Growth performance and digestive enzymes activities of Pacific white leg shrimp *(Litopenaus vannamei*) juveniles fed dietary mixtures of four medicinal plants

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Abstract

In present study the effect of dietary inclusion of equal amounts of four traditional medicines; *Matricaria chamomilla* L, *Zataria multiflora* L, *Mentha piperita* L and *Terminalia chebulo* L on growth performance and digestive enzymes activity in *Litopenaeus vannamei* was investigated. Two diets, including a control basal diet and an experimental diet with 30 g kg^{-1} herbal mixture supplementation were prepared. Juveniles (with average weight of 2.63±0.11 g) were fed control and supplemented diet for 60 days. At the end of the experiment, shrimp fed the experimental diet showed significant increase in SGR, WGR, compared with those of the control group. Shrimp fed with herbal mixture supplementation revealed significantly higher lipase, protease and amylase activities as compared with the control group in 60 days. The results indicated that using equal mixtures of four medicinal plants in *L. vannamei* diets can improve the growth parameters and digestive enzyme activities.

Keywords: Herbal, *Litopenaus vannamei*, Growth, Digestive enzyme activity

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Introduction

The Pacific white shrimp, *Litopenaeus vannamei*, is native to the eastern Pacific Ocean. It is commonly farmed in many countries (Campa- Cordova *et al*., 2002). Although aquaculture production of *L. vannamei* in Iran reached 22,000 metric tons a year, determining how to develop its resistance to various diseases including White Spot Disease, hematopoietic necrosis and vibrio infections has become very crucial to the continued and sustained growth of the shrimp culture industry (Sung *et al*., 2001; Hafezieh *et al*., 2017).

Although the use of hormones, antibiotics and drugs have positive effects on the growth and health of shrimp due to their residual effects and developing resistance in the muscle of shrimp, research for an alternative source of natural herbal origin is necessary (Jayaprakas and Sambhu, 1996).

Chamomile (*Matricoria chammilla*), *Zataria multiflora*, peppermint (*Mentha piperita*) and chebulic myrobalon (*Terminalia chebula*) belong to major groups of cultivated medicinal plants, which contain a large group of therapeutically interesting and active compound classes (Rani,1999; Sheikhzadeh *et al*., 2011; Singh *et al*., 2011; Talpur, 2014). Previous studies demonstrated that these plants are used as an anti-inflammatory, antiseptic, hypolipidemic and antinociceptive (Rani, 1999; Sheikhzadeh *et al*., 2011; Singh *et al*., 2011; Talpur, 2014). Several studies demonstrated that *Z. multiflora* and *M. piperita* are beneficial to improve growth and immune systems in aquatic animals (Shekhzadeh *et al*., 2011; Talpur, 2014). For example, the administration of peppermint can significantly increase weight gain, specific growth rates and resistance against *Vibrio harveyi* in Asian seabass (*Lates calcarifer*) (Talpur, 2014). Also, several commercial herbal products like trefoil containing ingredients such as *Tephrosia purpurea, Eclipta alba, Phyllanthus niruri, Andrographis paniculata, Ocimum sanctum* and *Terminalia chebula* improved the survival, molting efficiencies and growth in *Penaeus monodon* postlarvae (Rani, 1999). Also, biochemical changes and growth performance of black tiger shrimp larvae improved after using *Ricinus communis* extract (Sankar *et al*., 2011).

In this study, considering the beneficial effects of these four medicinal plants, equal amounts of these plant mixtures were used in Pacific white shrimp diets in order to investigate their effects on growth response and digestive enzyme activities in. *L. vannamei* juveniles

Materials and methods

Experimental diets and feeding conditions

Matricaria chamomilla L, *Zataria multiflora* L, *Mentha piperita* L and *Terminalia chebulo* L rhizomes were purchased from the local market in Shiraz, Iran. They were washed, shade-dried at room temperature and ground into a fine powder using a fine wire

mesh household sifter. Equal proportions of these powders were mixed together**.** Two experimental diets were prepared with the mixture of these powders at the inclusion level of 0 and 30 g kg^{-1}. For mixing well with the basal diet, 30% distilled water was added and further mixed. The wet dough was pelletized to a particle size of 9mm using a chopper machine. The experimental diets were freeze dried and stored at -20°C until use (Wu *et al.*, 2007). The feeding experiment was conducted at Fisheries Research Center, Chabahar, Iran. one hundred and eighty white leg shrimp with an initial mean weight of 2.63±0.11g were purchased from a Guatr farm, Chabahar, Iran and randomly distributed in 6 tanks (60 L) at a stocking density of 30 shrimp/tank (triplicates were used per treatment) and fed to satiation by hand four times (07:00 11:00, 15:00 and 19:00 hours) a day for 60 days. Feed intake was recorded on a daily basis. Temperature, dissolved oxygen concentration, ammonia nitrogen concentration, salinity and pH were measured to be about 24.8±1.5°C, 7.50±0.43 mg L^{-1}, 0.008±0.01 mg L^{-1}, 32.1±2.98 g L^{-1} and 8.2±0.2, respectively. The photoperiod was regulated to a 12:12 dark/light cycle. Feces and uneaten feed were removed daily. Biometry was done once a week.

Evaluation of growth performance

At the end of the feeding trial, each shrimp was individually weighted (±0.01). All parameters were corrected based on the ingested feed. Growth parameters, survival rate and nutritional efficiency indices were calculated as follows (Glencross *et al*., 2007; Khani *et al*., 2017)

Specific growth rate SGR; % day^{-1}= $[(\text{Ln } W_f - \text{Ln } W_I) /t] \times 100$

Survival rate=Final Individual Numbers/Initial Individual Numbers×100

Voluntary feed intake (VFI; % body weight day^{-1})=[(Feed $_{\text{consumed}}$ ($_{\text{dry matter (DM)}}$) /(Wmean×t)]

Feed conversion ratio (FCR)=(Feed consumed/W gain)

Protein efficiency ratio (PER)=(W gain/ Crude protein consumed)

In the above equations, WI, Wf, W mean and W gain, t and Feed consumed are the initial weight, final weight, mean weight, weight increment (g), time period (day) and consumed feed (g), respectively.

Digestive enzyme activities

The influence of the mixture of these medicinal plants on digestive enzyme (amylase, protease and lipase) activities in the digestive tract was measured by the method of Gamboa-Delgado *et al*. (2003). For preparation of enzyme extracts, shrimps were starved for 24 h immediately after 60 days and killed with a pair of sterile scissors. The digestive tracts were carefully removed, thoroughly washed with sterile distilled water, weighed and homogenized with cooled buffer phosphate (0.65%). The supernatant, extracted by centrifugation (3000 rpm for 20 min at 4°C), was used for enzyme assays. Unit amylase activity was calculated as the weight

(mg) of maltose liberated for duration of 10 min at 30°C. Unit protease activity was expressed as the amount of tyrosine liberated in 15 min under the assay conditions. Unit lipase activity was expressed as the amount of 0.025 N NaOH required to neutralize the fatty acids liberated during 18 h of incubation at pH 6.9 and temperature 30°C. Digestive enzymes were calculated as enzyme unit per gram tissue.

Statistical analysis

All data were analyzed by SPSS 16.0 for windows. Independent samples T-Test was used to compare the differences between control group and experimental shrimp (p<0.05). Normality was tested using the Kolmogorov–Smirnov test. Leven's test was carried out to verify the homogeneity of variance. Non homogenous data were arcsine transformed before further statistical analysis. Data were presented as (Mean±SD) of three replications.

Results

Growth performance

After 60 days of feeding, the final weight, specific growth rate (SGR) and voluntary feed intake of shrimp increased significantly in the experimental group (with 30 g kg^{-1} equal proportions of four medicinal plant supplemented diets) compared to those of the control group (p<0.05) (Table 1). Feed conversion ratio (FCR) was significantly lower in the experimental group compared with the control group (p<0.05). Using 30 g kg^{-1} dietary equal mixtures of four medicinal plants had significant effect on protein efficiency ratio (PER) and lipid efficiency ratio (LER) of shrimp compared with those fed the basal diet (Table 1).

Digestive enzyme activities

After 60 days of feeding, amylase, protease and lipase activities were significantly higher in the experimental group compared with the control group (p<0.05) (Table 2).

Table 1: The (Mean ± SD) of growth parameters of *Litopenaus vannamei* in the control and experimental group after 60 days (n=3).

Parameters	Groups	
	Control	Experiment
Initial weight (g)	2.65± 0.11[a]	2.64± 0.11[a]
Final weight (g)	11.27± 0.29[a]	14.61± 0.38[b]
Specific growth rate (% $day^{-1)}$	2.41±0.09[a]	2.85±0.09b
Voluntary feed intake (% BW day^{-1})	1.65± 0.07[a]	2.10± 0.10b
Feed conversion ratio	1.84±0.13[b]	1.32± 0.09[a]
Protein efficiency ratio	1.69± 0.12[a]	2.36± 0.16[b]
Lipid efficiency ratio	5.43±0.40[a]	7.58±0.54[b]

Different superscripts within a row indicate significant differences at p<0.05.

Table 2: Digestive enzyme activities in *Litopenaus vannamei* the control and experimental groups after 60 days (6 shrimp per treatment; n=3).

Enzyme (Unit g^{-1} tissue)	Groups	
	Control	**Experiment**
Amylase	221.90± 2.12[a]	250.63±2.54[b]
Protease	291.97± 5.32[a]	315±5[b]
Lipase	4.13±0.37[a]	5.13±0.45[b]

Different superscripts within a row indicate significant differences at $p<0.05$.

Discussion

The results of the present study indicated that using equal proportions of *M. chamomilla, Z. multiflora, M. piperita* and *T. chebulo* plays a positive role on the final weight, FCR, PER, LER, SGR and VFI in shrimp. Similar positive observations were reported by Jayaprakas and Euphrasia (1997) with improved digestion and growth in major carp *Cirrhinnus mrigala* fed with Livol (IHF-1000) which is a herbal growth promoter containing different plant products such as *Boerhavia diffusa, Solanum nigrum* and *Terminalia arjuna*, Also our results are comparable with those reported by Citarasu *et al*. (1998). They found that SGR (7.3 to 9.1%) and consumption rate (165 .7 to 171.9 mg/ g day) increased significantly in the post larvae of *P. indicus* fed with larval herbal diet, stressol II and stressol I enriched *Artemia* compared with those of the unenriched *Artemia* fed post larvae. In another study, post larvae of *P.monodon* fed herbal medicinal diet enriched *Artemia* showed increased SGR and consumption rate. The growth promoter characteristic herbals induced the transcription and lead to high protein synthesis (Citarasu *et al*., 2002).

Carbohydrate and protein as macronutrient can influence the digestive enzyme activities in shrimp (Gamboa-Delgado *et al.,* 2003). The presence of medicinal plants and their components even at low concentration in the gut can improve the activity of digestive enzymes and physiological status of farmed aquatic animals (Brito *et al*., 2004). In the present study, amylase, protease and lipase activities were significantly higher in the experimental group compared with those of the control group. This resulted in increased growth and better feed conversion ratio compared with the control group. Similarly, elevated levels of digestive enzymes resulted in better growth performance of black tiger shrimp fed *Ricinus communis* extract supplemented diets compared to the control group (Sanker *et al.,* 2011). Similar results were found on rohu (*L.rohita*) fed livol- incorporated diets which stimulated digestive enzyme activity and led to increased consumption. The use of different spices such as *Zingiber officinale, T.arjuna* and fenugreek have been demonstrated to increase pancreatic-secretions, protease, amylase and lipase activities in rat (Platel *et al*., 2002). The results suggest that the presence of the cell wall components and other unknown growth promoters stimulate

the production of endogenous digestive enzymes in the shrimp (Moss and Moss, 2004).

In conclusion, adding equal amounts of four medicinal plants *to L. vannamei* diets enhanced VFI, SGR, PER, LER, final weight and improved amylase, lipase and protease activities. Thereby our results revealed that using equal mixtures of these medicinal plants in shrimp diet can have potential effects on growth, feed intake and reduce feed costs.

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