

Effect of Dietary Polyphenol-Rich Feed Additive from Grape Pomace on Growth, Survival and Tolerance to *Vibrio* Infection in Pacific White Shrimp (*Litopenaeus vannamei*)

Hataitip Niyamosatha, Niti Chuchird and Tirawat Rairat*

ABSTRACT

The effect of Anta[®]Ox FlavoSyn, polyphenol-rich feed additive from grapes, on the health and tolerance to *Vibrio* infection in Pacific white shrimp was studied under laboratory conditions. In experiment 1, postlarvae 12 were stocked in an 18 x 500-L fiberglass tank at a density of 50 PL/tank. Water temperature and salinity were maintained at $29 \pm 1^\circ\text{C}$ and 20–25 ppt, respectively. Shrimp were distributed into three groups (with six replicates/treatment), then fed four times/day with normal pelleted feed (control group), or feed mixed with 400 and 800 ppm Anta[®]Ox FlavoSyn. After 60 days of feeding trials, the body weights of shrimp fed with 400 and 800 ppm Anta[®]Ox FlavoSyn were 3.42 ± 0.22 and 3.48 ± 0.18 g, respectively, both significantly higher than those of the control group (2.64 ± 0.43 g). However, there were no differences in the survival rates and FCR from the three experimental groups. In experiment 2, 30 shrimp from experiment 1 were randomly stocked in new 18 x 500-L fiberglass tanks with six replicate tanks per treatment. At the beginning of the trial and 14 days later, *Vibrio parahaemolyticus* was added into each tank to obtain a final concentration of 10^4 CFU/ml. Each treatment group was fed the aforementioned diets four times daily for 30 days. All water quality parameters were maintained as in experiment 1. At the end of this experiment, 800 ppm Anta[®]Ox FlavoSyn-fed shrimp had a survival rate of $78.33 \pm 1.92\%$, significantly higher than that of the control group ($64.17 \pm 1.67\%$). The hepatopancreas of the former showed less necrotic cells as well. Nevertheless, weight gain, total intestinal *Vibrio* spp., and total bacterial count were not significantly different among all experimental groups. The present study indicated that Anta[®]Ox FlavoSyn had a positive effect on their growth and survival.

Keywords: Anta[®]Ox FlavoSyn, polyphenol-rich feed additive, grape pomace, Pacific white shrimp

INTRODUCTION

Currently, Pacific white shrimp (*Litopenaeus vannamei*), native to the Pacific

coasts of Central and South America, is the major shrimp species cultured in China, Taiwan, and Thailand (Limsuwan and Chanratchakool, 2004). Since 2012, shrimp

*Aquaculture Business Research Center, Faculty of Fisheries, Kasetsart University, Bangkok 10900 Thailand
Corresponding author, email: ffishntc@ku.ac.th

farmers in Thailand have experienced Early Mortality Syndrome (EMS), causing major economic losses in many cultivation areas throughout the country. Affected shrimp show signs of pale coloration due to pigment loss as well as atrophy of the hepatopancreas. These signs may become apparent as early as four days post stocking (Munkongwongsiri *et al.*, 2013). *Vibrio parahaemolyticus* was reported to be the suspected agent causing mass mortality (Tran *et al.*, 2013). Many scientists have attempted to solve the problem by using probiotic bacteria or organic acids to reduce the pathogenic bacteria in the gut of the shrimp as a prevention method (Nayak *et al.*, 2012; Walla *et al.*, 2012; Jueliang *et al.*, 2013). The use of phytochemical compounds is one of the promising solutions as it has a good ability to inhibit pathogens or antioxidant properties and enhance activities of digestive enzymes and nutrient absorption, which causes better growth (Lee and Ahn, 1998; Lee *et al.*, 2004; Cross *et al.*, 2007; Steiner, 2009). Polyphenol-rich plant extracts are among the good candidates for use as feed additives as evidenced by the many reports showing the positive effects of polyphenol on health performance of both humans and animals (Sun *et al.*, 2012; Cardona *et al.*, 2013; Kemperman *et al.*, 2010). However, their use in shrimp is limited. The objective of this study was to evaluate the effects of dietary supplementation of a polyphenol-rich feed additive, namely Anta[®]Ox FlavoSyn (natural plant extracts from grape pomace), which has a total polyphenol content of 8.5% (Fiesel *et al.*, 2014), on growth, survival, intestinal bacteria, and tolerance to *Vibrio parahaemolyticus* infection in Pacific white shrimp in laboratory conditions.

MATERIALS AND METHODS

Experiment 1–The Effects of Anta[®]Ox FlavoSyn on the Growth and Survival of Pacific White Shrimp Postlarvae

Experimental animals

Pacific white shrimp postlarvae 9 (PL9) were used in this study. A total of 2,000 PL9 from a hatchery were transported to the Aquaculture Business Research Center (ABRC) Laboratory, Faculty of Fisheries, Kasetsart University. PL9 were acclimated in fiberglass tanks for three days, after which they were used in this experiment (at PL12). A total of 18 500-liter tanks were used for rearing PL12 in seawater of 20–25 ppt salinity. Temperature was maintained constantly at $29 \pm 1^\circ\text{C}$ with an aquarium heater. Postlarvae 12 were stocked at a density of 100 PL/m² or 50 PL/tank. Three experimental groups consisted of a control group in which shrimp were fed commercial pelleted feed and two treatment groups in which shrimp were fed pelleted feed mixed with either 400 or 800 ppm of Anta[®]Ox FlavoSyn. Each group had six replicates.

Growth and survival studies

Shrimp were fed four times daily until satiation. The feeding rate was adjusted according to shrimp weight throughout the 60-day experimental period. Water quality parameters such as pH, dissolved oxygen (DO), alkalinity, ammonia, and nitrite were maintained at optimal levels for rearing shrimp and analyzed weekly throughout the experiment. Survival rate, body weight, and

feed conversion ratio (FCR) of the shrimp were recorded at the end of the experiment.

Experiment 2—The Effects of Anta®Ox FlavoSyn on Growth, Survival and Intestinal Bacteria of Pacific White Shrimp Challenged with Vibrio parahaemolyticus

Experimental animals

Juvenile white shrimp from experiment 1 was used in this study. Eight hundred healthy shrimp were sampled and acclimated in fiberglass tanks at ABRC laboratory for seven days. A total of 18 500-liter tanks were used in this experiment. Salinity and temperature were maintained as in experiment 1. A virulent strain of *V. parahaemolyticus* cultured in Tryptic Soy Broth (TSB) with 1.5% NaCl (w/v) was added into the tanks at the dose of 10^4 CFU/ml before stocking, and 14 days after stocking. Shrimp were stocked at a density of 60 shrimp/m² or 30 shrimp/tank. Three experimental groups as in experiment 1 were carried out with six replicates. Four tanks from each group were used for growth and survival studies. Another two tanks from each group were used for histological and immune parameters studies.

Growth and survival studies

Shrimp were fed four times daily until satiation. Feeding rate was adjusted according to shrimp weight throughout the 30-day experimental period. Water quality parameters were maintained as in experiment 1. Survival rate and body weight of shrimp were recorded at the end of the trial.

Intestinal bacterial and histological studies

Five shrimp were sampled from each

group at 10, 20, and 30 days of the experiment. Intestines of each shrimp were removed, homogenized, and spread on TCBS (selective media for *Vibrio* spp. culture) or NA (general media for most bacteria culture) by spread plate method, then incubated at 37°C for 24 hours. Finally, all colonies of bacteria were counted and calculated to CFU/g unit.

At the end of the trial, five shrimp from each group were sampled, fixed with Davidson's fixative solution for histological examination of hepatopancreas, then embedded in paraffin and stained with haematoxylin and eosin (H&E) as describe by Bell and Lighter (1988).

Statistical analysis

At the end of the experiments, data from all experimental groups were statistically compared using one-way ANOVA and Duncan's New Multiple Range.

RESULTS

Experiment 1

After 60 days of dietary administration, the average body weights of 400 and 800 ppm Anta®Ox FlavoSyn-fed shrimps were 3.42 ± 0.22 and 3.48 ± 0.18 g, respectively, significantly higher than the control shrimp, which had an average body weight of 2.64 ± 0.43 g. However, the average survival rate and FCR of shrimp in all experimental groups were not significantly different from each other (Table 1).

Experiment 2

At the end of experiment 2, there

Table 1. Body weight, survival rate and feed conversion ratio (FCR) of Pacific white shrimp after 60 days of feeding with three different diets

Experimental groups	Body weight (g)	Survival rate (%)	FCR
Control	2.64 ± 0.43^b	86.67 ± 3.72^a	1.32 ± 0.07^a
Anta [®] Ox FlavoSyn 400 ppm	3.42 ± 0.22^a	89.33 ± 2.42^a	0.99 ± 0.09^a
Anta [®] Ox FlavoSyn 800 ppm	3.48 ± 0.18^a	90.33 ± 2.66^a	0.98 ± 0.06^a

Data are presented as means \pm standard deviation. Means in the same column with different superscripts are significantly different from each other ($P < 0.05$).

was no significant difference in weight gain among shrimp in all experimental groups. Nevertheless, the survival rate of shrimp from the 800 ppm Anta[®]Ox FlavoSyn ($78.33 \pm 1.92\%$) groups was significantly higher than those in 400 ppm Anta[®]Ox FlavoSyn ($67.50 \pm 1.67\%$) and control groups ($64.17 \pm 1.67\%$) (Table 2).

Table 2. Weight gain and survival rate of Pacific white shrimp fed with three different diets for 30 days after being challenged with *V. parahaemolyticus* at 10^4 CFU/ml

Experimental groups	Weight gain (g)	Survival rate (%)
Control	2.15 ± 0.61^a	64.17 ± 1.67^b
Anta [®] Ox FlavoSyn 400 ppm	2.00 ± 0.37^a	67.50 ± 1.67^b
Anta [®] Ox FlavoSyn 800 ppm	2.27 ± 0.47^a	78.33 ± 1.92^a

Data are presented as means \pm standard deviation. Means in the same column with different superscripts are significantly different from each other ($P < 0.05$).

For intestinal bacterial study, both experimental groups throughout the feeding trial (Tables 3 and 4). *Vibrio* spp. and total bacterial count were not significantly different among all

Table 3. Total number of *Vibrio* spp. (10^6 CFU/g) in the intestine of Pacific white shrimp after being challenged with *V. parahaemolyticus* at 10^4 CFU/ml and fed with three different diets for 30 days

Experimental groups	10 th day	20 th day	30 th day
Control	3.51 ± 3.33^a	8.99 ± 5.64^a	8.02 ± 7.82^a
Anta [®] Ox FlavoSyn 400 ppm	3.05 ± 1.85^a	8.65 ± 3.76^a	7.00 ± 3.00^a
Anta [®] Ox FlavoSyn 800 ppm	2.87 ± 1.56^a	7.68 ± 2.67^a	5.75 ± 2.29^a

Data are presented as means \pm standard deviation. Means in the same column with different superscripts are significantly different from each other ($P < 0.05$).

Table 4. Total number of bacteria (10^6 CFU/g) in the intestine of Pacific white shrimp after being challenged with *V. parahaemolyticus* at 10^4 CFU/ml and fed with three different diets for 30 days

Experimental groups	10 th day	20 th day	30 th day
Control	7.22 ± 6.98 ^a	17.48 ± 9.80 ^a	15.74 ± 9.94 ^a
Anta [®] Ox FlavoSyn 400 ppm	6.51 ± 3.84 ^a	16.88 ± 7.57 ^a	14.94 ± 6.17 ^a
Anta [®] Ox FlavoSyn 800 ppm	5.70 ± 2.57 ^a	15.33 ± 6.04 ^a	12.92 ± 7.28 ^a

Data are presented as means ± standard deviation. Means in the same column with different superscripts are significantly different from each other ($P < 0.05$).

A histopathological study of the hepatopancreas of the experimental shrimp revealed the occurrence of cell necrosis in the organ. However, the percentage of necrotic cells in the hepatopancreas of shrimp from each experimental group was varied. Shrimp from the control group showed 30% necrotic cells, while shrimp fed with 400 and 800 Anta[®]Ox FlavoSyn showed 25 and 5% necrotic cells, respectively (Figure 1).

DISCUSSION

Polyphenols are a major group of plant secondary metabolites which have one or several phenolic hydroxyl groups. They are commonly found in many plant foods and beverages such as fruits, vegetables, cereals, legumes, tea, wine, beer, etc. (Bravo, 1998; D'Archivio *et al.*, 2007; Wink and Schimmer, 2010). Their several biological effects include antioxidant, anti-inflammatory as well as antimicrobial activities, and make polyphenols useful in health promotion (Xia *et al.*, 2010; Neyestani, 2008; Landete, 2012). There are many reports about the positive effect of polyphenol-rich feed additive extracted from grape pomace (*Vitis vinifera*) on the health of pigs and chickens (Brenes *et al.*, 2010; Viveros *et al.*, 2011;

Gessner *et al.*, 2012, 2013; Fiesel *et al.*, 2014). To our knowledge, its effect on shrimp health has never been studied.

Given the fact that polyphenols have distinct anti-inflammatory effects and this activity may be responsible for growth promotion in many animals (Niewold, 2007), it is likely that the growth enhancing effects of Anta[®]Ox FlavoSyn (natural plant extracts from grape pomace) used in this study may be the result of the anti-inflammation property of grape seed and grape marc polyphenols that were proven to suppress NF-κB activity when their target genes were involved in inflammation in the duodenal mucosa of pigs and Caco-2 intestinal cells (Gessner *et al.*, 2012, 2013). Grape skin is rich in flavonols (e.g. quercetin, myricetin, kaempferol), flavanols (e.g. catechin, epicatechin, procyanidin), hydroxycinnamic acid, anthocyanins, and resveratrol, while flavanols (e.g. catechin, epicatechin, procyanidin) and hydroxybenzoic acid can be found in abundant amounts in grape seeds (Montealegre *et al.*, 2006; Xia *et al.*, 2010). One or several of these polyphenols in grapes may account for many biological effects, even if the identity of the active substances and their precise mechanism of action have yet to be investigated.

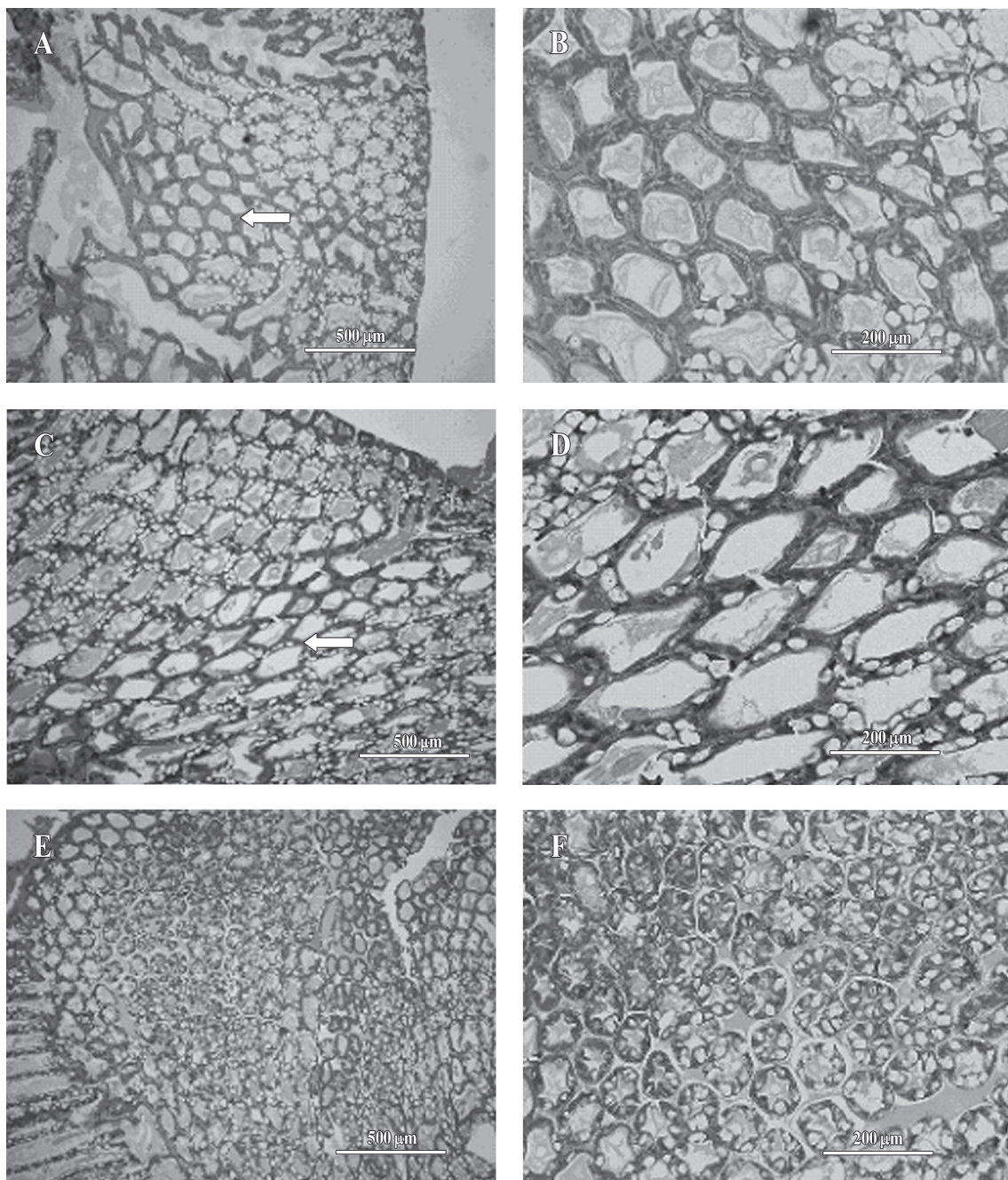


Figure 1. Necrotic cells in hepatopancreas of shrimp. Control shrimp (1A: 40x, 1B: 100x) with 30% cell necrosis; shrimp fed with Anta[®]Ox FlavoSyn 400 ppm (1 C: 40x, 1 D: 100x) with 25% cell necrosis; and shrimp fed with Anta[®]Ox FlavoSyn 800 ppm (1E: 40x, 1F: 100x) with 5% cell necrosis.

The survival rate of shrimps infected with *Vibrio parahaemolyticus* observed in the 800 ppm Anta®Ox FlavoSyn-fed groups was higher compared to the control group, while no difference in *Vibrio* counts indicated that the antimicrobial property of polyphenols was not the relevant mechanism. The other possible mechanism lies in the antioxidant and anti-inflammatory activity of polyphenols that protect gut mucosa against oxidative stress (Scalbert *et al.*, 2002; Gessner *et al.*, 2012, 2013). This explanation is also consistent with the histopathological study of hepatopancreas in which the 800 ppm Anta®Ox FlavoSyn-fed groups showed a lesser portion of necrotic cells compared to the control group. However, in contrast to the normal condition, the growth promoting effect of Anta®Ox FlavoSyn in *V. parahaemolyticus*-infected shrimps was diminished.

In conclusion, this study revealed the beneficial results of using polyphenol-rich feed additive in shrimp aquaculture. Anta®Ox FlavoSyn 800 ppm could improve growth and survival rate of shrimp infected with *V. parahaemolyticus*.

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