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SHORT COMMUNICATION

Dietary short-chain organic acids enhanced resistance to bacterial infection and hepatopancreatic structural integrity of the giant freshwater prawn, *Macrobrachium rosenbergii*

Wing-Keong Ng · Chia-Ling Lim · Nicholas Romano · Beng-Chu Kua

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Abstract The use of short-chain organic acids in the diets of aquacultured animals as a prophylactic to bacterial pathogens is receiving increasing research and commercial interest. After giant freshwater prawns, *Macrobrachium rosenbergii,* were fed diets supplemented with formic (FA), citric (CA), lactic (LA), propionic acid (PA), an organic acid blend (OAB) at 1% or a control diet (no additives) for 6 weeks, the prawns were subjected to *Vibrio harveyi* challenge for 2 weeks. From all remaining prawns, the hepatopancreatic histopathology was examined. Results showed that the survival of prawns to *V. harveyi* challenge was significantly higher ($P < 0.05$) when fed the LA diet, followed by the CA diet, than the other treatments. This was likely due to less bacterial-induced hepatopancreatic damage and higher energy reserves compared to those fed the other diets. This is the first report to compare the efficacy of different organic acids to a crustacean as well as showing hepatopancreatic protective properties to freshwater prawns when challenged with pathogenic bacteria. The present study indicated that dietary organic acids can be a viable alternative to the use of harmful antibiotics in the prawn farming industry.

Keywords Organic acids · Lactic acid · *Macrobrachium rosenbergii* · *Vibrio* challenge · Hepatopancreas

Introduction

An increasing trend in aquaculture is to reduce the reliance on antibiotics since these can be an effective prophylactic against disease as well as growth promoter when used at low doses, the long-term consequences can be harmful to the host animal, environment and potentially the human consumer (reviewed by Defoirdt et al. 2009; Marshall and Levy 2011). A promising alternative to antibiotics are organic acids, which include short-chain fatty acids (SCFA), which are "generally regarded as safe" compounds with one or more carboxyl groups and less than six carbons in their molecular formula. These have been used for many decades in the terrestrial animal feed industry as an antimicrobial and growth promoter (Thompson and Hinton 1997; Kluge et al. 2006). In more recent years, organic acids such as lactic, formic, citric and propionic acids and their

W.-K. Ng (✉) · C.-L. Lim · N. Romano
Fish Nutrition Laboratory, School of Biological Sciences, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia
e-mail: wkng@usm.my

N. Romano
Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

B.-C. Kua
National Fish Health Research Centre, Batu Maung, Penang, Malaysia

associated salts have been the focus of research for use in aquatic animal feeds (Ng and Koh 2011, 2016; Sukor et al. 2016).

The beneficial effects of dietary organic acids to the gastrointestinal tract of aquatic animals can include reducing potentially harmful bacteria in favor of more beneficial bacteria (e.g., lactic acid bacteria); however, the effectiveness of organic acids to disease resistance can be highly dependent on the host species and the type of organic acid used, often leading to contrasting findings (reviewed by Ng and Koh 2016). It is known that bacterial pathogens such as *Vibrio* spp. can be highly pathogenic to various crustacean species during the larviculture or post-larvae stages under stressful conditions (Tonguthai 1992; Lavilla Pitogo et al. 1998; Jayaprakash et al. 2006). Although *Vibrio* spp. is predominately found in brackish and marine waters, these bacteria have been detected in diseased *Macrobrachium rosenbergii* within some freshwater ponds in Thailand (Tonguthai 1992) even though the main route of infections is during the larval stages, which require brackish water (Jayaprakash et al. 2006). Moreover, when injected, *V. harveyi* was shown to be highly virulent to *M. rosenbergii* juveniles, which was suggested to become an increasing disease problem to freshwater hosts (Siripornadulsil et al. 2014). Apart from anti-bacterial properties, organic acids including SCFA can have a beneficial impact on farmed aquatic animals by enhancing nutrient availability, feed palatability, digestive enzyme activity as well as gut morphology (as reviewed in Ng and Koh, 2016). Dietary organic acids have also been reported to enhance the non-specific immune response of shrimp and have shown substantial promise in the control of vibriosis (Su et al. 2014; Romano et al. 2015; Ng et al. 2015).

The global farming of *M. rosenbergii* has been steadily growing over the past decade from 196,848 metric tons in 2004 to 230,333 metric tons in 2014 (FAO 2016). This industry has been limited, however, due to the threat of bacterial diseases leading to the widespread use of antibiotics in some areas to control bacterial diseases in *M. rosenbergii* farming (Paul et al. 2011). The exact extent of this practice is difficult to determine; nevertheless, finding eco-friendly prophylactics to bacterial disease would clearly benefit this industry. We recently reported that *Litopenaeus vannamei* and *Penaeus monodon* fed organic acid-treated diets had significantly enhanced resistance to *V. harveyi* (Romano et al. 2015; Ng et al. 2015); to date and to the best of our knowledge, no information exists that compares the efficacy of different dietary organic acids on the disease resistance and subsequent histopathology of a freshwater crustacean. The aim of the current study was to compare different dietary additions of organic acids at 1% that included formic acid (FA), citric acid (CA), lactic acid (LA), propionic acid (PA) or an organic acid blend (OAB), on susceptibility and hepatopancreatic histopathology of *M. rosenbergii* to *V. harveyi* challenge.

Materials and methods

Experimental diets

A total of six isonitrogenous and isolipidic practical diets were formulated to contain a 1% level of different organic acids which included formic acid (FA; HCOOH), citric acid (CA; $C_6H_8O_7$), lactic acid (LA; $C_3H_6O_3$), propionic acid (PA; CH_3CH_2COOH) and an organic acid blend (OAB). The proprietary OAB (Universiti Sains Malaysia) consisted of five organic acids (formic acid, lactic acid, malic acid, tartaric acid and citric acid; 75% w/w) adsorbed onto a silicon dioxide-based inert carrier (25% w/w) as previously described (Koh et al. 2016). Since LA, FA and PA were in a solution form, these were made into a powder by mixing with a silicon dioxide-based inert carrier at a 1:1 ratio. Therefore, when these were added in the experimental diets at 2%, this yielded 1% LA, FA and PA, respectively. The CA and the OAB were already in powder form and were added at 1% (Table 1). Since all the organic acids were made into powder form before use, they were added to the mixture of ingredients and mixed homogenously.

The main source of dietary protein was fishmeal (9.78%) and soybean meal (64.16%), while fish oil and soybean oil were included as the main lipid sources. All major dietary ingredients were purchased from local stores except otherwise indicated. Fish and soybean oils, corn starch and α-cellulose were purchased from Liang Traco Ltd. (Malaysia). Cholesterol (Solvay Ltd., The Netherlands) was added at 0.5% in all diets to satisfy the reported requirements for *M. rosenbergii* (Briggs et al. 1988). The proximate composition of the experimental diets was measured according to standard AOAC (1997) methods and values were generally similar among all diets (Table 1). Crude dietary lipid and protein were relatively constant at 7.7–8.3 and

Table 1 The ingredient and proximate composition (% dry matter) of the experimental diets for *Macrobrachium rosenbergii* with various added organic acids

	Experimental diets					
	Control	CA	LA	FA	PA	OAB
Fishmeal[a]	9.78	9.78	9.78	9.78	9.78	9.78
Soybean meal[b]	64.16	64.16	64.16	64.16	64.16	64.16
Fish oil	3.35	3.35	3.35	3.35	3.35	3.35
Soybean oil	2.70	2.70	2.70	2.70	2.70	2.70
Cholesterol	0.50	0.50	0.50	0.50	0.50	0.50
Corn starch	5.97	5.97	5.97	5.97	5.97	5.97
Vitamin premix[c]	3.00	3.00	3.00	3.00	3.00	3.00
Mineral premix[c]	4.00	4.00	4.00	4.00	4.00	4.00
CMC[d]	2.00	2.00	2.00	2.00	2.00	2.00
Citric acid[e]	0.00	1.00	0.00	0.00	0.00	0.00
Lactic acid[e]	0.00	0.00	2.00	0.00	0.00	0.00
Formic acid[e]	0.00	0.00	0.00	2.00	0.00	0.00
Propionic acid[e]	0.00	0.00	0.00	0.00	2.00	0.00
Organic acid blend[f]	0.00	0.00	0.00	0.00	0.00	1.00
α-Cellulose	4.53	3.53	2.53	2.53	2.53	3.53
Proximate composition						
Dry matter	97.70	93.40	94.00	94.3	91.80	91.70
Crude protein	35.22	35.50	35.07	35.18	35.18	35.42
Crude lipid	8.33	8.04	7.88	7.91	7.78	7.71
Ash	8.92	9.37	9.40	9.68	9.33	9.20
Crude fiber	1.94	2.22	1.33	1.81	2.38	2.71
pH	6.20	5.56	5.63	5.20	5.76	5.51

[a]Danish fish meal (Sri Putra Ltd., Malaysia) contained (% dry weight): 72.8 crude protein and 9.5 crude lipid

[b]Solvent-extracted soybean meal (Soon Soon Ltd., Malaysia) contained (% dry weight): 46.2 crude protein and 2.1 crude lipid

[c]Vitamin and mineral premix according to Kim et al. (2013)

[d]Carboxymethyl cellulose (Liang Traco Ltd., Malaysia)

[e]An inert carrier, silicone dioxide (SiO_2), was mixed with liquid organic acids (*LA* lactic acid, *FA* formic acid, *PA* propionic acid) at a 1:1 ratio, while citric acid (CA) was added in powder form. All organic acids were purchased from BioBasic Inc., Malaysia

[f]OAB, organic acid blend, consisted of five organic acids (formic acid, lactic acid, malic acid, tartaric acid and citric acid; Koh et al. 2016)

34.6–35.3%, respectively. This feed formulation was based on the known nutrient requirements for *M. rosenbergii* (D'Abramo and Sheen 1994). Dietary pH was measured according to Romano et al. (2015).

The experimental diets were prepared by thoroughly mixing the dry ingredients with oil and water in a Hobart mixer. The moist dough was then screw-pressed through a 2-mm die in a meat mincer and the feed pellets formed were fan-dried. After forced air-drying the pellets for at least 6 h to improve water stability, the diets were then crumbled into appropriate sizes (0.5–1.5 mm) and stored at −20 °C in airtight polyethylene bags until required.

Source of animals and acclimation

Giant freshwater prawn, *M. rosenbergii,* post-larvae were obtained from a commercial hatchery (Sitiawan, Perak) and acclimated in a one ton stocking tank that ran on a freshwater flow-through system for 3 weeks upon arrival to our laboratory. The water temperature was ambient (28 ± 2 °C) and the prawns were fed twice per day to apparent satiation with a commercial prawn feed (45% crude protein) (Gold Coin Ltd., Malaysia).

The water was sourced from the city water supply which was carbon and sand filtered prior to entering the tank and several air stones provided continuous aeration.

Experimental set-up and design

After the acclimation period, 360 prawns (initial weight 0.17 ± 0.01 g, mean ± SD) were randomly distributed into 18 glass aquariums [90-L capacity; 30 cm (W) × 70 cm (L) × 46 cm (H)]. Treatments were randomly assigned for a total of three replicates for each dietary treatment, and in each replicate there were 20 prawns. All aquaria ran on a freshwater flow-through system after being carbon and sand filtered and the set-up and conditions of the culture system were previously described by Kim et al. (2013). In each aquarium, one air stone provided gentle aeration and three groups of polyvinyl chloride (PVC) pipes (each pipe were 21 × 6.5 × 10.5 cm, radius × diameter × length), arranged as a pyramid, were allocated to minimize cannibalism by acting as shelters. The water temperature range was 29 ± 2 °C throughout the feeding period.

The prawns were fed with the experimental diets three times daily (08:30, 12:30 and 16:30 h) to apparent satiation (approximately 15% of wet body weight per day). To reduce nutrient leaching, the amount of feed given at each feeding time was dependent on feeding activity of the prawns and the introduction of feeds was stopped when it was observed that active prawn feeding activity had ceased. Each day, the aquaria were siphoned to remove feces and any molted exoskeletons and the feeding duration lasted 6 weeks. Growth and survival data were not monitored during the 6-week feeding period since it was known from an immediate prior 8-week feeding trial that prawns fed the same diets in the same experimental set-up elicited a percentage weight gain of 630–812% and a survival of 68–93% (unpublished data). The prawns were fed 6 weeks in the present experiment for the prawns to achieve sizes (1.2–1.5 g) suitable for the bacterial challenge test and subsequent hepatopancreatic histopathological analysis, which is the focus of the present study.

Bacterial challenge and histology

After 6 weeks, all prawns were transferred to the nearby National Fish Health Research Centre (Department of Fisheries, Malaysia) for hepatopancreas histology preparations and bacterial challenge test. For the bacterial challenge test, ten apparently healthy prawns of similar size (1.2–1.5 g) were distributed into each triplicate tank and fed their respective experimental diet twice daily (8:30 and 16:30 h) for 1 week. Prawns used for the bacterial challenge test were considered healthy prawns as they showed good growth and were actively feeding. All tanks had one air stone providing gentle aeration and PVC pipes acted as shelters. Each day, any uneaten feed or feces were siphoned out in the morning and approximately 30% of the water was exchanged. Remaining prawns from each dietary treatment not used for the disease challenge test were dissected and the hepatopancreas was immersion fixed in Davidson's formalin solution for 24 h. Samples were then transferred to 70% ethanol until processing using a Leica TP 1020 automatic tissue processor (Leica Microsystems, Nussloch, Germany). The samples were then embedded in paraffin wax and sectioned (5–6 μm thickness) using a Leica RM 2145 rotary microtome. Sections were mounted on poly-L-lysine (Sigma) frosted slides, stained with hematoxylin and eosin and examined under a light microscope.

In preparation for the bacterial disease challenge, *V. harveyi* (strain no. ATCC 14126), which was stored in a Marine broth 2216 containing beads and 20% (v/v) glycerol (Qrec, New Zealand) (pH 7.4) at −80 °C was grown on a TSA plate (Trypticase Soy Agar) at 28 °C for 24-h. A single colony was selected and cultured on a TSA plate for another 24-h at 28 °C before being transferred to a Tryptic Soy Broth for 24-h at 28 °C. The broth cultures were then stored in an incubator shaker for 12 h. The broth culture was then centrifuged at 10,000 rpm for 15 min. The supernatant was removed and the bacterial pellet was re-suspended in a saline solution (0.85% NaCl) and then diluted to a standard concentration equal to an optical density (OD) of 1.0 at 545 nm. As determined by standard dilutions and plating methods, this standard suspension of bacteria contained 1×10^9 CFU/ml.

Using this bacterial suspension, the prawns were injected intramuscularly with 50 μl between the third and fourth abdominal segments (Sahoo et al. 2006) at a concentration of 1×10^5 CFU/ml. This concentration was based on a pilot study to determine the relationship between prawn mortality and the challenge dose (10^7, 10^6, 10^5, 10^4 and 10^3 CFU/ml) and the most appropriate dose was chosen based on the occurrence of 50% mortality of prawns over a 2-week period. Each day the prawns were fed their respective diet twice to apparent satiation

along with a 15% water exchange. Mortalities were recorded daily and the challenge period lasted 2 weeks. After the 2-week bacterial challenge, the hepatopancreas from the remaining prawns were immersion fixed for histological examination. Three replicate prawns in each treatment were dissected for the hepatopancreas and examined for the histopathology. The prevalence of various hepatopancreatic cells (R cells, B cells and E cells) and tubule diameter (using a micrometer, 100 μm) was quantified within 20 randomly selected tubules from triplicates in each treatment.

Statistical analysis

All data were subjected to one-way ANOVA using SPSS 11.5 (SPSS, Chicago, IL, USA) after confirmation of homogeneity of variance. When a significant treatment effect was observed, a Duncan's multiple range test was used to compare means. Treatment effects were considered significant when $P < 0.05$.

Results

Histology of the hepatopancreas without pathogen challenge

The hepatopancreas of *M. rosenbergii* fed the control diet without pathogen challenge is shown in Fig. 1 and consisted of "star-like" tubules of varying sizes that contained different epithelial cells including E cells ("embryonalzellen" or embryonic cells), B cells ("blasenzellen" cells), R cells ("restzellen" cells) and F cells ("fibrenzellen" cells). The E cells are undifferentiated containing a relatively large nucleus, while the R cells are known to contain lipid droplets and these cells were generally located medially and distally from the lumen in each tubule. The B cells were the largest among the cells, but with high size variability, and often located medially and proximally from the lumen. The F cells were basophilic and were observed to be much less prevalent than the other cells. There was no significant difference ($P > 0.05$) to the prevalence of the R cells, E cells or B cells among the dietary treatments and ranged from 4.25 to 5.61, 16.88–19.42 and 7.42–8.41 (mean number/tubule), respectively. No significant differences in tubule diameter were observed.

Bacterial challenge

The cumulative mortalities of prawns fed different organic acid diets after the *V. harveyi* challenge test are shown in Fig. 2. Results showed that prawns fed the LA diet had significantly lower ($P < 0.05$) mortalities when challenged with *V. harveyi* than all other dietary treatments after 2 weeks. This was followed by the prawns fed the CA diet, which was significantly lower than those fed the control, FA, PA or OAB diet. The

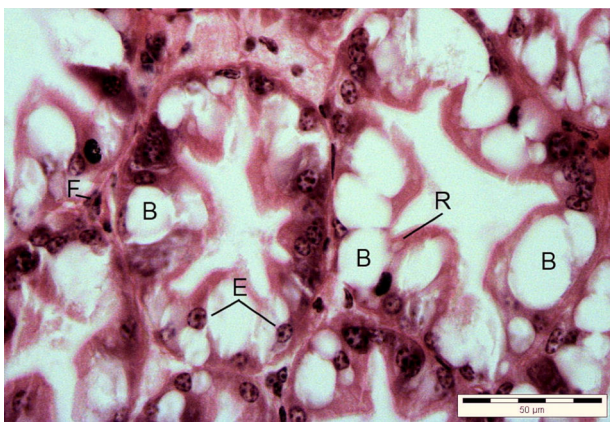

Fig. 1 Section of the hepatopancreas of giant freshwater prawn fed the control diet and not challenged with bacteria showing B cells (B), R cells (R), F cells and predominately E cells (E) within the tubules. H&E stain. Magnification ×40; bar 50 μm

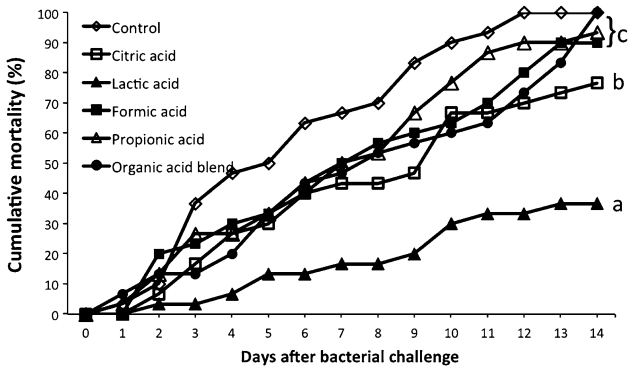

Fig. 2 The mean cumulative mortality (%) of the giant freshwater prawn fed diets with different added organic acids and subsequently challenged with *Vibrio harveyi* over a 14-day period. Values are mean of triplicate groups of ten prawns. Different letters indicate significant difference ($P < 0.05$)

mortality of prawns from the remaining dietary treatments was not significantly different from each other ($P > 0.05$).

Histology of the hepatopancreas after bacterial challenge

It was observed that substantial morphological changes occurred in all treatments; however, the amount of changes appeared to be dependent on the dietary treatment. It should be noted that since the control and OAB diets resulted in 100% mortality, near the end of the challenge period, freshly dead prawns were removed within about 1 h of death and only these prawns were used for histological analysis. Near the end of the disease challenge trial when there were only a few prawns remaining in the tanks, hourly observations were made to ensure that only freshly dead prawns were used. Results showed that prawns fed the control, PA, FA or OAB diets often had increased hemocytes within the interstitial spaces, separation of the myoepithelial layer and epithelium, a near collapse of the tubule lumen and, in some cases, tubule rupture. These effects appeared to be less pronounced for prawns fed the LA or CA diet since, aside from some cases of abnormally shaped lumen, the tubule structure appeared more intact and normal with less hemocyte infiltrations. The histopathology of the hepatopancreas of *M. rosenbergii* fed the control diet is shown in Fig. 3a, b and the LA diet in Fig. 3c, d.

After quantifying the various hepatopancreatic epithelial cells, it was found that prawns fed the LA or CA diets had significantly more R cells in their hepatopancreas than those fed the other diets while no significant changes were detected for the B cells or E cells among treatments (Table 2). Moreover, the R cells from prawns fed the LA or CA diet appeared to have a more rounded shape compared to the other treatments. The tubule diameter of the hepatopancreas of the prawns was not quantified since many individuals had tubules that were too severely compressed and/or distorted to accurately measure using a micrometer.

Discussion

In the present study, it was demonstrated that prawns fed the LA diet or the CA diet had significantly enhanced resistance to *V. harveyi* infection than all other treatments. Significantly lower prawn mortalities were also observed between prawns fed the LA diet compared to the CA diet. To the best of our knowledge, in terms of comparing different dietary organic acids on the disease resistance of a host, this is the first study to demonstrate this with a freshwater aquatic animal.

The use of different SCFA was shown to improve the resistance of *Artemia franciscana* to *V. campbelli* and was suggested that SCFA were ingested and thus inhibited the growth of *V. campbelli* (Defoirdt et al. 2006). This study by Defoirdt et al. (2006) demonstrated a significant step to the potential prophylactic protection of SCFA, but was noted that this would likely become cost-prohibitive in large-scale aquaculture operations if SCFA were to be added into the culture water. The use of organic acids, including SCFA, as prophylactics in

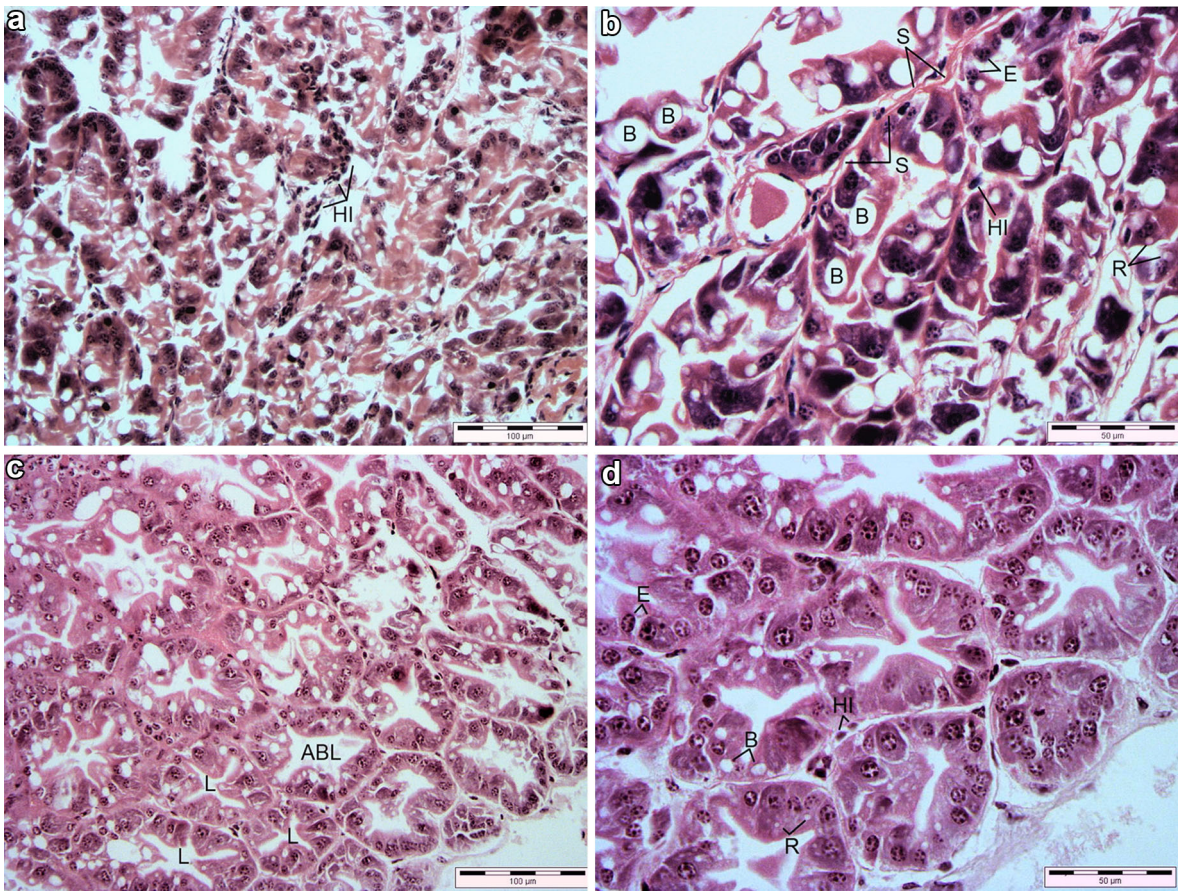

Fig. 3 Section of the hepatopancreas from the giant freshwater prawns fed the control diet (**a**, **b**) or LA diet (**c**, **d**) after being challenged with *Vibrio harveyi* for 2 weeks. The hepatopancreatic sections of the prawns fed the control diet show hemocyte infiltrations (HI) within the interstitial sinuses, a near collapse of the tubules (**a**) and separation of myoepithelial layer and epithelium (S) (**b**). The hepatopancreatic sections of the prawns fed the LA diet show a more normal tubule structure and lumen (L), but with some instances of abnormal lumen (ABL) (**c**) along with some hemocyte infiltrations (**d**). E cells (E), B cells (B), R cells (R). H&E stain. Magnification ×20; bar 100 μm (**a**, **c**). Magnification ×40; bar 50 μm (**b**, **d**)

Table 2 The prevalence of R cells, B cells and E cells (number/tubule) within the hepatopancreas of the giant freshwater prawn fed different organic acids added to the diets after 2 weeks of *Vibrio harveyi* challenge

Experimental diets[a]	Cell prevalence		
	R cell	B cell	E cell
Control	1.25 ± 1.42[b]	7.41 ± 1.87	10.42 ± 2.30
CA	5.48 ± 2.45[a]	6.54 ± 2.19	12.88 ± 3.11
LA	4.45 ± 1.85[a]	6.42 ± 3.42	13.22 ± 4.08
FA	0.89 ± 0.28[b]	7.22 ± 1.51	11.14 ± 2.77
PA	1.78 ± 0.34[b]	8.14 ± 2.48	10.07 ± 1.18
OAB	1.29 ± 0.17[b]	6.27 ± 1.85	13.85 ± 1.29

Cell prevalence is the mean number from 20 randomly selected tubules from three replicates and all values in the same column with different superscripts letters indicate significant differences ($P < 0.05$)

[a]Refer to Table 1 footnote for description of diets

the feeds of crustaceans has been met with good success recently and can be a cost-effective option (Ng et al. 2009; Park et al. 2011; Romano et al. 2015; Ng et al. 2015). It is believed that organic acids confer prophylactic protection from pathogenic bacteria by acting as antimicrobials, which includes their dissociation

when entering the cell membranes of bacteria and lowering their cytoplasm pH (Ng and Koh 2016). Furthermore, the bacteria that are more resistant to low pH are more likely to survive. Such bacteria include lactic acid bacteria, since these are tolerant to low-pH conditions, and are known to act as beneficial probiotics to some aquatic animals.

Interestingly, Silva et al. (2013) found that the in vitro inhibitory abilities of LA or CA salts on three different *Vibrio* species, including *V. harveyi*, were significantly less compared to PA, butyrate and acetate acid salts. Dietary additions of butyrate and acetate acids were not investigated in the present study, although the fact that the LA and CA diets led to significantly higher resistance to *V. harveyi* challenge than the PA diet indicates that the antimicrobial effectiveness may rely on complex interactions with the host species and diet. In particular, different organic acids may alter the microbiota of the host animal in different ways. In addition, a review on the current knowledge and application of organic acids in the feeds of aquatic animals have revealed that the potential beneficial effects on growth performance and health status of fish and shrimp seem to depend on the aquatic animal species, type and concentrations of organic acids and the culture conditions used (Ng and Koh 2016).

The addition of organic acids to the diets of white and tiger shrimp significantly increased their resistance to *V. harveyi* challenge and was suggested that increased phenoloxidase activity as well as less hepatopancreatic damage were likely contributors to this finding (Ng et al. 2015; Romano et al. 2015). This is because the hepatopancreas of crustaceans is known to be targeted by *V. harveyi* (Lavilla Pitogo et al. 1998). Typically, *V. harveyi* is a brackish and marine bacterium, but this has been detected in diseased *M. rosenbergii* within some freshwater ponds in Thailand (Tonguthai 1992) and continues to be a major disease problem in *M. rosenbergii* hatcheries where the larval and post-larval stages are cultured in brackish water to complete their life cycle (Jayaprakash et al. 2006). Recently, it was demonstrated that *V. harveyi* is highly virulent to *M. rosenbergii*, but was dependent on the strain (Siripornadulsil et al. 2014). The present experiment also demonstrated that *V. harveyi* was pathogenic when present in sufficient numbers in *M. rosenbergii*, and caused substantial damage to the hepatopancreas which included a separation of myoepithelial layer and epithelium, hemocyte infiltrations within the interstitial sinuses, abnormal lumen and, in severe cases, tubule rupture and collapse.

These findings are consistent with reports of *V. vulnificus*-infected *M. rosenbergii* (Sharshar and Azab 2008) and *V. harveyi*-infected *P. monodon* (Soonthornchai et al. 2010). However, prawns fed the LA diet or the CA diet showed significantly higher survival to *V. harveyi* infection as well as substantially less hepatopancreatic damage/alterations and significantly more R cells compared to the other treatments. It is worthy to note that Siripornadulsil et al. (2014) showed that *V. harveyi*-infected *M. rosenbergii* had degraded lipid droplets in the hepatopancreas, which was suggested to be the result of phospholipase activity from the bacterium, compared to control prawns. Therefore, this greater protection to the hepatopancreas of prawns fed the CA or LA diet in the present study, along with indications of higher energy reserves, may have contributed to their enhanced bacterial resistance. Considering *Vibrio* spp. can lead to substantial mortalities, particularly in the hatchery and nursery stages (Tonguthai 1992; Jayaprakash et al. 2006), these findings may have important implications to controlling bacterial disease in this freshwater prawn aquaculture industry.

Finally, it has been reported that synergistic activities among organic acids may exist or provide a broader spectrum of antimicrobial protection than the sole use of one organic acid type (Thompson and Hinton 1997; Chaveerach et al. 2002). However, the inclusion of dietary OAB in the present study had little effect on the resistance of the prawns from *V. harveyi* infections despite our previous investigation demonstrating that a similar organic acid blend significantly improved the resistance of tilapia to *S. agalactiae* (Ng et al. 2009; Koh et al. 2016). This appears to indicate that the effectiveness of such blends are dependent on the host species and/or target pathogenic bacteria. Therefore, since CA and LA dietary additions to the diets of *M. rosenbergii* provided the best resistance to *V. harveyi* infections, the use of these organic acids at optimal levels should be further explored, especially for LA. The utilization of these two organic acids as a prophylactic against a potentially broader range of pathogenic bacteria should also be investigated. The results of the present study are certainly encouraging, and while more research directions should be made, dietary organic acids showed beneficial properties that may help reduce the reliance on antibiotics in prawn farming and thus produce a more environmentally friendly seafood product acceptable to a wider consumer market.

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