Effects of heat killed *Lactobacillus plantarum* (HK L-137) supplemental diets on growth, survival and health of juvenile striped catfish, *Pangasianodon hypophthalmus*

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Abstract: The present study was conducted to determine the effects of dietary heat killed *Lactobacillus plantarum* (HK L-137) on growth, survival, and immune response of juvenile striped catfish (*Pangasianodon hypophthalmus*). Feeding trial was carried out and four replicates groups of experimental fish (initial wt. 0.06 g) were fed with respective test diets containing four different concentrations of HK L-137 (0 ppm-control diet), 10 ppm, 20 ppm and 50 ppm, respectively. At the end of the feeding trial, results showed that the dietary supplementation of HK L-137 significantly enhanced the final weight (W_f), weight gain (WG) and specific growth rate (SGR) of juvenile striped catfish fed the diets containing 20 ppm and 50 ppm HK L-137 ($p<0.05$). Simultaneously, the survival rate of experimental fish fed the diet contains 50 ppm HK L-137 was significantly higher than the HK L-137 free group of fish fed with control diet ($p<0.05$). The highest protein efficiency ratios (PER) were also recorded in HK L-137 supplemented groups. Moreover, feed conversion ratio (FCR) of HK L-137 supplemented groups were also significantly lower than the control group. Furthermore, the dietary supplementation of HK L-137 improved and augmented the immune parameters including lysozyme activity, the number of RBC and WBC of juvenile striped catfish. Thereafter, a 14-day bacterial challenge experiment was also carried out and the highest mortality rate (100%) was recorded in positive-control group of fish fed with control diet. Consequently, post challenge survival rates (PCSR) of HK L-137 supplemented groups were higher than the control group. Present study demonstrated that the dietary supplementation of HK L-137 has the tremendous positive-effects on SR, FCR, WG, SGR, FI, PER, immune response and disease resistance of juvenile striped catfish.

Keywords: Heat-killed *Lactobacillus plantarum,* HK L-137, striped catfish, *Pangasianodon hypophthalmus.*

I. INTRODUCTION

Vietnam is the world's largest producer country of striped catfish *(Pangasianodon hypophthalmus)* (Sauvage 1878) all over the world, produced 1.252 million tons in 2017. Striped catfish is commercially important species belongs to the family Pangasiidae, one of the major fish species and native of Mekong River, spread to the rivers in Southeast Asia. Disease is one of the most serious constraint and obstacles to sustainable striped catfish farming (Phuong *et al*., 2009), caused by bacteria and parasites that lead to massive losses for farmers, the farming of striped catfish has also been struggling with several diseases. Moreover, striped catfish are also vulnerable and susceptible to bacterial diseases that would result in loss of appetite and lethal weakness to fish. Striped catfish farming are also being intensified for maximizing profits. Consequently, intensifying stocking densities are having devastating effects on water quality as well as pond's environment that would be a perfect place or paradise for bacteria, parasite, fungi, virus and such kinds of pathogens and diseases. Most common diseases in striped catfish farming are Bacillary Necrosis Disease (BNP- the causative agent- *Edwardsiella ictaluri)*, Motile *Aeromonad septicemia* (MAS) - caused by *Aeromonas* spp. (mainly *A. hydrophila, A. sobria and A. caviae*) and other Parasitic Diseases-caused by *Trichodina* spp. and *Epistylis* spp. Probiotics are being used in aquaculture in order to protect against pathogens such as bacteria, virus, fungi and parasites (Balcazar *et al*., 2006). More recently, the use of prebiotic and probiotic have been increasing significantly in aquaculture. Probiotics are microorganisms that are associated with the host animals and get beneficial effects for the host (Salmien *et al*., 1999) while prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon (Ringo *et al*., 2010). The supplementation and administration of prebiotics and probiotics have become one of the most important preventive and control measures for environment-friendly sustainable aquaculture (Pandiyan *et al*., 2013) instead of using antibiotics and chemicals.

In modern aquaculture, good aquaculture practices and managements are the most crucial for sustainable aquaculture industry (Dawood *et al*., 2016). Heat killed *L. plantarum* L-137 was used as an immunostimulant to induce interleukin-12 production and antitumor effect in mice, and to enhance gamma interferon production, which stimulates a substance that suppresses virus reproduction and other T-cells and activates macrophages cells (Murosaki *et al*., 2000). Accordingly, previous studies have found and reported that the dietary administration and supplementation of dietary feed HK L-137 enhanced, improved and augmented the survival rate, growth rate, immune responses, disease and stress resistances of marine and freshwater aquatic species (Duc *et al*., 2016, 2017; Doan *et al*., 2016; Nguyen *et al*., 2019; Dawood *et al*., 2015a; Tung *et al*., 2009). HK L-137, can increase the performance oxidative status in a specific manner compared to the control regimen by elevating the levels of antioxidative enzymes. Blood immune markers upregulated by supplementation with HK L-137 could have a significant effect on preserving fish health and could in turn enhance defense against infectious pathogens (Dawood *et al*., 2016).

Thus, HK L-137 has been shown as an immunobiotic, and plays an important role in enhancing growth, innate immune responses and resistances against diseases and stress of aquatic animal. Up to now, there are no studies on the effectiveness of HK L-137 of juvenile striped catfish. Hence, the present study aims to determine the effects of HK L-137 on the growth performances and immune responses of juvenile striped catfish Moreover, the juvenile striped catfish were challenged with *Edwardsiella ictaluri* (BNP- Bacillary Necrosis of Pangasius) in order to examine and evaluate their immune response and disease resistance of juvenile striped catfish.

II. MATERIALS AND METHODS

2.1 Experimental fish

The newly hatched striped catfish larvae were purchased from a private hatchery at Dong Thap Province in Mekong Delta of Vietnam. The larvae were reared and acclimated for 21 days in 500-L plastic quarantine tanks at 28±2°C. All the fish were fed with control diet during nursing in RAS before feeding trial.

2.2 Experimental diet

The formulated feed (45% crude protein) was prepared which followed Hien *et al*. (2003), the composition and ingredients of formulated diets are shown in (Table 1). Heat-killed *Lactobacillus plantarum*, strain L-137, HK L-137 with 20% heat killed *L. plantarum* L-137 and 80% dextrin in dried-weight basis was made by House Wellness Foods Corporation in Itami, Japan. HK L-137 was added and mixed thoroughly with the ingredients of formulated diets before pellet extrusion. Experimental feed was produced following the procedure described by Hien *et al*. (2017). Experimental ingredients were homogeneously mixed and pelleted at 80°C without steam to produce feed with a diameter of 2 mm. The experimental feed was then sterilized at 105°C for 10 min and dried at 45°C for 24 h to reach 11–12% moisture. Experimental diets were stored and kept at 4°C until use. Four experimental diets were prepared including control diet (without HK L-137) and each diet contains different supplemented doses of HK L-137 at 0 ppm (control diet), 10 ppm, 20 ppm and 50 ppm, respectively.

Table 1 The formulation and chemical composition of experimental diets (dry matter basis)

Items	**Experimental diet**
Ingredients (g.kg^{-1} dry diet)	
Fish meal*	357
Soybean meal	332
Cassava	74
Rice bran	150
Premix mineral and vitamin**	20
Carboxymethyl cellulose	3.8
Butylated hydroxytoluene	0.2
Methionine	2.8
Lysine	4.0
Shrimp solution	15
Phytase	0.2
Squid oil	7.4
Soybean oil	32.5
Total	**1000**
Chemical composition (%)	
Crude protein	45.0
Crude fat	9.0
Ash	12.5
NFE	29.9
Crude fiber	2.16
Gross energy (KJ g^{-1})	19.5

** Ca Mau fishmeal, Vietnam; cassava and local rice bran were supplied by Viet Thang feed mill.*

***Premix mineral and vitamin were supplied by Vemedim Company (unit.Kg^{-1}): Vitamin A. 2.000.000 IU; Vitamin D. 400.000 IU; Vitamin E. 6g; Vitamin B_1. 800mg; Vitamin B_2. 800mg; Vitamin B_{12}. 2mg; Calcium D. Pantothenate. 2g; Folic acid. 160mg;Vitamin C. 15g; Choline Chloride. 100g; Iron (Fe^{2+}). 1g; Zinc (Zn^{2+}). 3g; Manganese (Mn^{2+}). 2g; Copper (Cu^{2+}). 100mg; Iodine (I^-). 20mg; Cobalt (Co^{2+}). 10mg.*

2.3. Experimental design

The experiments were assigned 4 treatments with 4 replicates into 16 plastic tanks (100 liter/tank) in indoor mini Recirculating Aquaculture System with appropriate stocking density of 3 ind./L (initial weight 0.06 g) with density 300 ind./tank. A feeding trial was conducted for 60 days.

Fish were fed with 4 diets twice a day and the amount of feed consumed by the fish in each tank was recorded daily by removing and weighing (dry weight) excess feed to ascertain intake. Amounts of feed provided per replicate were recorded so that feed conversion ratio (FCR) and protein efficiency ratio (PER) could be calculated at the end of the experiment.

During experiment, water temperature ranged from 28.5-30.6°C, dissolved oxygen from 5.3-6.6mg·L^{-1}, pH from 6.7-8.0, NO_2^- from 0.4-0.5 mg·L^{-1} and TAN<0.1 mg·L^{-1}, so the water quality parameters in all treatments were in a suitable range for the normal growth and development of this species.

The experiment lasted eight weeks, at the end of which fish were measured, weighed and then used in a bacterial challenge experiment. Blood samples of a subset of experimental fish were taken at the end of experiment and examined for white blood cell count, white blood cell differential, red blood cell count, lysozyme activity.

Growth parameter and carcass analysis

Initial (W_i) and final weights (W_f) of individual fish were determined before and after the experiment, respectively. Survival Rate (SR, %), Specific Growth Rate (SGR; %/d), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), Net Protein Utilization (NPU), and Hepatosomatic Index (HSI) were calculated as follows (where t = time in days):

SR = [(number of fish at the end of experiment)/(number of initial fish)] x 100

SGR = $(\ln(W_f) - \ln(W_i))*100./t$

FCR = (amount of consumed feed in dry matter)/(weight gain)

PER = $(W_f - W_i)$/(protein intake)

Initial fish (ten fish/tank), final fish (ten fish/tank) and feed (100 g) were collected, minced and stored at -20°C until analysis of their chemical composition following methods of AOAC (2000).

Sample collection and Immune parameters analysis

Blood sampling (9 fish/treatment) of juvenile striped catfish were conducted at the end of the feeding trial for haematological parameters and lysozyme analysis. The red blood cells (RBC) count was determined in duplicate for each sample using Neubauer hemocytometer after dilution with Natt-Herrick solution (Natt and Herrick, 1952). White blood cells (WBC) count and white blood cell differential were also analyzed according to the method previous described by Chinabut *et al*. (1991). The lysozyme activity was evaluated from a standard curve generated by the lysis of a Gram-positive bacterium (*Micrococcus lysodeikticus*) according the method previous described by Ellis (1990); One unit of lysozyme activity was defined as the amount of enzyme producing a decrease in absorbance of 0.001 min^{-1} mL^{-1}. Gram-positive bacteria are attacked and destroyed by fish lysozyme. The lysis rate of (*M. lysodeikticus*) was measured by the serum lysozyme. This is a turbid- metric method based on the fact that absorbance is a linear function of (*M. lysodeikticus*) concentration.

Bacterial challenge test

The bacterial challenge experiment was conducted at the end of the feeding trial. Total of 30 fish from each feeding treatment (10fish/replicate) were intraperitoneally (IP) injected with 0.1 mL (10^5 CFU/mL) *Edwardsiella ictaluri* at a dose causing 50% mortality (LD_{50}). Fish from control feeding treatment was also IP injected with 0.1 mL of physiological saline (0.85%) as negative control treatment. Ward et al. (2016). During the 14 d post-inoculation, fish continued to be fed their respective diets, and clinical signs and cumulative mortality were noted daily. Moribund fish were sampled for bacteria confirmation by PCR method (Panangala *et al*., 2007). The data were analyzed by the Kaplan-Meier log-rank survival test, with pairwise Holm-Sidak multiple comparison procedure where appropriate (Ward et al. 2016).

2.4 Statistical analysis

Data were analyzed at the end of the experiment. SPSS-16 (SPSS Inc., Chicago, Illinois, USA), One –way analysis of variance (ANOVA; $p<0.05$), followed by Duncan test was applied to determine the significant differences.

III. RESULTS

3.1 Growth performance

The results showed that the final weight (W_f), weight gain (WG) and specific growth rate (SGR) of experimental fish fed the diets contain 20 ppm and 50 ppm HK L-137 were significantly higher than the control groups and fish fed 10 ppm HK L-137 diet (p<0.05). FCR of control group was significantly higher than the three other groups (p<0.05). On the other hand, the protein efficiency ratio (PER) of experimental fish fed the diets contain 10 ppm, 20 ppm and 50 ppm HK L-137 were significantly higher than the HK L-137 fed the control diet (p<0.05). Besides, the highest survival rate (SR) was recorded juvenile striped catfish fed the 50 ppm diet is significantly higher than those of fish fed control diet ($p<0.05$). Meanwhile, the lowest survival rate was recorded in without HK-LP treatment.

Table 2. Growth parameters, survival rate and nutrient utilization in striped catfish fed experimental diets for 60 days. W_i: initial mean weight, W_f: final mean weight, Wg: mean weight gain, SGR: specific growth rate, FI: feed intake, PER: protein efficiency ratio, FCR: feed conversion ratio, SR: survival rates

Parameters	Treatments			
	HK L-137 (0ppm)	HK L-137 (10 ppm)	HK L-137 (20ppm)	HK L-137 (50ppm)
W_i (g/fish)	0.06±0.01	0.06±0.00	0.06±0.00	0.06±0.00
W_f (g/fish)	14.3±1.10^b	15.4±0.98^b	17.5±1.06^a	18.1±0.77^a
WG (g)	14.2±1.10^b	15.4±0.98^b	17.4±1.06^a	18.1±0.78^a
SGR (%/day)	9.10±0.13^b	9.2±0.10^b	9.43±0.10^a	9.49±0.06^a

FI (%fish/day)	29.9±1.29[a]	26.7±3.86[ab]	25.5±2.87[b]	24.9±0.98[b]
FCR	1.06±0.05[a]	0.90±0.11[b]	0.81±0.09[b]	0.78±0.02[b]
PER	2.11±0.09[b]	2.48±0.28[a]	2.76±0.34[a]	2.84±0.07[a]
SR (%)	41.8±16.9[b]	56.2±28.5[ab]	57.8±18.4[ab]	74.2±4.72[a]

Values (mean±SD) in a row with different superscripts letter are significantly different ($p<0.05$).

3.2 Immunity

Lysozyme: A 60-day feeding trial was carried out and lysozyme activity of striped catfish was analyzed at the end of the feeding trial. The lysozyme activity of experimental fish fed the HK L-137 diets were significantly higher than fish fed with HK L-137 without diet ($p<0.05$). Whereas, the highest serum lysozyme activity was found in the group of experimental fish fed the diet contains 50 ppm HK L-137. There was no significant difference between the groups of fish fed the diets contain 10 ppm and 20 ppm HK L-137.

Red blood cell (RBC): the number of erythrocytes of experimental fish fed the diet that contains 50 ppm KH L-137 are significantly increased at the end of the feeding trial ($p<0.05$). However, there were no significant differences among the control group and two other groups of fish fed with the diets contain 10 ppm and 20 ppm HK L-137 ($p>0.05$).

White blood cell (WBC): white blood cells were counted and results showed that the leukocyte number of juvenile striped catfish fed the diets contains 20 ppm and 50 ppm HK L-137 were significantly increased compared to the HK L-137 free group fed with control diet ($p>0.05$). By comparison, the greatest number of WBC was found in the group of fish fed with diet 50 ppm HK L-137. The dietary supplementation of HK L-137 significantly increased and improved the number of white blood cells that results in higher immune response of juvenile striped catfish.

Table 3. The immunity of Lysozyme activity, Red blood cell (RBC), White blood cell (WBC) of striped catfish after 60 days culture with dietary supplementation in different concentration of HK L-137.

Parameters	**Treatments**			
	HK L-137 (0ppm)	**HK L-137 (10ppm)**	**HK L-137 (20ppm)**	**HK L-137 (50ppm)**
Lysozyme (µg/ml)	344.5±31.5[c]	410.0±23.7[b]	449.8±13.4[b]	496.8±33.3[a]
RBC (x 10^6 cell/mm^3)	3.47±0.34[b]	3.97±0.20[b]	3.99±0.39[b]	4.69±0.42[a]
WBC (x 10^5 cell/mm^3)	2.48±0.47[c]	3.26±0.69[bc]	3.60±0.49[b]	5.52±0.57[a]

Values (mean±SD) in a row with different superscripts letter are significantly different ($p<0.05$).

3.3 Bacterial challenge

The null hypothesis that HK-137 levels in the feeding trial above do not lead to significantly different survival in a post-trial bacterial challenge was tested. The highest mortality (100%) was recorded in positive-control group of fish fed the control diet. The first mortality of positive control group and 10 ppm HK L-137 supplemented group were found on the first day of bacterial challenge experiment. Afterwards, the first mortality of 20 and 50 ppm HK L-137 supplemented group were noted on the third and fourth day respectively. On the other hand, there was no mortality occurred in negative-control group (Fig. 1). There were no significant differences among the HK L-137 supplemented groups. In contrast, the cumulative mortality of fish fed with diet contain 20 ppm was significantly lower than the positive control group of fish fed control diet. On average, cumulative mortality

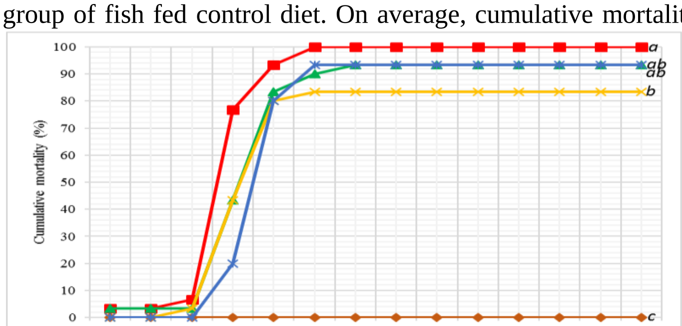

Figure 1. Cumulative mortality of juvenile striped catfish after 14 days challenged with *E. ictaluri*.

of HK L-137 supplemented groups were lower than the Positive-control group fed control diet (Fig. 1).

IV. DISCUSSION

In aquaculture, antibiotics and chemicals have been applied for many decades in order to protect against pathogens. The use of prebiotic and probiotic have been increasing in aquaculture in order to enhance and promote immune response and growth performance of animals. In environment-friendly sustainable aquaculture, prebiotic and probiotic have become alternative method to antibiotic treatment. As in other developing aquaculture ventures, health problems that include disease outbreaks from parasites and bacteria have been encountered. For pangasius, the most important disease problems are caused by the bacteria *(Edwardsiella ictaluri), (Aeromonas hydrophila), (A. sobria) and (A. caviae).* At the end of the 60-day feeding trial, growth performance, protein efficiency ratio, feed conversion ratio of juvenile striped catfish were evaluated and compared. The experimental fish fed with the test diets contain 20 and 50 ppm HK L-137 grew faster than the without HK L-

137 diet. However, there was no significant difference was found between the control group and 10 ppm HK L-137 supplemented group ($p>0.05$). Similarly, the highest weight gain of white leg shrimp (*L. vannamei*) was found in 100 ppm HK L-137 supplemented group (Duc *et al*., 2016).

Similar previous studies reported that the dietary administration of HK L-137 enhanced the survival rates, growth rates, immune parameters, disease, and stress resistance of experimental animals. Murosaki *et al*. (1998, 2000) summarized that HK L-137 induces the interleukin (IL-12) production that enhances the production of specific interferon (IFN) and T-cells growth. Besides, Tung *et al*. (2009) also reported that the growth rate of juvenile Kuruma shrimp (*Marsupenaeus japonicas*) fed with diets contain 100 and 1000 mg/kg HK L-137 significantly higher than the HK L-137 free group. Dawood *et al*. (2015b) found that HK L-137 supplemented groups grew significantly faster than the control group in red seabream (*Pagrus major*). Moreover, Duc *et al*. (2016, 2017) reported that the dietary administration of HK L-137 enhanced the growth performance and improved immune response of juvenile white leg shrimp (*L. vannamei*). In addition, the higher growth rate, phagocytic and lysozyme activity of Nile tilapia (*Oreochromis niloticus*) were also recorded in HK L-137 supplemented groups (Nguven *et al*., 2019).

In the study, the highest survival rate (SR) of experimental fish was recorded in 50 ppm HK L-137 supplemented group and that is significantly higher than fish fed 10 and 20 ppm HK L-137 diets ($p<0.05$). However, there were no significant differences among control group and two other groups fed with 10 ppm and 20 ppm HK L-137 diets ($p>0.05$). According to the result of present study, the different concentration of HK L-137 can strongly affect the survival rates of juvenile striped catfish *i.e.* the higher the concentration of HK L-137 results in the higher survival rate of experimental fish. Duc *et al*. (2016) also reported that the highest survival rate of white leg shrimp (*L. vannamei*) was recorded in shrimp fed the diet containing 100 ppm HK L-137. As a result, the survival rate of juvenile striped catfish was also obviously increased in fish fed the diet contains 250 ppm HK L-137. Likewise, the feed conversion ratio (FCR) of HK L-137 supplemented groups were significantly lower than the control group fed control diet ($p<0.05$). Although, the highest feed intake (FI) was also found in control group, the protein efficiency ratio (RER) were significantly higher in HK L-137 supplemented groups ($p<0.05$). Feed costs were also calculated and compared among the groups at the end of the feeding trial, the lowest feed cost was recorded in fish fed the diet contain 20 ppm HK L-137 ($p<0.05$). Nonetheless, there were no significant differences among the three other groups ($p> 0.05$).

The immune parameters of juvenile striped catfish were also evaluated and compared including lysozyme activity, red blood cell and white blood cell count. The obtained results showed that the lysozyme activity of experimental fish fed with the HK K-137 diets significantly higher than fish fed control diet ($p<0.05$). Among them, the highest lysozyme activity was recorded in 50 ppm HK L-137 supplemented diet ($p<0.05$). According to Nguyen *et al*. (2019), the higher lysozyme activity of Nile tilapia (*O. niloticus*) was also found in HK L-137 supplemented groups. Lysozyme, known as an important defense molecule of fish innate immune system antibacterial agent that attack and destroy the outer cell wall of bacteria. As a result, the higher lysozyme activity can be able to strongly against pathogenic bacteria. Likewise, the higher lysozyme activity of red seabream (*Pagrus major*) was also found in HK L-137 supplemented groups (Dawood *et al*., 2015b). For this reason, dietary administration of HK L-137 enhanced the innate immune response of juvenile striped catfish. Afterwards, blood samples of experimental fish were taken and examined for red blood cell and white blood cell count. Red blood cells, known as erythrocyte carries and transport oxygen to body tissues. Lower level of red blood cells cause anemia that weaken animal. In this study, the number of red blood cells were significantly increased in 50 ppm HK L-137 supplemented group ($p<0.05$). Nevertheless, there were no significant differences among the three other groups ($p>0.05$). Furthermore, the white blood cells of experimental fish were also counted at the end of the feeding trial. The dietary administration of 20 ppm and 50 ppm HK L-137 significantly increased the number of white blood cells compared with control group fed with control diet ($p<0.05$). White blood cells are known as the immune cells of animal's body that against and attack the foreign invaders. Accordingly, dietary supplementation of 20 ppm and 50 ppm HK L-137 tremendously enhanced the immune response of juvenile striped catfish. Hirose *et al*. (2006) also reported that the daily intake of HK L-137 augmented the acquire immunity in healthy adults.

Furthermore, a 14-day bacterial challenge experiment was also carried out at the end of the feeding trial in order to evaluate the disease resistance of juvenile striped catfish. The first mortalities were recorded in positive control group and 10 ppm HK L-137 supplemented group on the first day of bacterial challenge experiment. The first mortalities of 20 ppm and 50 ppm HK L-137 supplemented groups were noted on the 3rd and 4th day respectively (Fig. 1). After the 14-day bacterial challenge experiment, the highest mortality (100%) was found in positive control group. The post challenge survival rate of HK L-137 supplemented groups were higher than the positive control group. Interestingly, the lowest cumulative mortality was recorded in 20 ppm HK L-137 supplemented group ($p<0.05$). There was no mortality in negative control group. In comparison, Duc *et al*. (2017) also reported that the highest cumulative mortality of white leg shrimp (*L. vannamei*) was recorded in positive control group fed without HK L-137 diet after challenged with (*Vibrio parahaemolyticus*). Likewise, 1g/kg HK L-137 supplemented group showed the highest tolerance against low salinity stress in red seabream (*Pagrus major*) (Dawood *et al*., 2015b).

According to the results of present study, the juvenile striped catfish fed the diets contain 20 ppm and 50 ppm HK L-137 showed the significant specific growth rate, survival rate, feed conversion ratio and weight gain. Furthermore, the dietary supplementation of HK L-137 enhanced and improved the immune response of juvenile striped catfish including lysozyme activity, the number of white blood cells and red blood cells. Moreover, the post-challenge survival rates were also higher in HK L-137 supplemented groups. In this study, HK L-137 was applied as an immuno-stimulant and growth promoter by enhancing feed conversion efficiency, innate immune response and growth of experimental fish. Accordingly, the dietary supplementation of HK L-137 has tremendous positive effect on specific growth rate (SGR), weight gain (WG), survival rate (SR), feed conversion rate (FCR), protein efficiency ratio (PER), immune response and disease resistance of juvenile striped catfish. In conclusion, the dietary supplementation and administration of HK L-137 can be applied as growth promoter and alternative method to antibiotic treatment for environment-friendly sustainable aquaculture.

V. CONCLUSION

The study demonstrated and proved that the dietary supplementation of HK L-137 tremendously enhanced the weight gain (WG) and specific growth rate (SGR) of juvenile striped catfish, improved the survival rate and feed conversion ratio (FCR) of juvenile striped catfish. Dietary supplementation of HK L-137 obviously augmented and enhanced the hematological immune parameters. The requirement of HK L-137 by juvenile striped catfish was determined as 10-50 $mg.kg^{-1}$ feed in the laboratory condition.

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