ผลของสารสกัดจากฟ้าทะลายโจร (*Andrographis paniculata* Wall. Ex Nees) ต่อการเจริญเติบโตและความต้านทานโรคแบคทีเรียในกุ้งขาวแวนนาไม (*Litopenaeus vannamei* Boone)

Effects of Creat (*Andrographis paniculata* Wall. Ex Nees) Extract on Growth Performance and Bacterial Disease Resistance in Pacific White Shrimp (*Litopenaeus vannamei* Boone)

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บทคัดย่อ

เนื่องด้วยปัญหาด้านโรคที่เกิดจากแบคทีเรียในการทำฟาร์มเลี้ยงกุ้ง ประกอบกับมีการควบคุมการใช้ยาปฏิชีวนะที่อาจก่อให้เกิดผลกระทบทางด้านลบตามมา การใช้พืชสมุนไพรจึงเป็นอีกทางเลือกหนึ่งที่สามารถนำมาป้องกันการเกิดโรคในการเพาะเลี้ยงกุ้งได้ ในปัจจุบันมีการนำฟ้าทะลายโจร (*Andrographis paniculata* Wall. Ex Nees) ซึ่งเป็นพืชสมุนไพรชนิดหนึ่งมาใช้ในการเพาะเลี้ยงสัตว์น้ำ โดยใช้เป็นสารเสริมการเจริญเติบโตเสริมภูมิคุ้มกัน ต้านจุลชีพ และด้านอื่นๆ อีกมากมายเพื่อที่จะทดสอบประสิทธิภาพของสารสกัดจากสมุนไพรชนิดนี้ที่มีต่อการส่งเสริมการเจริญเติบโต และการเพิ่มความต้านทานโรคติดเชื้อแบคทีเรียในกุ้งขาวแวนนาไม (*Litopenaeus vannamei* Boone) จึงได้ทำการเลี้ยงกุ้งและทดสอบความต้านทานโรคติดเชื้อแบคทีเรีย *Vibrio harveyi* VHAQ001 โดยทำการเลี้ยงกุ้งน้ำหนักเฉลี่ย 3.58 กรัม เป็นระยะเวลา 56 วัน ด้วยอาหารกุ้งที่มีสารสกัดจากฟ้าทะลายโจรในระดับความเข้มแตกต่างกัน คือ 0, 10, 20, 30, 40, 50 และ 60 ppm จากนั้นทำการทดสอบความต้านทานโรคที่เกิดจากเชื้อ *Vibrio harveyi* VHAQ001 ด้วยวิธีการฉีดเชื้อที่ระดับความเข้มข้นซึ่งทำให้กุ้งตาย 50% เป็นระยะเวลา 7 วัน เมื่อสิ้นสุดการทดลอง พบว่าปัจจัยต่างๆ ด้านการเจริญเติบโตที่ตรวจวัดไม่มีความแตกต่างทางสถิติที่ระดับความเชื่อมั่น 95% ระหว่างชุดควบคุมและชุดการทดลอง อย่างไรก็ตามหลังจากการทดสอบความต้านทานโรคพบว่าชุดทดลองที่เลี้ยงกุ้งด้วยอาหารซึ่งผสมสารสกัดจากฟ้าทะลายโจรที่ระดับความเข้มข้น 60 ppm มีค่าอัตราการรอดตายสูงที่สุดอย่างมีนัยสำคัญทางสถิติ

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(P< 0.05) จากผลการศึกษาครั้งนี้ อาจสรุปได้ว่าสารสกัดจากฟ้าทะลายโจรไม่มีผลต่อการเสริมการเจริญเติบโต แต่จะมีผลดีในการเสริมความต้านทานโรคที่เกิดจากเชื้อแบคทีเรียในกุ้งขาวแวนนาไม

คำสำคัญ: ฟ้าทะลายโจร, กุ้งขาวแวนนาไม, การเจริญเติบโต, ความต้านทานโรค

ABSTRACT

Since bacterial disease-related problems in shrimp farming and prohibition of antibiotics leading to negative effects has occurred, the use of medical plant may be effective alternative approach for disease management in shrimp aquaculture. Creat (*Andrographis paniculata* Wall. Ex Nees), one of traditional medical plant presenting various biomedical effects, is currently applied in aquaculture operations as growth promoter, immunostimulator, antibacterial, and others. In order to study the efficacy of this plant on growth performance and bacterial disease resistance in Pacific white shrimp (*Litopenaeus vannamei* Boone), feeding trial and bacterial challenge test with *Vibrio harveyi* VHAQ001 were carried out in this trial. Initially, experimental shrimp (average weight of 3.58 g) were fed for 56 days with diets containing Creat extract at graded levels of 0, 10, 20, 30, 40, 50, and 60 ppm, and then challenged with LD_{50} of *Vibrio harveyi* VHAQ001 through injection method for 7 days. At the end of the trial, the obtained results indicated that there were no significant ($P \geq 0.05$) differences between control and treatment groups in growth parameters investigated; however, treatment group using dietary supplemented Creat extract at the concentration level of 60 ppm had shown the significant ($P<0.05$) highest survival rate after challenge test. According to these results, it can be concluded that Creat extract has no effect on growth promotion, but it can increase bacterial disease resistance in Pacific white shrimp.

Key words: *Andrographis paniculata*, *Litopenaeus vannamei*, growth, disease resistance

INTRODUCTION

Marine shrimp aquaculture industry is rapidly expanding, and produces the largest single commodity in value terms, accounting for 15% of the total value of internationally traded seafood products in 2012 (FAO, 2014). Pacific white shrimp (*Litopenaeus vannamai* Boone) is one of the economically important Penaeid shrimp, currently cultured around coastal area in several countries, especially in South America and Southeast Asia (Alcivar-Warren *et al.*, 2007; Hsu and Chen, 2007). With intensification of

shrimp aquaculture industry, occurrence of disease outbreak has increased, and brings about major constraint of shrimp aquaculture, resulting in significant socio-economic losses in affected areas (Kumar *et al.*, 2014).

Disease outbreak is induced from aquatic environment deterioration, stress condition and multiplication of various pathogens such as parasite, virus and bacteria (Lavilla-Pitogo *et al.*, 1998; Johnson *et al.*, 2010). In 2012 and particularly in 2013, early mortality syndrome, one of bacterial disease-related problems, has found in some countries of Asia and Latin America, leading to decrease of world farmed shrimp production volumes (FAO, 2014). The most common approach for treating bacterial diseases and promoting growth is the application of antibiotics. This leads to rejection of consignment during export due to accumulation of antibiotics both in the environment and in the shrimp flesh is overregulated level (Alderman and Hasting, 1998). Actually, the primary approach of shrimp disease management should be based on prevention through promoting health status without negative consequences. For this reason, the use of herbal plants may be alternative way for reducing disease problems in shrimp farming because the biological substances in these plants can function as growth promotion, immunostimulation, antistress, antibacterial, antifungal, antiviral, appetite stimulation and aphrodisiac (Citarasu, 2010).

Creat (*Andrographis paniculata* Wall. Ex Nees), one of alternative medical plant, belongs to family Acanthacea. This medical plant is an annual herbaceous plant, extensively cultivated in Southern Asia, China and some parts of Europe (Joselin and Jeeva, 2014). Creat is a traditional herb widely used for body heat elimination, dispelling toxins from the body, common cold prevention, protection of respiratory tract infections including sinusitis and fever (Gabrielian *et al.*, 2002) moreover, it shows various mode of biological activities such as anti-inflammatory (Chao *et al.*, 2010), antioxidant (Koul and Kapil, 1994), antibacterial (Abubacker and Vasantha, 2010; Roy *et al.*, 2010), antiviral (Wiart *et al.*, 2005), Immunomodulation (Wang *et al.*, 2010) and anticancer (Iruretagoyena *et al.*, 2005; Li *et al.*, 2007). This is because it contains effective phytochemicals in cluding diterpenes-diterpenoids of the ent-labdane type, diterpene glucosides and diterpene dimers (Matsuda *et al.*, 1994), lactones-deoxyandrographolide, andrographolide, neoandrographolide and 14-deoxy-11, 12 didehydroandrographolide (Chang and But, 1987) and flavonoids -5,7,2′,3′-tetramethoxyflavanone and 5 - hydroxyl -7,2′,3′-trimethoxyflavone (Koteswara *et al.*, 2004). In present aquaculture operations, this medical plant has been applied for growth promotion (Basha *et al.*, 2013), immunostimulation (Vanichkul, 2010) and prevention of viral and bacterial diseases in aquatic animals (Direkbusarakom, 1998). Hence, the aim of this investigation was to evaluate the efficacy of Creat (*A. paniculata*) extract on growth performance and bacterial disease resistance of Pacific white shrimp

(*L. vannamei*) in order to acquire new effective approach for improvement of shrimp aquaculture in Thailand.

MATERIALS AND MATHODS

1. Experimental animal

Healthy Pacific white shrimp (*L. vannamei*) in post larvae 12 stage were obtained from a commercial farm in Thailand, acclimated in 8,000-L concrete ponds with suitable condition of ambient environment, and fed with commercial control diet 4 meals daily for 2 months in order to increase shrimp size around 3-4 gused in this trial. When had been grown to anus able size, shrimp were graded and transferred to concrete ponds containing 1,000-L of sea water (30 ppt) for setting the experiment.

2. Plant materials and extraction

Creat (*A. paniculata*) derived from grown source in Tha Sala district, Nakhon Si Thammarat province, Thailand. For crude extract preparation, whole plants were thoroughly washed with distilled water to remove dirt and contaminants, and then oven-dried at 45 ^{o}C for 5 days. The dried plant was chopped into small pieces, and finely ground with the mortar, afterward macerated in 95 % ethyl alcohol for 5 days. The derived crude extract was filtered and concentrated at 40 ^{o}C by using a rotary evaporator under low pressure, then stored at 4 ^{o}C until used (Herunsalee and Direkbusarakom, 1993 modified method)

3. Inoculum preparation for challenge test

The inoculum of *Vibrio harveyi* VHAQ001 causing Vibriosis, provided by Kasetsart University, was used as representative of shrimp pathogenic bacteria for this trial, and was prepared following the published method of Sritunyalucksana *et al.* (2005). This bacterial strain was cultured in Brain Heart Infusion (BHI) agar supplemented with 1.5% NaCl, incubated at 35 ^{o}C for 18-24 hr, collected, and washed in 1.5 % NaCl solution by centrifuging at 4,500 rpm for 15 min at 4 ^{o}C. In final process, the bacterial suspension was adjusted as Median lethal dose (LD_{50}) estimated though the modified method of Reed and Muench (1938).

4. Experimental diets preparation

The commercial formulated feed purchased from Charoen Pokphand Co., Ltd. (Thailand) was used to prepare experimental diets in this trial. This diet was thoroughly mixed with Creat extract in different levels by top-coating, then air-dried and re-coated with fish oil at 1.5 % of shrimp feed so as to protect leaking of effective substance from pellets and to enhance shrimp palatability. The diets were air-dried again, and stored in the fridge at 4 ^{o}C until used. All experimental diets were prepared twice weekly.

5. Experimental design for feeding trial

The experiment was conducted as complete randomized design (CRD) in which every treatment was randomly assigned to

different concrete ponds containing 1,000-L of sea water. There were 7 treatments with triplicate in this trial, divided through various levels of Creat extract supplemented to commercial diet at the concentration levels of 0 (control), 10, 20, 30, 40, 50 and 60 ppm. In each replicate, 70 shrimp with equal size (average weight of 3.58 g) were stocked in the concrete pond and fed with experimental diets 4 meals daily for 56 days. Initially, daily feeding rate was 6% of total body weight, after that it was re-adjusted daily, considered from feed intake of shrimp in each pond. In case of trial system management, uneaten feed as well as waste matter were removed daily; the cultured water was monitored and adjusted to maintain an appropriate condition through installation of aeration and water circulation system. In the end, all data from feeding trial were recorded for assessment of growth performance, feed utilization and survival rate of experimental shrimp; moreover, the shrimp from each treatment were randomly collected for further bacterial challenge test.

6. Parameters assessment from feeding trial

After 56-days feeding period, there were three parts of data collection for evaluating the efficacy of Creat extract on shrimp growth performance, feed utilization and survival rate. Firstly, individual weight of shrimp in each treatment were recorded at the beginning and the end of feeding trial in order to determine growth parameters including average body weight (g), percent weight gain (%), specific growth rate (%/day), and average daily growth (g/day). Secondly, amount of diet used in each treatment were daily recorded for calculation of feed conversion ratio and feed efficiency ratio. Finally, the survival rate of shrimp was estimated through enumeration of remaining shrimp in the concrete pond. In brief, all parameters had been calculated using the equations as follows:

Average body weight (g) = final biomass / final shrimp number

Specific growth rate (%/day) = [natural log (final mean biomass) - natural log (initial mean biomass)] / duration of feeding (days) x 100

Percent weight gain (%) = [(final mean biomass - initial mean biomass) / initial mean biomass] x 100

Average daily growth (g/day) = (final mean biomass - initial mean biomass) / duration of feeding (days)

Feed conversion ratio = total feed intake (g) / shrimp weight gain (g)

Feed efficiency ratio = shrimp weight gain (g) / total feed intake (g)

Survival rate (%) = (initial shrimp number / final shrimp number) x 100

7. Bacterial challenge test

After 56-days feeding trial, the shrimp from each treatment were collected for bacterial challenge test by injection (Supamattaya *et al.*, 2000 modified method), observed for 7 days, in order to evaluate the bacterial disease resistance

of the shrimp received different concentrations of Creat extract. In this part, the challenge test had been conducted as two groups. In group 1, ten shrimp from each treatment were transferred to 30-L aquaria, and then injected with LD_{50} of *V. harveyi* inoculum (3.63 x 10^6 CFU/ml), conducted in triplicate. In group 2, ten shrimp from each treatment were also transferred to 30-L aquaria with triplicate, and injected with 1.5% NaCl solution for setting as control group. During challenge test, the shrimp were still fed with each experimental diet at 4 meals daily similar to feeding trial, and dead shrimp were also removed from the aquaria daily. To ensure cause of mortality from bacterial infection, the dead shrimp were diagnosed by microbiological technique. After 7 days post-injection, results of survival rate (%) and relative percent survival, RPS (%), were statistically compared. The relative percent survival (RPS) was used for comparing additional level of effect on disease resistance among control and treatment groups, which could be calculated following the formula:

RPS (%) = [1- (percent of treatment mortality / percent of control mortality)] x 100 (Amend, 1981)

8. Statistical analysis

All data obtained from this experiment were statistically analyzed using One-way ANOVA in statistical program (IBM SPSS Statistics 21), and Duncan's multiple range test (DMRT) was used to determine the significant differences between the means. The comparisons were done at 5% probability level (Duncan, 1995).

RESULTS

1. Growth performance

As the result shown in Table 1, growth parameters including average body weight (g), percent weight gain (%), specific growth rate (%/day) and average daily growth (g/day) had been analyzed in order to assess growth performance of the shrimp fed with various experimental diets containing graded levels of Creat extract. In this trial, there were no significant ($P \geq 0.05$) differences among control and treatment groups in every parameter estimated (Table 1).

2. Feed utilization

The feed conversion ratio and feed efficiency ratio, the parameters for estimation of feed utilization of the shrimp fed with various experimental diets containing graded levels of Creat extract, had been evaluated at the end of feeding trial. The obtained results specified in Table 2 indicated that feed conversion ratio and feed efficiency ratio had been presented similar trend; however, there were no significant ($P \geq 0.05$) differences among control and treatment groups in both parameters estimated.

3. Survival rate

In term of survival rate, the shrimp fed

with diet containing 60 ppm of Creat extract showed survival rate higher than shrimp fed with control and other experimental diets in this trial; nevertheless, the significant ($P \geq 0.05$) differences among control and treatment groups did not present in this parameter (Table 2).

4. Bacterial disease resistance

After having been challenged with LD_{50} of *V. harveyi* by injection route, tested shrimp from each experimental group showed the disease tolerance presenting as survival rate (%) and relative percent survival (%) at 7 days post-injection. Survival rate (%) of shrimp fed with control diet and diets supplemented with Creat extract at 10, 20, 30, 40, 50 and 60 ppm were 40.00±0.00, 46.67±5.77, 50.00±0.00, 56.67±11.55, 56.67±11.55, 60.00±17.32 and

Table 1 Effect of Creat extract-supplemented diets on growth performance of Pacific white shrimp after 56-days feeding period (Mean ± SD)

Concentrations	Average body weight (g)		Percent weight gain (%)	Specific growth rate (%/day)	Average daily growth (g/day)
	Initial	Final			
0 ppm	3.58±0.61	26.21+3.76	632.49±22.49	3.56±0.05	0.40±0.02
10 ppm	3.58±0.61	26.21+3.46	633.37±12.39	3.56±0.03	0.40±0.01
20 ppm	3.58±0.60	27.06+4.07	656.37±23.81	3.61±0.06	0.42±0.02
30 ppm	3.58±0.62	27.12+3.76	659.06±42.95	3.62±0.10	0.42±0.03
40 ppm	3.58±0.63	27.28+4.58	635.07±9.49	3.56±0.02	0.41±0.01
50 ppm	3.58±0.61	27.31+4.52	663.25±13.43	3.63±0.03	0.42±0.01
60 ppm	3.58±0.63	26.31+3.89	635.51±24.64	3.56±0.06	0.41±0.02

Table 2 Effect of Creat extract-supplemented diets on feed utilization and survival rate of Pacific white shrimp after 56-days feeding period (Mean ± SD)

Concentrations	Feed conversion ratio	Feed efficiency ratio	Survival rate (%)
0 ppm	1.15±0.06	0.87±0.05	92.38±2.18
10 ppm	1.09±0.07	0.92±0.06	94.76±5.41
20 ppm	1.14±0.20	0.89±0.14	95.71±0.00
30 ppm	1.01±0.04	1.00±0.04	94.76±5.77
40 ppm	1.05±0.06	0.95±0.06	95.71±4.95
50 ppm	0.99±0.07	1.01±0.07	96.19±5.41
60 ppm	1.01±0.03	0.99±0.03	99.05±1.65

66.67±15.28, respectively. Relative percent survival (%) of aforementioned treatments were 0.00±0.00, 11.11±9.62, 16.67±0.00, 27.78±19.25, 27.78±19.25, 33.33±28.87 and 44.44±25.46, respectively. The derived results indicated that the treatment group conducted by using dietary supplemented Creat extract at the concentration level of 60 ppm presented the highest values of survival rate, and significantly ($P<0.05$) differed when compared with control group; however, the relative percent survival (RPS) did not exhibited the significant ($P\geq0.05$) difference among treatment groups. In addition, trend of these parameters detected was found to be increased in accordance with increasing of Creat extract levels mixed in experimental diets (Figure 1 and 2).

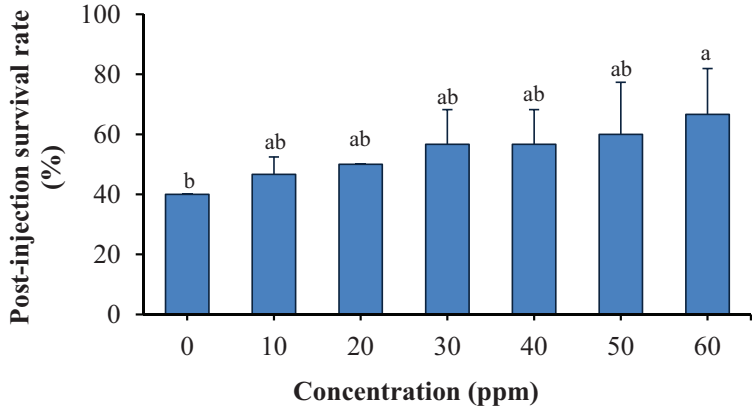

Figure 1 Survival rate (%) at 7 days post-injection of the Pacific white shrimp fed with diets containing different levels of Creat extract after challenging with LD_{50} of *Vibrio harveyi* VHAQ001 (3.63 x 10^6 CFU/ml) through injection method. Different letters presented significant difference at $P<0.05$

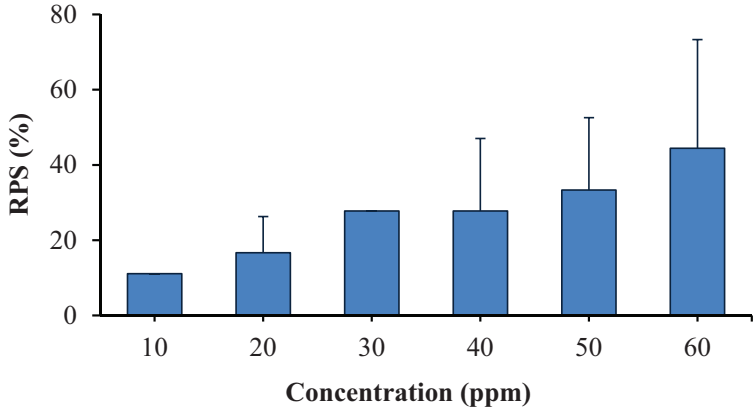

Figure 2 Relative percent survival, RPS (%) at 7 days post-injection of the Pacific white Shrimp fed with diets containing different levels of Creat extract after challenging with LD_{50} of *Vibrio harveyi* VHAQ001 (3.63 x 10^6 CFU/ml) through injection method

DISCUSSION

Herbal plants containing biomedical compounds have been widely applied in veterinary and human medicines. They are natural products that can be used as safe for mankind, and used in aquaculture operations for various remedies including growth promotion, appetite enhancement, immunostimulation, antistress, antibacterial, antifungal, antiviral and aphrodisiac (Citarasu, 2010). Previously, there were several studies revealing the positive effects of herb application in aquatic animals, for instance, Direkbusarakom *et al.* (1995) reported that *Phyllanthus amarus* and *P. urinaria* showed the positive effect against yellow head virus infection in black tiger shrimp (*Penaeus monodon*). Next, Chatchawanchaipan *et al.* (2004) reported that numbers of black tiger shrimp (*P. monodon*) infected with gregarines were 100 % reduced after fed with fresh garlic paste at the level of 10g/kg of feed for 4 weeks. Besides, 100 and 200 mg/kg supplementation of *Withania somnifera* and *Ocimum sanctum* could improve growth, non-specific immune response, and resistance against *V. harveyi* in juvenile greasy grouper (*Epinephelus tauvina*) (Sivarama *et al.*, 2004). By the same token, many kinds of herb can be utilized instead of antibiotics and other chemicals causing residues and side effects in aquatic animals (Citarasu, 2010).

In this study, the efficacy of Creat (*A. paniculata*) extract on growth and bacterial disease resistance had been evaluated in Pacific white shrimp (*L. vannamei*). The obtained results indicated that dietary supplemented Creat extract at the concentration level of 60 ppm can increase resistance against *Vibrio harveyi* in experimental shrimp. This result was consistent with the investigation of Vanichkul (2010), who reported that dietary supplemented Creat extract at the concentration level of 62.50 ppm could reduce the mortality of Pacific white shrimp (*L. vannamei*) after *V. harveyi* infection. In addition, Citarasu *et al.* (2003) had reported that methanolic extracts of *A. paniculata* bioencapsulated in *Artemia* before feeding to *P. monodon* post larvae, performed well in control of pathogenic bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Vibrio* spp., inoculated in culture media of shrimp larvae. As has been noted, the elevation of resistance against *V. harveyi* infection may be due to the phytochemical entity of Creat, angrographolide in particular, activating non-specific cell receptors of vital immune cells (Basha *et al.*, 2013). Moreover, some bioactive substances of this plant may be related to antibacterial activity, supported by the research of Direkbusarakom *et al.* (1995) specified that 10 ppm of *A. paniculata* showed positive effect on inhibition of fish and shrimp pathogenic bacteria.

Nevertheless, Creat did not present effective mode of shrimp growth promotion in this trial because growth parameters of each treatment had shown similar trend. On the other hand, some researches had revealed that this plant could increase growth performance in fish;

for example, Prasad and Mukthiraj (2011) found that the dietary inclusion of methanolic extracts of *A. paniculata* at a dosage of 1,000 mg/kg of feed had presented good effect on increase of growth performance and cellular immunity functions of *Oreochromis mossambicus* ingesting this diet for 45 days. Then, Basha *et al.* (2013) reported that the andrographolide (EC50%), principal medical compound in *A. paniculata*, had a stimulatory effect on non-specific immune parameters along with improved growth performance, and increased resistance against *Aeromonas hydrophila* in *Labeo rohita* fingerlings ingesting diet containing this substance at the concentration level of 0.10% for 42 days. As a result, this finding may be because the development of digestive mechanismin shrimp is lower than that in fish.

All things considered, the use of Creat extract may contribute to the advantage on immune enhancement, but this plant cannot use as growth promoter in shrimp. Accordingly, the characteristic of some biological compounds suppressing shrimp palatability or growth-related mechanism should be further in vestigated in order to obtain more effective approach for administration.

CONCLUSION

As had been indicated, Creat extract cannot promote growth performance of Pacific white shrimp in this trial, but it can be used for supporting shrimp health because application of Creat extract as dietary supplement at concentration level of 60 ppm can increase survival rate of shrimp infected with *V. harveyi* causing Vibriosis.

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