Effects of Activate DA on Growth, Survival and the Total Number of Bacteria and *Vibrio* spp. in Rearing of Pacific White Shrimp (*Litopenaeus vannamei*)

Worrawut Walla[1], Watchariya Purivirojkul[2], Niti Chuchird[1] and Chalor Limsuwan[1]

ABSTRACT

A study on the effects of activate DA on growth and survival of Pacific white shrimp (*Litopenaeus vannamei*) was conducted under laboratory conditions. Tests were carried out in seven treatments (with five replicates/treatment). Post larvae 12 (PL12) were stocked into 500-liter fiberglass tanks at a density of 50 PL/tank. Salinity during the experimentation period was 25 ppt. Shrimp were fed four times daily with pelleted feed containing activate DA at different levels, namely 0.1, 0.2, 0.4, 0.8, 1.2 and 1.6%, and a control treatment which did not contain activate DA. After 60 days of dietary administration, shrimp fed with 1.2% of activate DA had an average body weight of 6.87±0.27g, which was not significantly higher (P>0.05) than those of shrimp fed with 1.6% (6.47±0.24g) of activate DA. However it was significantly higher (P<0.05) than the control group (5.14±0.76g) and the other treatments with 0.1% (4.61±0.47g), 0.2% (5.10±0.13g), 0.4% (5.42±0.24g) and 0.8% (5.92±1.04g) of activate DA. Shrimp fed with 1.2% of activate DA had the highest survival rate at 95.60±3.58 %, followed by those fed with 1.6% (92.00 ±2.83%), 0.8% (88.40±2.61%), 0.4% (87.20±4.60%), 0.2% (80.40±1.67%) and 0.1% (78.80±5.02%) of activate DA. Shrimp in the control group had the lowest survival at 67.20±6.73%. Total bacterial and total *Vibrio* spp. counts in the intestines of shrimp fed with 1.2% of activate DA were 5.48±1.74 x 10^2 CFU/g and 2.90±0.85 x 10^2 CFU/g, respectively.

Keywords: Activate DA , *Litopenaeus vannamei*, *Vibrio* spp.

INTRODUCTION

Currently, Pacific white leg shrimp, *Litopenaeus vannamei*, native to the Pacific coast of Central and South America, is the major shrimp species cultured in China, Taiwan and Thailand (Limsuwan and Chanratchakool, 2004). Since 2010, shrimp farmers in Thailand have experienced white feces syndrome which caused major economic losses among shrimp farmers throughout the country. Bacterial pathogens including *Vibrio vulnificus*, *V. fluvialis*, *V. parahaemolyticus* and *V. alginolyticus* were reported to be the

[1]Aquaculture Business Research Center, Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand
[2]Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

suspect agents causing mass mortalities (Montagan *et al*.,2012). Many scientists have attempted to solve the problems by using immunostimulants such as Beta-glucan and Peptidoglycan to enhance the non-specific immune response of shrimp against diseases (Purivirojkul *et al*. 2006).

Acidification of animal and aquaculture feeds by adding organic acids and organic salts can help to control the growth of bacteria and fungi which can reduce feed quality and produce toxins (Iba and Berchieri 1995; Franco *et al*. 2005). The reduction of bacterial numbers in the gut and improvement in the balance of gut microfloral communities can potentially have an important prophylactic effect, reducing opportunity for infection associated with the proliferation of dangerous pathogens in the gut (Eidelsburger *et al*. 1992; Roth *et al*. 1992; Hansen *et al*. 2007; Castro 2005). Although organic acids and organic acid salts have been widely used in poultry and swine, their use in aquaculture is new. The objective of this study was to evaluate the effect of organic acid namely activate DA on growth and survival rate of Pacific white shrimp in laboratory conditions. The effect of this organic acid on the total number of bacteria and specifically on *Vibrio* spp. in the intestine of shrimp was also observed.

MATERIALS AND METHODS

Seven treatments consisting of six levels of activate DA in pellet feed (0.1, 0.2, 0.4, 0.8, 1.2 and 1.6%) and a control (no activate DA added) were used in the experiment (with five replications per treatment). Each replicate consisted of 50 postlarvae in 500-liter tanks. Growth and survival rates were recorded after 60 days of experiment.

Experimental animals

Litopenaeus vannamei post larvae (PL9) were obtained from a commercial shrimp hatchery in Chachoengsao province, Thailand. A total of 5,000 PLs from the hatchery were transported and acclimated in 500-Lfiberglass tanks at the Aquaculture Business Research Center Laboratory, Faculty of Fisheries, Kasetsart University. After 3 days of acclimatization, the shrimp were used for the experiment. Salinity during the acclimation period and experiment was maintained at 25 ppt. Dissolved oxygen was maintained continuously to achieve optimal level above 4 mg/l (ppm), and temperature was maintained at 29±1^{O}C by an aquarium heater throughout the trial. PL12 were stocked into each aquariumata density of 100 PL/m^2 or 50 PL/tank.

Growth and Survival Study

Shrimp in all treatment groups were fed four times daily at 8.00 am, 12.00 am, 16.00 pm and 20.00 pm. Feeding rate was adjusted according to shrimp weight following Limsuwan and Chanratchakool (2004) throughout the 60 days experimental period. The types of feed used in this study are shown in Table 1.

Table1. Type of feeds used during the experiment

Days	0-10	11-27	28-44	45-60
Feeds	Artemia	Pellet feed No.1	Pellet feed No.2	Pellet feed No.3

Activate DA for each treatment was applied by dissolving activate DA in water (i.e. 5-10 g in 150-200 cc water), mixing it with guar gum then applying on top of the feed.

Water quality parameters such as pH, dissolved oxygen (DO), alkalinity, ammonia and nitrite were analyzed weekly throughout the experiment to maintain the optimal levels for rearing shrimp following Limsuwan and Chanratchakool (2004).

Growth, feed conversion ratio (FCR) and survival rate were recorded after 60 days experiment period.

The following equations were used in calculations:

$$\text{Survival rate (\%)} = \frac{\text{Number of surviving shrimp}}{\text{Total shrimp}} \times 100$$

$$\text{Average daily growth (ADG)} = \frac{\text{(Final weight) - (Initial weight)}}{\text{Days}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed consumed}}{\text{(final weight – initial weight)}}$$

The average concentration of total number of bacteria and total number of *Vibrio* spp. in the intestine of shrimp were determined at the end of the 60 days trial. All treatment used 100 shrimp/treatment and intestines were collected and placed into the eppendorf tubes. Intestines were homogenized with 1.5% NaCl into the eppendorf tubes and 100 µl supernatant was spread on Thiosulfate Citrate Bile Salt Sucrose Agar (TCBS) and Nutrient Agar (NA). Samples were incubated at 35°C for 24 hours, after which the colonies were counted.

Statistical analysis

The data were analyzed using the software SPSS v 13.0. One way ANOVA and the Duncan's New Multiple Range test were used to compare data among treatments. Differences were considered significant if $P<0.05$

RESULTS AND DISCUSSION

After 60 days of feeding trial, shrimp fed with 1.2% of activate DA had the highest average body weight of 6.87±0.27g, followed by 6.47±0.24g from the group fed with 1.6% activate DA. The average body weight of these two groups were significantly higher ($P<0.05$) than the control group (5.14 ±0.76g), and groups fed with 0.1% (4.61± 0.47g), 0.2% (5.10±0.13g), 0.4%(5.42± 0.24g) and 0.8% (5.92±1.04g) of activate DA (Figure 1).

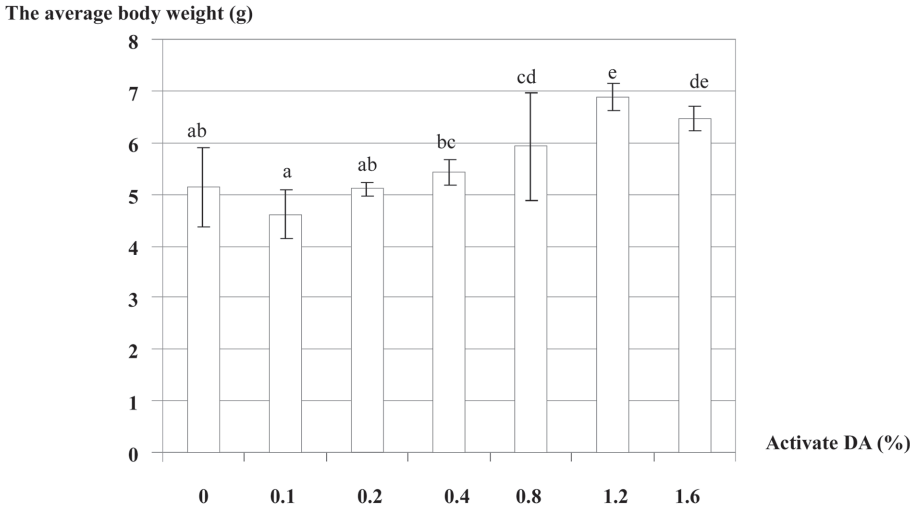

Figure 1. The average body weight of *L. vannamei* after 60 days of feeding with 0%, 0.1%, 0.2%, 0.4%, 0.8%, 1.2% and 1.6% of activate DA.

At the end of the feeding trial, shrimp in the group fed with 1.2% of activate DA showed the highest average percentage survival rate of 95.60±3.58 % followed by 92.00±2.83% from the group fed with 1.6% activate DA. The average survival rates of these two groups were significantly higher (P<0.05) than the control group (67.20±6.73%), 0.1% (78.80± 5.02%), 0.2% (80.40±1.67%), 0.4% (87.20± 4.60%), 0.8% (88.40±2.61%) of activate DA (Figure 2).

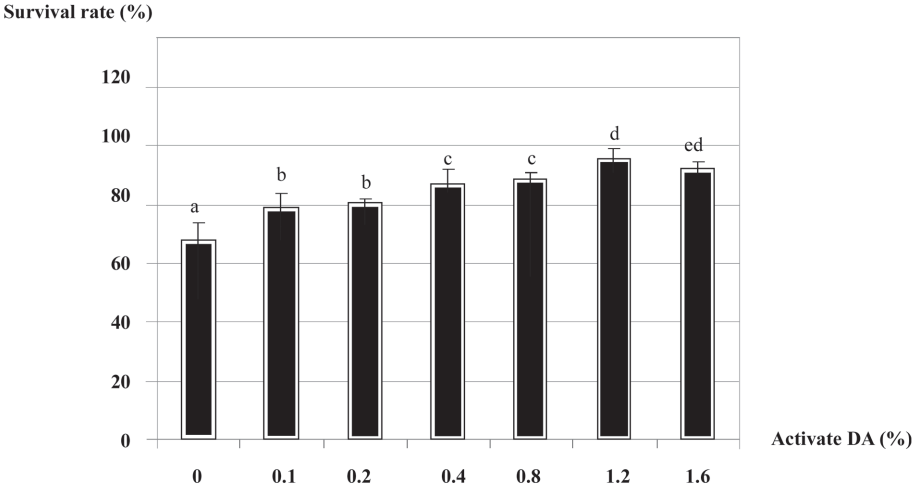

Figure 2. The survival rate of *L. vannamei* after 60 days of feeding with 0%, 0.1%, 0.2%, 0.4%, 0.8%, 1.2% and 1.6% of activate DA.

The average daily growth rate (ADG) of experimental shrimp is shown in Figure 3. After 40 days, shrimp fed with 1.2% and 1.6% of activate DA had the average daily growth rate of 0.0618±0.0067g and 0.0597 ±0.0044g, respectively. The average daily growth rate of shrimp from these two groups were significantly higher (P<0.05) than the control group, the group fed 0.1%, 0.2%, 0.4% and 0.8% of activate DA ,which showed the average daily growth rate of 0.0534± 0.0043g, 0.0453±0.0061g, 0.0473±0.0061g, 0.0532±0.0083g and 0.0543±0.0051g, respectively. At day 60, shrimp fed 1.2% of activate DA had the highest average daily growth rate of 0.1144±0.0044g. The average daily growth rate of shrimp in this group was not significant difference (P>0.05) form the group that fed 1.6% activate DA which was 0.1079±0.0039g but was significantly higher (P<0.05) than the group fed 0.8% (0.0986± 0.0173g), 0.4% (0.0903±0.0040g), 0.2% (0.0849 ±0.0021g), 0.1% (0.0769±0.0079g) activate DA and the control group (0.0856±0.0127g).

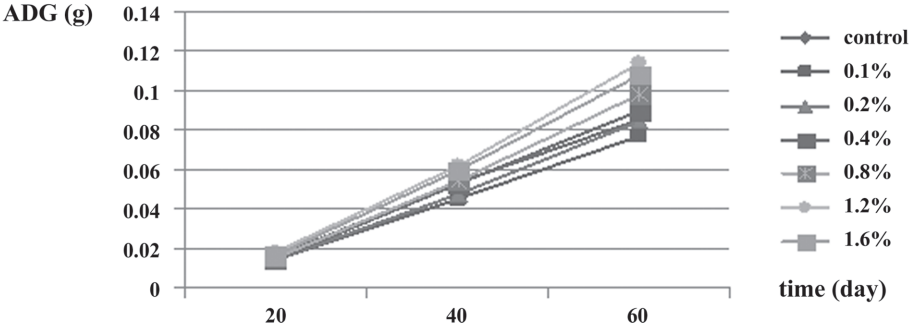

Figure 3. The average daily growth (ADG) after 20, 40 and 60 days of feeding with 0%, 0.1%, 0.2%, 0.4%, 0.8%, 1.2% and 1.6% of activate DA.

At the end of the trial,feed conversion ratio (FCR) of shrimp fed with 1.2% of activate DA was 0.76 which was lower than the group fed with 1.6% (0.82) , 0.8% (0.85), 0.4% (0.87), 0.2% (0.86), 0.1% (0.91) of activate DA and the control group (1.13) (Figure 4).

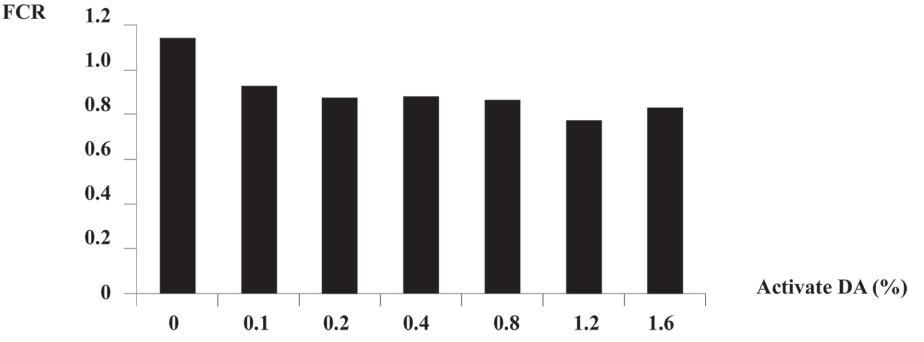

Figure 4. Feed conversion ratio (FCR) after 60 days of feeding with 0%, 0.1%, 0.2%, 0.4%, 0.8%, 1.2% and 1.6% of activate DA.

Similar studies in hybrid tilapia and Nile tilapia found improvements in weight gain when feed was supplemented with organic acids or their salts (Ramli *et al*. 2005; Zhou *et al*. 2008; Petkam *et al*. 2008). Improvements in mineral absorption have been reported for organic acid supplemented diets, thus resulting in a reduction of phosphorus in fish faeces (Vielma and Lall 1997; Sugiura *et al*. 1998). The positive growth effects of the organic acid may be attributed to the gut physiology and anatomy of the shrimp, allowing for a more direct gut acidification effect which may potentially improve digestive enzyme function which leads to better nutrient utilization (Yangomut, 2009; Roth *et al*. 1998; De Freitas *et al*. 2006)

The average total number of bacteria and the average total number of *Vibrio* spp. in the intestine of shrimp are shown in Figures 5 and 6. Shrimp fed with 1.6% of activate DA showed the lowest average total number of bacteria and total number of *Vibrio* spp. in the intestine at 4.53±1.53 x 10^2CFU/g and 2.32±0.87 x 10^2CFU/g , respectively when compared with the average total number of bacteria (5.48±1.74 x 10^2CFU/g) and the average number of *Vibrio* spp. (2.90±0.85 x 10^2CFU/g) from the intestine of shrimp fed with 1.2% activate DA. The average total number of bacteria and the average total number of *Vibrio* spp. in the intestine of shrimp from these two groups were significantly lower ($P<0.05$) than the control group (58.1±7.81 x 10^2CFU/g and 33.17± 11.9 x 10^2CFU/g), and the groups fed with activate DA at 0.1% (50.9±11.4 x 10^2CFU/g and 30.3±8.32 x 10^2CFU/g), 0.2% (41.0± 9.82 x 10^2CFU/g and 22.7±5.71 x 10^2CFU /g), 0.4% (27.9±8.15 x 10^2CFU/g and 13.7 ±4.33 x 10^2CFU/g) and 0.8% (19.05±7.71 x 10^2CFU/g and 7.64±2.17 x 10^2CFU/g).

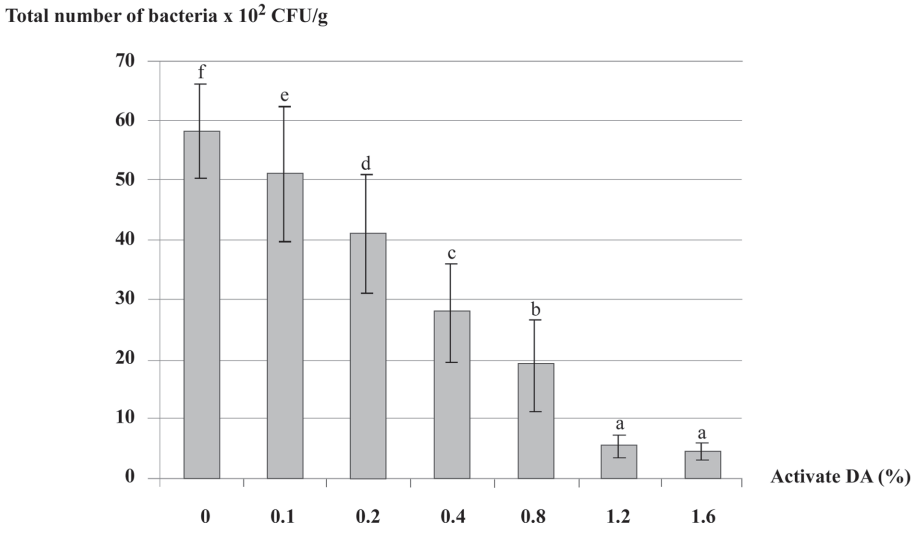

Figure 5. The average total number of bacteria in intestine of *L. vannamei* after 60 days of feeding with 0%, 0.1%, 0.2%, 0.4%, 0.8%, 1.2% and 1.6% of activate DA

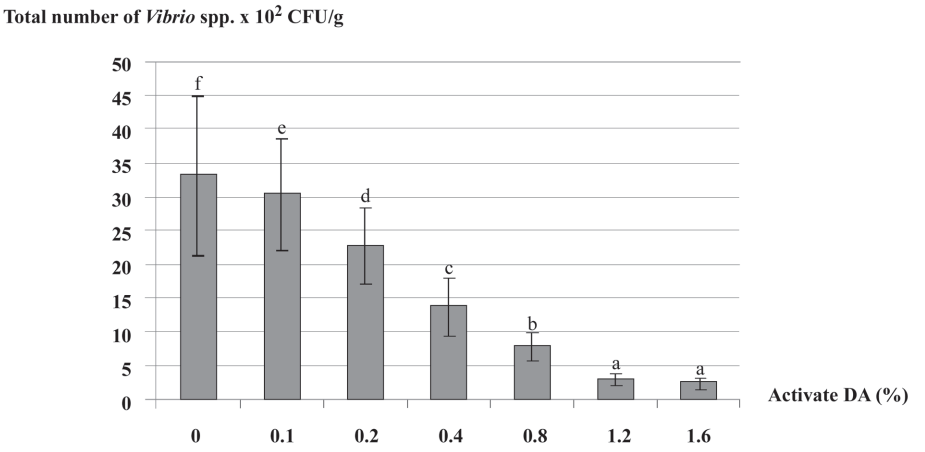

Figure 6. The average total number of *Vibrio* spp. (x 102 CFU/g) in intestine of *L. vannamei* after 60 days of feeding with 0%, 0.1% , 0.2%, 0.4%, 0.8%, 1.2% and 1.6% of activate DA.

Previous studies reported that some organic acids are the natural metabolic products of organisms, and they have been used as feed additives to control pathogens in animal husbandry (Franco *et al*., 2005). Several studies have proven that inclusion of organic acids in diets could suppress pathogenic bacterial growth in gastrointestinal tracts of poultry and swine. (Iba and Berchieri, 1995). Organic acids can improve gut health by lowering gastric pH or by diffusing into bacterial cells, then dissociating to release H+ cations to perturb cellular pH which results in bacteria cell death. Lowering of gut pH with the use of in-feed acidifiers has been observed in tilapia and *Labeo rohita* (Ng *et al*. 2009; Baruah *et al*. 2005). A recent study by Mine and Boopathy (2011) revealed the inhibitory effects of short-chain fatty acids, namely formic acid, acetic acid, propionic acid and butyric acid, on *V. harveyi*. Among the four acids, formic acid showed the strongest inhibitory effect with the minimum inhibitory concentration (MIC) of 0.035% followed by acetic acid, propionic acid and butyric acid. Previous studies (Mine and Boopathy, 2011) also indicated that the major inhibitory mechanism seems to be the pH effect of organic acids. This study revealed the potential use of organic acids as a diet supplement in shrimp feed to control Vibriosis in shrimp culture.

CONCLUSION

Oral administration of activate DA at the concentration of 0.2, 0.4, 0.8, 1.2 and 1.6% for 60 days could increase growth rate, survival rate and ADG and decrease FCR in rearing of Pacific white shrimp. Shrimp fed with 1.2% activate DA had the highest growth rate, survival rate, lowest FCR, number of total bacteria , *Vibrio* spp. in the intestine of shrimp though it is not significantly higher compared to adding 1.6% activate DA.

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