/g (LY); and D; T4, commercial Pacific white shrimp diet supplementation with autolyzed yeast at 35% protein (AY)

5. Discussion

In this study, sand worms (*P. nuntia*) were cultured and fed with four different diets for 90 days. During the first 60 days, the mean weight of sand worms were not significantly different among four treatments ($p>0.05$). These happened because in this period the sand worms were in larval stage with light initial weight of 0.052 ± 0.004 g. After 60 days, the sand worms developed into adult, as a difference of weight was observed.

At the end of experiment, it was observed that the weight of the sand worms in the control group (0.568 ± 0.081 g) was significantly lower than sand worms fed on feed supplementation with astaxanthin (AS, 0.626 ± 0.075 g), live cell yeast (LY, 0.694 ± 0.072 g) and autolyzed yeast (AY, 0.674 ± 0.091 g) ($p<0.05$), which corresponds to the study of the effect of astaxanthin increased growth performance of *Litopenaeus.vannamei* (Rajabi et al., 2012).

Weight gain (WG) of the sand worms fed on LY and AY diets (133.577 ± 1.015 and 128.467 ± 1.877 g/ sand worm) was significantly higher than the control groups and AS treatments, indicating that feed supplemented with yeast strongly stimulated growth of the sand worms. This result agreed with the study of Xiong et al. (2018) who reported that Pacific white shrimps fed on commercial diet supplementation with yeast had growth higher than those in the control group. Yeast contains a significant amount of protein, polysaccharide, nucleic acid and lipid. Beta glucan, mannan oligosaccharide and chitin are main compounds that are found in yeast cell wall. Mannan oligosaccharide has properties like prebiotics that is not digesting in the digestive system of sand worms and it also benefits for some intestinal bacteria. Moreover, digestive enzymes were released by yeast cells, result in the breakdown of polysaccharide, protein and lipid (Chanchaichaovivat, 2015). Thereby, efficiency of digestion and absorption in digestive system of the sand worms fed on yeast increased.

Astaxanthin is an antioxidant that prevents cell damage, stimulates immune response and inhibits stress in aquatic animals (Lim et al., 2017). In this study, sand worms fed on diet supplementation with astaxanthin had WG and ADG was significantly higher than the control group ($p<0.05$), indicating that feed added astaxanthin had effect on growth of the sand worms. Our result agreed with the findings of Chuchird (1995), who stated that black tiger shrimp (*Penaeus monodon*) at post-larva15 stage fed on astaxanthin supplement diet (0.625 g/kg) had growth higher than those fed on the control diet. Moreover, Paibulkichakul (2002) reported that black tiger shrimp fed on diet supplementation with astaxanthin at the concentration of 300 ppm had weight higher than shrimps in the control group, whereas survival rate was not different.

In this study, commercial shrimp feeds were used in all treatments. While LY, AY, and AS treatments were supplemented with live cell yeast, autolyzed yeast and astaxanthin (respectively). Thus, yeast cells affected protein and carotenoids contents in LY, AY, and AS treatments were higher than the control group. These resulted in the sand worms in the control group having a specific growth rate (SGR), feed intake (FI), feed convention ratio (FCR) and survival rate (SR) significantly lower than the other treatments ($p<0.05$), while none were significantly different when the parameters were observed between LY, AY, and AS treatments ($p>0.05$).Pacific white shrimp fed with yeast cells (Srimarksuk, et al., 2011) and astaxanthin had a significantly higher pigment growth rates than the control group (Salarzadeh & Rajabi, 2015).

Carotenoid contents in the control group, AS, LY and AY treatments were 0.07 ± 0.01, 0.55 ± 0.02, 0.09 ± 0.02 and 0.11 ± 0.01 µg/g, respectively. At 90 days of this experiment, carotenoid contents of sand worms in the control group, AS, LY, and AY treatments were 0.049 ± 0.001, 0.313 ± 0.019, 0.109 ± 0.019 and 0.114 ± 0.114 µg/g, respectively. It was indicated that carotenoid content in the diet strongly correlated with carotenoid content in the sand worms. The highest carotenoid content was observed in AS treatments (sand worms fed with astaxanthin supplement diet), followed by AY and LY treatments (sand worms fed with yeast supplement diet). Yeast is a significant source of carotenoids that are nutritionally essential for sand worms (Chanchaichaovivat, 2015).

During 90 days of the experiment, water quality of all treatments were not significantly different ($p>0.05$), which salinity, pH, alkalinity, DO and total ammonia are suitable for aquatic animals. Thus, water quality of this study was not affected in terms of growth and survival rate of the sand worms.

The result of study showed the ability of coelomic fluid in sand worms to resist *Vp* $_{AHPND}$ against shrimp virulence pathogen were fed by a diet in each treatment that found the annular zone of treatment (LY) were fed by instant shrimp mixed with live cell yeast diet be able to resist *Vp* $_{AHPND}$ because live cell yeasts were able to inhibit and improve the digestibility and stimulates immunity and produces antimicrobial peptides (AMPs) in inhibiting or killing bacteria (Techaprempreecha, 2007). The immune system of the antifouling sandbox is an innate immunity (Buyuksirit & Kuleasan, 2014). While the experimental group was using instant food, instant shrimp mixed with autolyzed yeasts and instant shrimp and astaxanthin diet were not significantly different to inhibit the growth of *Vp* $_{AHPND}$ intense species pathogenic *Vp* $_{AHPND}$. In the experimental group, the ready-mixed shrimp feed with prebiotic autolyzed yeasts helped to stimulate growth or promote microbial activity in the digestive system and stimulates the immune system. While instant shrimp feed with Astaxanthin is an antioxidant improving the immune, the control group's diet lowered the infection of *V.parahaemolyticus*.

6. Conclusion and recommendation

For 90 days of the experiment, the sand worms (*P. nuntia*) fed on diet supplementation with astaxanthin and yeast had growth, weight gain, average daily gain, specific growth rate and survival rate significantly higher than the control group, while feed intake and feed conversion ratio were significantly lower than the control group ($p<0.05$). Sand worm diets supplementation yeast also resulted in protein and lipid contents of sand worms were significantly higher than the control group ($p<0.05$). Therefore, feed supplementation with live cell yeast and autolyzed yeast affected more growth of sand worms because they are containing a high level of protein and lipid. It was observed in this study that shrimp feed supplementation with live cell yeast distinctly stimulated growth of the sand worms. Astaxanthin is an antioxidant that prevents cell damage, stimulates immune response and inhibits stress in aquatic animals that indirectly affected the growth of sand worms in AS (feed supplementation with astaxanthin) were higher than the control group. This research can be used to further study and should be investigated for the enhancement of the nutritional value of sand worm feed, which are used as feed to generate egg maturation and the health of broodstocks.

From the study of the property of coelomic fluid in the sand worm fed on diets for supplementation with astaxanthin and yeast to inhibit the growth of virulent strains of *Vp* $_{AHPND}$ showed to have a bigger annular radius of the zone of inhibition surrounding well more than the experimental group without dietary supplementation ($p<0.05$). The feed on diet supplementation with live cell yeast exhibited the highest activity against the virulent strain of *Vp* $_{AHPND}$ was statistically significant ($p<0.05$).

Therefore, sand worms (*P. nuntia*) fed on diet supplementations with astaxanthin and live cell yeast or autolyzed yeast improved the growth rate better than fed without supplementations. In terms of inhibiting the pathogenic bacteria, the live cell yeast should be best mixed with white shrimp, since live cell yeast is a living yeast that is a high protein source. Qualified as probiotics, it helps to increase digestion efficiency. There are nucleotide and B-Glucan which has immune stimulating properties.

Living yeast cells can produce potent enzymes. These enzymes are released in the intestines, which helps to improve digestion of the barnacle sand. As a result, the sand worms can absorb nutrients efficiently.

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