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Effects of Vinegars and Sodium Acetate on the Growth Performance of Pacific White Shrimp, *Penaeus vannamei*

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Keywords: organic acids; sodium acetate; alternative growth promoter; attractability; leaching; feed acidity

Abstract

Vinegars and their salts have the potential to act as growth promoters and prophylactics against bacterial pathogens. This study aims to evaluate the effects of various vinegars and sodium acetate on the growth performance of white shrimp *Penaeus vannamei*. Groups of shrimps were fed diets containing 2% of either apple cider vinegar (ACV), coconut sap vinegar (CSV), sugar cane vinegar (CaV), or sodium acetate, and a diet with no vinegar (i.e. a control diet). Total acidity data of the diet showed that only the sodium acetate diet resulted in the highest total acidity after 60 min immersion in salt water (20 ppt). Attractability tests using customized repartitioned aquaria showed that the CSV diet attracted the highest significant percentage of shrimps after 10 min of feed placement in the feeding chamber. In the feeding trial that lasted for 60 days, results showed that the ACV and CSV groups of shrimps consumed significantly more feed than the other groups. All vinegar groups exhibited significantly higher final average body weight, weight gain, and specific growth, rate than either the control or sodium acetate group. The CSV group exhibited the significantly best feed conversion ratio and protein efficiency ratio. Survival was statistically similar among all groups. Conclusion: the CSV group exhibited the significantly best growth performance and efficiency while both the control and sodium acetate groups exhibited the poorest.

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Introduction

The increasing demand for aquaculture products has led to the intensification of aquaculture systems causing degradation of the aquatic environment thereby resulting in slower growth or total inhibition of growth of cultured shrimp. The inclusion of organic acids or their salts have been observed to increase the nutritive value of diets for aquatic animals (Ng and Koh, 2011) and growth of shrimp. One reason for this could be that organic acids are components of several metabolic pathways for energy generation (Luckstad, 2008). These also improve digestibility in shrimp diets as in fish (Morken et al, 2011). Supplementing the diet of *Penaeus monodon* with 1% commercial acidifiers with sodium butyrate enhanced the digestibility of dry matter, crude protein, and energy (Nuez-Ortin, 2011). In addition, organic acids and their salts increase nitrogen and phosphorus retention and bioavailability of calcium and phosphorus, as in fish (Hossain et al, 2007; Sarker et al, 2005; Khajepour and Hosseini, 2012; Sarker et al 2012a, 2012b).

Studies on evaluation of the effects of organic acids or their salts on growth performance of shrimp are limited. *L. vannamei,* fed diets supplemented with butyrate and propionate, showed improved growth (da Silva et al, 2014). In another study, formic, lactic, malic, and citric acids were incorporated in shrimp diets and results showed that they enhanced the growth of *L. vannamei* (Romano et al, 2014) and of *Penaeus monodon* (Ng et al, 2015). A range of a commercial acid (0.4 to 2%) was added to diets of *Penaeus vannamei* however there was no improvement in weight gain and survival, but immune response improved and there was a change in intestinal microbiota (Anuta et al, 2011). Diets of *L. vannamei* supplemented with 0.5% potassium diformate resulted in increased productivity over that of the control diet.

In our study we selected various natural vinegars such as apple cider vinegar, coconut sap vinegar, sugar cane vinegar, and a chemically produced sodium acetate salt to find potential beneficial growth promoters for white shrimp. The criteria used for the selection were feed acidity and attractability, growth performance, feed efficiency.

Materials and Methods

Experimental shrimps

Healthy Pacific white shrimp (*Penaeus vannamei*), which tested negative for white spot syndrome virus (WSSV) using one-step detection by PCR, were used. They were acclimatized in the laboratory for 3 days by feeding them the control diet at 20% body weight prior to the start of the feeding trial. A total of 450 shrimps (ABW=0.08 g) were used for the feeding experiment.

Table 1. Formulation of the experimental diets.

Feed Ingredient	*Treatments (% Composition)*				
	Diet 1 (control)	*Diet 2 (ACV)*	*Diet 3 (CSV)*	*Diet 4 (CaV)*	*Diet 5 (NaOAc)*
Peruvian Fish Meal	200.0	200.00	200.00	200.00	200.00
Shrimp Meal	340.0	340.00	340.00	340.00	340.00
Soybean Meal	210.0	210.00	210.00	210.00	210.00
CMC	54.8	54.80	54.80	54.80	54.80
Vitamin Mix	10.0	10.00	10.00	10.00	10.00
Mineral Mix	10.0	10.00	10.00	10.00	10.00
BHT	0.2	0.20	0.20	0.20	0.20
Lecithin	5.0	5.00	5.00	5.00	5.00
Cod Liver Oil	40.0	40.00	40.00	40.00	40.00
Starch	130.0	130.00	130.00	130.00	130.00
Apple Cider Vinegar	-	20.00	-	-	-
Coconut Sap Vinegar	-	-	20.00	-	-
Cane Vinegar	-	-	-	20.00	-
Sodium acetate	-	-	-	-	20.00
Distilled water	20.0	-	-	-	-
TOTAL	1000.0	1000.0	1000.0	1000.0	1000.0

ACV=apple cider vinegar; CSV=coconut sap vinegar; CaV=cane vinegar; NaOAc=sodium acetate salt; CMC=carboxymethylcellulose;BHT=butylated hydroxytoluene.

Experimental Diet

A basal diet with 42% crude protein was formulated containing 2% each of distilled water (control), apple cider vinegar (ACV), coconut sap vinegar (CSV), and cane vinegar (CaV) herein called Diet 1, Diet 2, Diet 3, and Diet 4, respectively (Table 1). Diet 5 (without vinegar) contained 2% organic salt, sodium acetate which was supplemented as dry ingredient in the basal diet ingredients.

Total acidity test

Total acidity test was conducted prior to the feeding trial by placing each experimental diet (2 g) in the water medium with salinity similar to the culture water medium (20 ppt). Total acidity was measured at 10, 30, and 60 min after placement by titration with sodium hydroxide with methyl orange indicator for the determination of mineral acidity. Following titration to the yellow endpoint, phenolphthalein indicator was checked and titration continued until the pink endpoint. Total acidity was calculated from the total volume of the standard NaOH to reach the phenolphthalein endpoint.

Attractability tests

Six randomly selected runs were conducted using rectangular glass tanks with multiple chambers adapted from the design of Suresh et al (2011) (Figure 1). Each tank consisted of 3 major chambers (acclimatization chamber, middle chamber, and feeding chamber). The three chambers were separated with a removable glass shutter. The feeding chamber consisted of 5 sub-chambers, each with a 6 x 5 cm opening so the shrimp would have access to the feeding chamber.

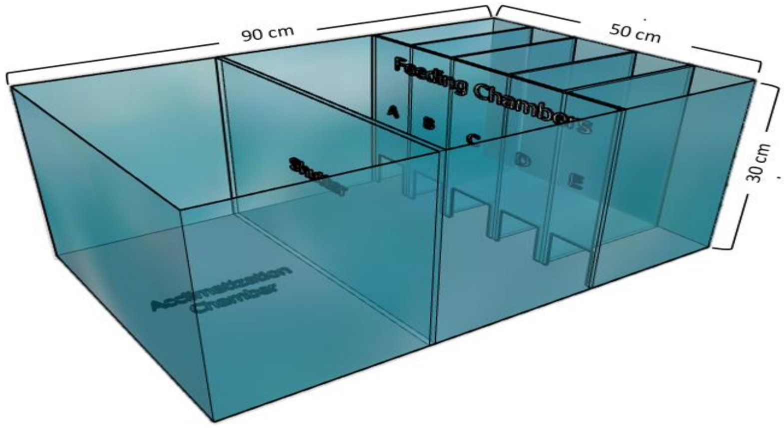

Figure 1. Schematic diagram of the tank used for the attractability test.

Each tank was filled with 40 L seawater (20 parts per thousand, ‰) and was set up in a closed laboratory where it received artificial light. Ten shrimps (ABW=1.02 g) were randomly stocked in the acclimatization chamber and allowed to acclimatize for 1 h. 2 g of each test diet were placed in each of the 5 chambers randomly. The shutter was removed 2 min after feed placement to allow access to feed. Feed preference was quantified visually by counting the number of shrimp in the feeding chamber at 1, 5, and 10 min following shutter raising. Six tests were conducted over 2 days with 3 simultaneous tests a day commencing at 9 am. Total percentage of shrimp within the feeding chamber were determined.

Feeding trial

A total of 450 *L. vannamei* (ABW of 0.08 g) were randomly distributed into 15 X 50L plastic aquaria filled with 40 L seawater (20 parts per thousand, ppm) in a closed water recirculating system. The system consisted of a reservoir (500 L fiberglass container), a physical filter, and a biological filter made up of oyster shells previously cleaned and disinfected. Each experimental diet, in triplicate, was randomly assigned to each experimental unit. Feed was given 3 times daily (8:00, 13:00, and 16:00 h) for 60 days. The amount of feed to reach the apparent satiation was measured on the first day of the week and this was used as the basis for the feeding rate for the rest of the week; uneaten feed was collected, oven-dried, and the weight subtracted from the total feed offered. All experimental aquaria were provided with aeration and uniform flow of water

(11L/h/container). Water parameters such as temperature, pH, and salinity were monitored daily, while ammonia, and nitrite was monitored weekly. About thirty percent of the total water volume of the recirculating system was changed twice a week. Bulk weighing of the shrimps from each container was done every 20th day.

Response Parameters

Survival rate, weight gain, specific growth rate, and feed conversion ratio were calculated using the following formulas:

Survival Rate (%)= (Total no. of live shrimp)/(Total no. of shrimp stocked) ×100
Weight Gain (g)= Final ABW- Initial ABW
Specific Growth Rate (SGR)= [Ln (Final ABW in g)- Ln(initial ABW in g)]/(No. of Days)
Feed Conversion Ratio (FCR) = (Total Individual Feed Intake)/(Weight Gain)

Statistical Analyses

Statistical Package for Social Sciences (SPSS) version 16 software was used to perform statistical analysis. Data (survival rate, weight gain, specific growth rate, and feed conversion ratio) were presented as mean ± standard error of the mean (SEM) and were tested for normality using Shapiro-Wilk test and variance homogeneity using Levene's test. The one-way analysis of variance (ANOVA) was used on data which passed the tests while those that did not were subjected to transformation until they passed the tests, then one-way ANOVA tests were used at α=0.05. Post hoc analyses were done using Duncan's Multiple Range Test (DMRT) to identify differences between independent factors. The percentage number of shrimp from attractability test as well as the survival rates were arcsin-transformed before subjecting to ANOVA.

Results

Water Quality

The mean (±SEM) of water quality parameters were recorded as follows: average water temperature at 29.0 ± 0.9 °C, total ammonia nitrogen (TAN) at 0.157 ± 0.074 mg/L, nitrite-N at 0.033 ± 0.013 mg/L, and pH at 7.9 ± 0.1.

Leaching Test

Leaching test of the diets were conducted at three time intervals (10, 30, and 60 min). In the first 10 to 30 min, results showed no significant differences between diets (Figure 2). After 60 min, sodium acetate diet showed significantly higher total acidity of 0.053 mg/L than that of the other diets which ranged from 0.043 to 0.045 mg/L ($p < 0.05$).

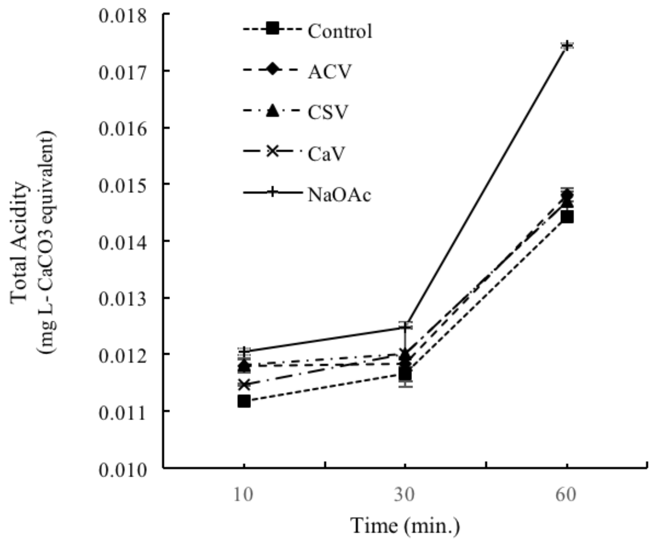

Figure 2. Leaching equivalent of experimental diets in the water measured in total acidity. ACV=apple cider vinegar; CSV=coconut sap vinegar; CaV=cane vinegar; NaOAc=sodium acetate

Attractability Test

Cumulative percentages of shrimp attracted to CSV diet and ACV diets were significantly higher than attraction to CaV, sodium acetate, and control diets (Table 2).

Table 2. Cumulative percentage of shrimp attracted to experimental diet.

Diet	*Shrimp (%) ± SEM*
Control	8.33 ± 3.07 [a]
ACV	26.67 ± 12.02 [ab]
CSV	41.67 ± 5.43 [b]
CaV	13.33 ± 6.15 [a]
NaOAc	10.00 ± 5.16 [a]

Different superscripts indicate significant differences between diets ($p < 0.05$). ACV=apple cider vinegar; CSV=coconut sap vinegar; CaV=cane vinegar; NaOAc=sodium acetate.

Feeding Trials

Periodic sampling data showed that at 20 and 40 days of feeding, weight gain was significantly higher in shrimp fed diets containing coconut sap vinegar (CSV) and apple cider vinegar (ACV) than in those fed the other diets (Figure 3). At the termination of the experiment, shrimp fed the vinegar diets exhibited significantly higher ABW than those fed the control diet. Specifically, the CSV and ACV groups exhibited significantly higher ABW than those fed the sodium acetate diet.

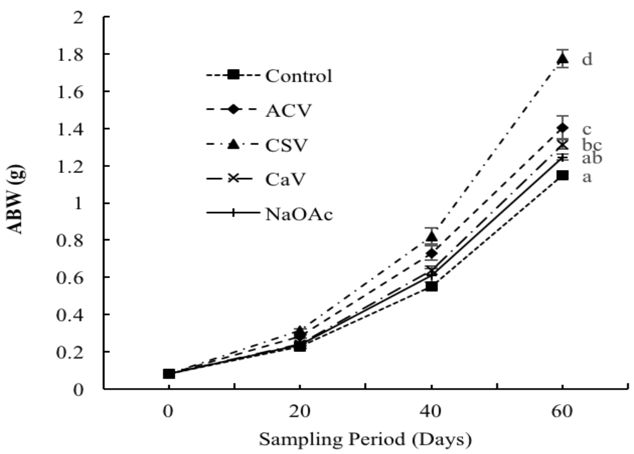

Figure 3. Periodic body weight of shrimp fed the control diet and diets containing apple cider vinegar (ACV), coconut sap vinegar (CSV), sugar cane vinegar (CaV) and sodium acetate (NaOAc). ABW=average body weight.

The final average body weight (FABW), weight gain (WG), and specific growth rate (SGR) were significantly higher in vinegar groups than those in the control group (Table 3). Specifically, the CSV group exhibited significantly the highest WG and SGR followed by the ACV group, sodium acetate group, and the control diet group.

Shrimp fed the vinegar and sodium acetate diets exhibited significantly better feed utilization than those fed the control diet. Shrimp fed the CSV diet exhibited significantly the best (i.e. lowest) feed conversion ratio (FCR) among dietary treatments (Table 3). The ACV, CaV, and sodium acetate groups exhibited statistically similar FCR values.

Table 3. Growth performance and feed utilization of shrimp fed with control, organic acid, and organic salt diets in 60 days.

	I_{ABW} (g)	F_{ABW}±SEM(g)	WG±SEM(g)	TIFI±SEM(g)	SGR±SEM(% bw/day)	FCR±SEM	PER±SEM	SURV±SEM
Control	0.08	1.15±0.02[a]	1.07±0.02[a]	1.97±0.04[a]	4.49±0.03[a]	1.84±0.04[c]	1.27±0.03[a]	90.00±1.92[a]
ACV	0.08	1.41±0.06[c]	1.33±0.06[c]	2.31±0.05[b]	4.82±0.07[c]	1.75±0.05[bc]	1.34±0.03[a]	88.89±1.11[a]
CSV	0.08	1.78±0.05[d]	1.70±0.05[d]	2.49±0.07[b]	5.21±0.05[d]	1.47±0.01[a]	1.59±0.01[b]	88.89±1.11[a]
CaV	0.08	1.31±0.03[bc]	1.24±0.03[bc]	2.11±0.04[a]	4.71±0.03[bc]	1.71±0.02[b]	1.37±0.02[a]	90.00±0.00[a]
NaOAc	0.08	1.25±0.01[ab]	1.17±0.0[ab]	2.06±0.08[a]	4.63±0.02[ab]	1.77±0.05[bc]	1.32±0.03[a]	90.00±1.92[a]

Superscripts indicate significant differences between diets ($p < 0.05$). SEM=standard error of the mean; I_{ABW}=initial average body weight; F_{ABW}=final average body weight; WG=weight gain; TIFI=total individual feed intake; FCR=food conversion ratio; PER=protein efficiency ratio; SURV=survival rate; ACV=apple cider vinegar; CSV=coconut sap vinegar; CaV=cane vinegar; NaOAc=sodium acetate.

Discussion

Feed effectors of shrimp that have been identified include fish oil, fish solubles, complex of amino acids, and different protein source ingredients (Smith et al, 2005; Nunes et al, 2006; Suresh et al, 2011). In general, major characteristics of feed effectors for shrimp and fish were of relatively small size, water soluble, and non-volatile (Olsén & Lundh, 2016). Attractant may also include nitrogen bearing compounds such as organic acids, nucleotides, and sugars (Derby and Sorensen, 2008). The differences in the attractability of diets containing the various vinegars in the present study probably differed in their non-volatile and volatile compounds emitted even after oven-drying the pellets. Although we did not measure the volatile compounds from each vinegar, there was a report that coconut sap vinegar contained 9 essential amino acids and 8 nonessential amino acids (Lee and Meyers, 1996). Some of these corresponded those reported by Nunes et al (2006) for having better attractability for *Penaeus vannamei* such as alanine, valine, glycine, proline, serine, histidine, glutamic acid and tyrosine. Apple cider vinegar which was similar in attractability to coconut sap vinegar contains key amino acids such as glycine, alanine, aspartate, glutamate, tryptophan and proline (Qi et al, 2017). Information on the composition of the sugarcane vinegar is scarce and probably the low attractability of the diet containing CaV might stem from the high content of volatile compounds. The attractability of sodium acetate and control diets exhibited similarly low attractability. This corresponds with the observation of da Silva et al (2012) in which sodium acetate showed a repulsive effect on *P. vannamei*.

The results of the present study demonstrated that vinegars substantially improved the growth performance and feed utilization of *P. vannamei*. There have been reports on the improvement brought about by the addition of organic acids due to improved nutrient and mineral digestibility which reduce the excretion of phosphorus and/or dephosphorylate phosphorus from phytate (Baruah et al, 2007; Pandey and Satoh, 2008; Ng et al, 2009). Several reports also showed a correlation between more rapid growth of shrimp and improved hepatopancreatic condition including increased tubule diameter and lipid storage R-cells when fed diets containing organic acids (Vogt et al, 1985, Johnston et al, 2003, Romano et al, 2014). The effects of dietary organic acids appear to be dependent on the type of organic acid and host species (Ng and Koh, 2011); this may be the reason that the three types of dietary vinegar in the present study differed in the extent of improvement of growth performance of white shrimp. Other organic acids that exhibited beneficial effects on growth of white shrimp included formic, lactic, malic, and citric acid, and salts such as propionate, butyrate, fumarate, and succinate (Ng and Koh, 2011)

We hypothesize that growth improvement of white shrimp in the present study could be attributable to the positive effect of vinegars on nutrient digestibility as well as their organic acids content (i.e. intermediate substrate in various biochemical pathways), vitamins and minerals (Horiuchi et al, 1999). Organic acid blend containing acetic acid has been demonstrated to promote the growth performance of South African abalone *Haliotis midae* (Goosen et al, 2011). Vinegar also contains anthocyanins, flavonoids, vitamins, minerals and other organic acids (Johnston et al, 2006; Shahidi et al., 2008).

To the best of our knowledge, this is the first report on the use of vinegars in the diet of white shrimp. Dietary apple cider vinegar (ACV) on the growth performance of *P. vannamei* was previously investigated and showed no significant effect after 60 days of feeding (Pourmozaffar et al, 2016). This result is in contrast with that of the present study in which dietary coconut sap vinegar improved the growth rate (weight gain by 159%) of white shrimp. This means that either marketable size could be attained in a shorter time, or shrimp production could be much greater at harvest time.

In conclusion, supplementation of different organic vinegars in the diet improved growth performance and feed utilization of *Penaeus vannamei* postlarvae. Among the evaluated vinegars, coconut vinegar had the highest potential as feed additive followed by sugarcane vinegar. Both supplements increased attractability of the diet to white shrimp.

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