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Effect of Dietary Essential Oils on Growth, Feed Utilization and Meat Yields of White Leg Shrimp *L. vannamei*

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ABSTRACT : Effect of dietary essential oils on growth, feed utilization and meat yields of white leg shrimp *L. vannamei* was investigated. White shrimp fry weighing 0.62 g were kept in one of 12 tanks (75 head/500 L holding tank) in a closed recirculation system. Four experimental diets, a commercial diet (control), phytoncide oil (PO), oregano oil (OO) and fermented garlic liquid (GL) were fed for 16 weeks. The mean water quality values for the whole experimental period were 27.8±1°C, 7.6±0.3, 15.5±0.3 g/L and 6.1 ±0.3 mg/L for water temperature, pH, salinity and dissolved oxygen, respectively. At the end of the trial, 10 shrimp per tank were randomly sampled and meat yields (%) were evaluated after peeling the shell and removing the head. After a 16 week feeding trial, final weight of shrimp ranged from 21.9 g to 23.6 g. Feed conversion was not significantly different among groups ($p>0.05$), which was the lowest (1.95) in the control and highest (2.30) in the PO. Specific growth rate was also not significantly different ($p>0.05$) and ranged from 3.18% to 3.25%. Average daily gain of 0.2 g was obtained in all treatments. Mortality varied from 35.1% for control to 44.9% for OO. Meat yields maintained constant at 52.1% for control to 53.0% for PO. The study suggested that natural essential oils could not exert any improvement in growth performance, mortality and meat yields of white leg shrimp. (**Key Words :** White Shrimp (*L. vannamei*), Essential Oils, Growth, Mortality, Meat Yields)

INTRODUCTION

The pacific white shrimp, *Litopenaeus vannamei*, also known as white leg shrimp, inhabits waters that range in salinity from 1 to 40 ppt (Bray et al., 1994). *L. vannamei* is the most commonly cultured shrimp in the western hemisphere and has been grown in inland saline waters ranging in salinity from 28.3 ppt (Smith and Lawrence, 1990) to 0.5 ppt (Samocha et al., 2001). White leg shrimp was first introduced into Vietnam between 1997-2000. Since then the production of white leg shrimp has increased rapidly, mainly in the central and northern provinces of Vietnam, under the strict control by the Ministry of Fisheries. In January 2008, the culture of white leg shrimp in the Mekong Delta region was approved for intensive pond systems in safe aquaculture zone areas approved by the provincial authorities (Tien and Griffiths, 2009).

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With an increase in public concern regarding the use of antibiotics in animal production, the current trend is to seek alternatives with aromatic plants and the essential oils extracted from them claimed to be strong candidates having both anti-microbial activity and the stimulating effect on animal digestive systems. Essential oils from aromatic plants are presently used extensively in medicine and in the food and cosmetic industries (Lewis and Elvin-Lewis, 2003).

Essential oils basically consist of two classes of compounds, the terpenes and phenylpropenes (Lee et al., 2004). It was suggested that terpenoids and phenylpropanoids can penetrate the membrane of bacteria and reach the inner part of the cell because of their lipophilicity (Helander et al., 1998). However, the composition and concentration of main components, thymol and carvacrol, which were classified as isoprenoids and cinnamaldehyde as phenylpropanoids respectively, are variable, therefore the biological effects of essential oils may differ (Janssen et al., 1987; Deans and Waterman, 1993; Hammer et al., 1999). Ching (2008) reported that dietary Oregano oil improved not only growth and feed utilization but also survival rate of shrimp. Recently, Kim (2010) reported that dietary addition of 0.1% phytoncide oil from *Pinus koraiensis* improved feed conversion and fat digestibility in broiler chicks.

Garlic is probably one of the earliest known medicinal plants. Garlic has been proved to be hypolipidemic (Yaoling et al., 1998), antimicrobial (Kumar and Berwal, 1998), antioxidant (Sivam, 2001), antihypertensive (Suetsuna, 1998), hepatoprotective (Horie et al., 1989) and anticarcinogenic (Agarwal, 1996). These are mainly attributed to the bioactive components of garlic such as alliin, diallysulphides and allicin (Amagase et al., 2001). Recently, Metwally (2009) reported that garlic improved the growth performance and cell protection against the effects of oxidative substances in Nile tilapia. It was also suggested that dietary garlic extracts could improve glucose utilization through insulin secretion resulting in better growth and feed utilization of juvenile starlet sturgeon (Lee and Kim, 2010).

Even though microbial digestion seems to be less significant in shrimp than in fish and terrestrial animals, the application of the essential oils to shrimp production as an antibiotic replacer has been little studied. The present study, therefore, was carried out to investigate the effect of dietary essential oils on growth, feed utilization and meat yield of *L. vannamei*.

MATERIALS AND METHODS

Shrimp and the experimental conditions

Postlarvae 15 of white leg shrimp were purchased from a private hatchery. They were active, body shining, uniform in size and free of disease. Prior to stocking in the experimental tanks, postlarvae 15 were nursed for 15 days during which commercial fry feed was fed. Brine water with salinity of 90-120‰ was transported from a salt pan in Vinh Chau, Soc Trang. The water was diluted with tap water to make brackish water of 15 ppt for the feeding experiment. Sea water was disinfected with chlorine at 30 ppm, neutralized thiosulphate and aerated for 48 hours to eliminate chlorine residues. The recirculation system consisted of two plastic tanks of 250 L each for submerged biofilter, one plastic bucket of 70 L for trickling biofilter and 3 grey conical composite tanks (500 L/tank). Four sets of the system were employed to carry out the feeding trial. Sand, gravel and portioned PVC pipes were used as substrates for the nitrification and nitrifying bacteria in the recirculation system. All the materials were disinfected by chlorine at 200 ppm for 48 hours before installation. Alkalinity and pH were controlled by adding Dolmite at 50 g/m^3 weekly. The mean water quality values for whole experimental period were 27.8±1°C, 7.6±0.3, 15.5±0.3 g/L and 6.1±0.3 mg/L for water temperature, pH, salinity and dissolved oxygen, respectively. Tank water was exchanged at the rate of 200% a day. Postlarvae of white leg shrimp with initial weight and length of 0.62 g and 4.25 cm, respectively, were randomly distributed into twelve of 500 L tanks (75 PLs/tank) with the density of 150 PLs/m^3.

Experimental design and feeding management

Three natural oils, phytoncide oil (PO) from *Pinus koraiensis*, oregano oil (OO) from *Origanum heracleoticum L*. and garlic liquid (GL) produced by fermenting with Kimchi lactic acid bacteria (*Weissella koreensis*) for 72 hours, were supplemented to a commercial shrimp diet (control) at the level of 0.1%, 0.025% and 0.1%, respectively. The addition level was made following the previous studies for PO (Kim, 2010) and OO (Ching, 2008). The level of GL was recommended by the product company. Pellet size of the control diet, which was commercially produced through triple conditioner and post-conditioning system, was changed every 4 weeks. The aliquots of the oil extracts were mixed with 30 ml of distilled water and sprayed on the required amounts of the control diet every 4 weeks. Then, diet was mixed well by hand, dried in the sunlight and stored in cool place. Chemical composition of the control diets was shown in Table 1. Shrimp were fed 5 times a day for first 4 weeks at the level of 10% of body weight. Feeding frequency and feeding level were then reduced 4 times, 3 times and twice a day and 8%, 5% and 3% for second, third and fourth 4 weeks, respectively. Leftover feed was siphoned out of the tank before the first meal every morning. Feeding was made for 7 days a week. All feed fed was considered as consumed. Shrimp activities were carefully observed and mortality was daily recorded. Feed intake and weight gain were checked every 4 weeks. All shrimp in each tank were bulk-weighed at the end of every 4 weeks after they were starved for a day and counted to investigate the survival rate.

Table 1. Chemical composition of the control diets employed for every 4 weeks*

Diet	Moisture	C. protein	C. fat	C. ash	Ca	P
1st	11.25±0.12	40.35±0.31	6.45±0.03	10.50±0.11	1.82±0.03	1.12±0.04
2nd	11.26±0.03	39.60±0.23	6.63±0.02	10.95±0.14	1.69±0.02	1.09±0.02
3rd	11.43±0.16	39.14±0.09	6.58±0.03	10.83±0.20	1.71±0.04	1.12±0.02
4th	11.72±0.14	38.61±0.12	6.51±0.04	9.97±0.08	1.68±0.07	1.10±0.03

* Values are means±SD of 2 determinations.

Table 2. Growth performance of shrimp fed the experimental diets for 16 weeks*

Diet	Final wt g/shrimp	Wt. gain g/shrimp	Feed intake g/shrimp	FCR[1] feed/gain	Mortality %	SGR[2] %	DFR[3] %/Av.wt/d	ADG[4] g/shrimp	PER[5]
Control	23.55±0.77^{ns}	22.93±0.77^{ns}	44.67±2.79^{ns}	1.95±0.17^{ns}	35.11±1.26^{ns}	3.25±0.03^{ns}	3.31±0.28^{ns}	0.20±0.01^{ns}	1.20±0.10^{ns}
PO	21.93±1.12	21.31±1.12	48.54±5.81	2.30±0.38	42.22±1.66	3.18±0.05	3.88±0.63	0.19±0.01	1.08±0.18
OO	23.08±0.73	22.46±0.73	48.27±2.27	2.15±0.05	44.89±13.30	3.23±0.03	3.64±0.08	0.20±0.01	1.12±0.19
GL	22.50±0.17	21.88±0.17	47.30±5.60	2.16±0.26	38.22±12.99	3.21±0.01	3.65±0.44	0.20±0.00	1.16±0.06

* Values (means±SE of 3 replicates) in the same column sharing a common superscript are not significantly different (p>0.05); ns = Non-significant, PO = Phytoncide oil, OO = Oregano oil and GL= Garlic liquid.

[1] Feed conversion ratio = Feed fed, dm/weight gain of fish.

[2] Specific growth rate = (Ln final wt-Ln initial wt)/experimental days×100.

[3] Daily feeding rate = Feed intake×100/((initial wt+final wt)/2×experimental days).

[4] Average daily gain = Weight gain/feeding days. [5] Protein efficiency ratio = Weight gain/protein fed.

Analytical methods

The experimental diets were sampled before spraying the essential oils every 4 weeks for the analysis of moisture, crude protein, crude fat, crude ash, Ca and P. Ten shrimps per tank at the end of the feeding trial were sampled for meat yield determination. The meat was homogenized for the analysis of moisture, crude protein and crude ash. The chemical composition was determined following procedures (AOAC, 1984): crude protein (N×6.25) by the Kjeldahl method (Kjeltec 2100 Distillation Unit, Foss Tecator, Hoganas, Sweden), crude fat using an ether-extraction method, moisture by oven drying at 105°C for 24 h, ash using a muffle furnace at 550°C for 4 h, Ca by a wet ash method and titration with $KMnO_4$ and P by the vanado-molybdate method.

Calculation and statistical analysis

Calculations were made as follows: feed conversion ratio (FCR) = feed fed, DM/weight gain, specific growth rate (SGR, %) = (Ln final weight-Ln initial weight)/ experimental days×100, daily feeding rate (DFR, %) = feed intake/((initial weight+final weight)/2×days of the experiment)×100, average daily gain (ADG, g) = weight gains of shrimp/experimental days, protein efficiency ratio (PER) = weight gain/protein fed, meat yields (%) = fresh meat weight/fresh body weight×100.

Data were analyzed using one-way analysis of variance (ANOVA) and significant differences among treatment means were compared using Duncan's multiple range test (Duncan, 1955). Significance was tested at 5% level and all statistical analyses were carried out using the SPSS Version 10 (SPSS, Michigan Av. Chicago, IL, USA).

RESULTS

Chemical composition of the control diet (Table 1) showed that the protein level decreased from 40.35% to 38.61% while moisture content increased from 11.25% to 11.72%. After the 16 week feeding trial, shrimp weight reached 21.9 g to 23.6 g for PO and control groups, respectively (Table 2). Dry feed intake for 16 weeks ranged from 44.7 g for control to 48.5 g for PO groups. Lowest feed conversion ratio (FCR) was found in shrimp fed control diet, while highest one was found in shrimp fed diet PO. However, there were no significant difference (p>0.05) in weight gain, feed intake and FCR among treatments. Mortality varied from 35.1% for control group to 44.9% for OO group, which were not significantly different (p>0.05). Shrimp showed the specific growth rate (SGR) of 3.18% to 3.25% with average daily gain (ADG) of 0.2 g. Shrimp fed diet PC showed the highest daily feeding rate (DFR) of 3.9%, while the lowest DFR of 3.31% was found in control group (Table 2). Protein efficiency ratio (PER) ranged from 1.08 for PO group to 1.20 for control group. Meat yields of shrimp fed the experimental diets maintained constant at 52.1% to 53% for control and PO groups, respectively. There were no significant differences (p>0.05) in chemical composition of shrimp meat among treatments (Table 3).

Table 3. Meat yields (%) of shrimp and chemical composition of meat*

Diet	Body wt (g)	Shell (g)	Meat (g)	Yield (%)	Moisture	Protein	Ash
Control	24.57±1.51^{ns}	2.82±0.17^{ns}	12.78±0.28^{ns}	52.12±2.01^{ns}	75.41±0.80^{ns}	20.90±0.43^{ns}	1.41±0.05^{ns}
PO	21.37±1.17	2.43±0.19	11.33±0.77	52.98±0.72	74.72±0.53	21.24±0.33	1.46±0.09
OO	23.25±0.82	3.29±0.79	12.14±0.46	52.26±2.11	74.74±0.34	21.69±0.34	1.47±0.08
GL	22.54±1.40	2.30±0.29	11.85±0.73	52.58±1.07	74.51±0.58	21.55±0.29	1.43±0.10

* Values of meat yields (meat/body wt×100) and chemical composition of meat are means±SE of 3 replicate tanks with each 10 determinations and 2 determinations, respectively; ns = Non-significant (p>0.05).

DISCUSSION

Even though white leg shrimp could be adapted to a wide rage of salinity (Bray et al., 1994), survival rate at low salinity (3‰) was significantly lower than at 17‰ or 32‰ (Li et al., 2007). In the present study, the effect of dietary essential oils on growth, feed utilization and meat yields of the shrimp was investigated at 15‰. Shrimp fed control diet showed the highest weight gain (22.9 g), the lowest feed conversion (1.95) and mortality (35.1%) though there were not significant differences ($p>0.05$) among treatments. The present results revealed that dietary essential oils did not exert any beneficial or detrimental effect on growth performance and survival rate of white leg shrimp. As a medicinal plant, oregano has been used as a antimicrobial (Burt and Reinders, 2003), anticoccidial (Giannenas et al., 2003), antifungal (Pina-Vas et al., 2004) and antioxidant (Miura et al., 2002). Ching (2008) reported that an addition of oregano oil improved feed conversion by about 20% and survival rate by 6.5% compared to control group without any antimicrobials. It remains to be investigated whether this difference from the present study comes from diet composition, its preparation and/or experimental scale (pond or laboratory tank trials). Although dietary garlic improved growth and feed utilization of fish (Metwally, 2009; Lee and Kim, 2010), such an effect was not found in present study. Unlike Kim (2010)'s finding in broiler chicks, phytoncide oil addition also did not show any improvement on growth performance and survival rate.

In general, a feed additive acts as a growth booster for animal production. However, such an effect could be exerted only when the diet has a requirement for the additive. Shrimp cultured in extensive and semi-intensive production systems depend on natural pond biota as a direct nutritional source (Moss, 2002). Moss et al. (2006) found that the growth enhancing effect of pond water was more pronounced when shrimp were fed diets of inferior quality. In the present study where indoor tank water was recirculated, natural food organisms did not seem to be significant. Therefore, growth of shrimp would be mainly dependant on the food supplied. Shrimp of initial weight of 0.31 g were fed diets containing 36% crude protein and 8.5% crude fat with various levels of fish meal in ponds for 18 weeks and reached 18.4 to 20.7 g after 18 weeks (Amaya et al., 2007a). Even though shrimp in present study were reared in indoor tanks, good growth was achieved reaching final weight of 21.9 g (PO group) to 23.6 g (control) after 16 weeks, though the initial weight of shrimp in present study was comparatively high (0.62 g). SGR of 3.2% and ADG of 0.2 g also were obtained in all treatments (Table 2). This is in agreement with the result reported by Argue et al. (2002) who reported a weekly growth rate of 1.39 g in a 21 week trial. In the present study, SGR decreased from 5.70, 3.65, 2.14 to 1.50% during the 1st to 4th weeks, while ADG increased from 0.09, 0.19, 0.25 to 0.29 in the control group (data not shown). A similar trend was also found in the other treatment groups. The results suggest that a control diet containing crude proteins of 38.6% to 40.4% and crude fat of 6.5% well met the requirements for the shrimp as recommended by Akiyama (1992). Then, it seemed that any improving effect on growth and feed utilization could not be obtained by the addition of tested essential oils. However, FCR and mortality in the present study were higher than those found in Moss et al. (2006) and Amaya et al. (2007a,b).

FCR ranges from 1.1 to 1.4 (Amaya et al., 2007a,b) and from 1.3 to 1.4 (Moss et al., 2006) for *L. vannamei* in pond water rearing, while the value of 2.0 (control) to 2.3 (PO) was obtained in the present study. Since seven day feedings per week were carried out and all feeds fed were considered as ingested in the present study the higher FCR seemed to be due to this higher feeding rate. On the other hand, high mortality in all treatments (35.1% to 44.5%) was found to be due to cannibalism. However, it is unclear whether the cause was due to stocking density (150 PLs/m^3), dietary composition or water quality. Cannibalism among postlarvae and juveniles of *Penaeus monodon* was found to be positively density dependent, significantly suppressed by shelter and negatively correlated to food availability and feeding frequency (Abdussamad and Thampy, 1994). Moss et al. (2006) stocked 0.68 g of white leg shrimp at a density of 24 head/52 L aquaria and obtained 88.3 to 93.3% of survival rate using pond water during 10 weeks. Boyd (1998) stated that a temperature range of 25-30°C was optimum for the growth of shrimp. Too high or too low a temperature could have a negative effect on the molting of shrimp (Huong, 2010). According to Hai (2009), the optimal ranges of pH and DO in shrimp culture are from 7.5 to 8.5 and from 4.5 to 6 mg/L, respectively, while the concentration of total ammonia nitrogen (TAN) and nitrite should not be higher than 1 mg/L and 0.1 mg/L, respectively. Li et al. (2007) documented that white leg shrimp can survive for 96 hours when the concentration of TAN is 6.7 mg/L. Lin and Chen (2003) stated that the maximum concentration of nitrite for white leg shrimp (cultured at 15 ppt) is 6.1 mg/L. Ray et al. (2010) reported that white leg shrimp can develop well at the temperature of 26-28°C and the concentration of TAN and nitrite were 0.4 and 1.1 mg/L, respectively. Water quality parameters (temperature, pH, DO, TAN and nitrite) in this study are optimal for stocking of white leg shrimp.

The meat yield values obtained in the present study, ranging from 52.1% (control) to 53.0% (PO), were much lower than those (66 to 68%) reported by Briggs et al. (2004). This difference seems to be due to the presence of shell and the size of shrimp measured. Dietary addition of essential oils also showed no significant difference ($p>0.05$) in moisture, protein and ash contents of meat. In conclusion, dietary essential oils did not have any beneficial or detrimental effects on growth, feed utilization, survival rate, meat yields and chemical composition of the meat of white leg shrimp. However, it remains to be clarified whether any promising effect of essential oils could be obtained through a large scale field study. Furthermore, adding different levels of essential oils in shrimp diets could be researched.

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