



Dietary potassium diformate (Formi) supplementation on juvenile white shrimp (*Litopenaeus vannamei*) diets for growth and survival support.

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ABSTRACT

Objective: This feeding trial was designed to assess the effects of formi (**Dietary potassium diformate**) supplementation in shrimp diet on growth and survival of white shrimp, *Litopenaeus vannamei*.

Methodology and results: Seven isonitrogenous diets were formulated with graded formi levels of 0, 1, 2, 4, 8, 14 and 20 mg kg⁻¹ of dry diet, respectively. Shrimps were randomly allocated to twenty four (24) 50 l aquariums (10 shrimps of 0.82 ± 0.08 g per aquarium, eight treatments and three replicates). Water salinity was set at 17 g L⁻¹ and the flow rate of each aquarium maintained at 2 L min⁻¹ and decreased to 1 L min⁻¹ at day 41th. Shrimps were exposed to 18:6 hours light/dark photoperiod. The results showed that water quality parameters did not vary significantly with the dietary supplementation and was optimal for growth and survival of *L. vannamei*. The maximum weight gain and survival occurred at 14 mg kg⁻¹ formi diet. However, no significant differences were observed in percent weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) between diets with different levels of formi supplementations. The survival rate data varied between 73.33 % and 100 %. Survival rate revealed that the commercial diet had the lowest survival. At the end of the study no significant effects on the growth, survival, feed efficiency and water quality of white shrimp, *Litopenaeus vannamei* was found.

Key words: *Litopenaeus vannamei*, Potassium diformate, growth performance, survival

INTRODUCTION

The search for new feed additives is still a very important point for aquaculture researchers (Cho and Lee, 2012). A wide variety of natural growth promoters, including plant extracts, probiotics and organic acids, have been applied worldwide with reasonable success. Potassium diformate (KFD) known as Formi, is generally appears in nature and in the digestive tract of pigs. KDF acts as antimicrobial which reduces the general bacterial population in the gut, especially harmful bacteria

such as *E. coli* and *Salmonella*, and promotes a more favourable microflora in the gut (Hebeler *et al.* 2000). Moreover, KDF improves growth performance of piglets by an average of 11% (Øverland *et al. et al.*, 2000), which have a similar effect commonly obtained by antibiotic growth promoters. Organic acids are receiving increasing attention as a potential means to improve the growth as well as nutrient utilization of aquatic animals. Oral administration of potassium

diformate (KDF) significantly improves the feed intake, the live weight gain, the feed conversion ratio and the protein efficiency ratio of various tilapia species Ramli *et al.* 2005; Cuvin-Aralar 2010; Liebert *et al.* 2010 and Lim *et al.* 2010. The supplementation of potassium diformate added to the raw material in Atlantic salmon (*Salmo salar*) diet promoted a significantly increased body weight (Christiansen and Lückstädt, 2008). The Pacific white shrimp, *Litopenaeus vannamei* is originated from the Western Pacific coast of Latin America stretching from the south of Peru to the north of Mexico (Briggs *et al.* 2004). This species was introduced commercially into China and Taiwan in

1996 and other coastal Asian countries started to culture this species in early 2000 (Briggs *et al.*, 2004). Since then, *L. vannamei* became one of the world's three major farmed shrimp (Balakrishnan *et al.* 2011). Most of the KDF study in terrestrial animal shows positive effects on aquatic animal growth performance. There is lack of information on the effects of potassium diformate (Formi) supplementation on growth and survival of white shrimp, *Litopenaeus vannamei*. This study was conducted to examine the influence of the effects of potassium diformate (Formi) supplementation on growth and survival of white shrimp, *Litopenaeus vannamei*.

MATERIALS AND METHODS

Experimental diet: Seven (7) experimental diets supplemented respectively with graded Formi level at 0, 1, 2, 4, 8, 14 and 20 mg kg⁻¹ substituted wheat flour (Table 1) were formulated to contain 40% crude protein. Superior standard of Chilean fish meal (El golfo fishmeal) were used as major protein source, wheat flour as binder and wheat gluten were used to adjust the protein level. A commercial diet was used as a control. Ingredients were blended and screened individually through a 40 µm mesh die to obtain a fine particle size before mixing. All ingredients were

homogenized by a mixer for 15 minutes, fish oil were sprayed and homogenized for 15 minutes and water added after. Two pellet sizes with a diameter of 2 mm, length of 3.5 and 4.5 mm were extruded using a pelleting machine. These wet basis diets were dry under 70 °C for 2 hours to gelatinize. The diets were then dry in oven under 40 °C for 24 hours. The pelleted diets were stored in plastic bags at 4 °C until further use.

Table 1. Formulations and proximate composition of experimental diets (dry matter)

Ingredient (g Kg ⁻¹)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Commercial diet
Fish meal	240	240	240	240	240	240	240	-
Wheat gluten	30	31	31	31	32	33	34	-
Wheat flour	307	305	304	302	297	290	283	-
Basal mixture ^a	423	423	423	423	423	423	423	-
Formi	00	01	02	04	08	14	20	-
Proximate composition (g kg ⁻¹)								
Dry matter	969	908	901	955	900	972	987	969
Crude protein	392.7	391.8	393.5	394.2	392.7	388.4	390.8	406.5
Lipid	59.7	62.9	62.3	62.7	60.6	63.1	62.0	72.9
Ash	119.0	123.0	123.6	123.2	125.0	124.9	121.2	123.0

^a Basal mixture: Soybean meal, 20.0; Full fat soybean, 2.5; Shrimp meal, 7.0; Squid meal, 3.0; Torulla yeast, 3.0; Cholesterol, 0.1; Lecithin, 1.0; Fish oil, 1.0; Vitamin premix^c, 0.2; Mineral premix, 4.0, Choline chloride, 0.5.

Culture conditions and feeding trial: White shrimp (*Litopenaeus vannamei*) were obtained from a local commercial fish farm (South of Taiwan) and transported to the Aquaculture Department of National Taiwan Ocean University. After acclimation, shrimps were randomly allocated to twenty four (24) 50 l aquariums (10 shrimps of 0.82 ± 0.08 g per aquarium, eight treatments and three replicates). Water temperature, dissolved oxygen, pH, ammonia, and nitrite were monitored daily. During the rearing experiment, all aquarium were covered by net to avoid shrimp jump out. Water salinity was set at 17 g L^{-1} and the flow rate of each aquarium maintained at 2 L min^{-1} and decreased to 1 L min^{-1} at day 41th. Shrimps were exposed to 18:6 hours light/dark photoperiod. Experimental groups were handfed at 7 % of their body weight per day divided into four times at 06:30; 12:00; 18:00 and 24:30 h for 80 days.

Calculations and statistical analyses: The following parameters were calculated:

Per cent weight gain (WG, %) = $100 \times (W_t - W_i)/W_i$;
Specific Growth Rate (SGR, % day⁻¹) = $100 \times (\ln W_t - \ln W_i)/t$,

Feed Conversion Rate (FCR) = feed consumed (g, dry weight)/weight gain (g, wet weight)

Survival rate (%) = $100 \times (\text{final amount of shrimp})/(\text{initial amount of shrimp})$.

W_t is the final body weight (g), W_i is the initial body weight (g); and t is the experimental duration in days. Results are presented as mean \pm SEM. Data were subjected to one-way analysis of variance (ANOVA) to test the main effect of formi supplementation. Treatment effects were considered significant at 5%; Duncan's new multiple range tests was used to compare significant difference among treatments. The survival data were transformed into a normal distribution using the arcsine square root prior to analysis of variance. All statistical analysis was carried out using the SAS/PC statistical software.

RESULTS

Water quality parameters: Water temperatures, dissolved oxygen, pH, ammonia and nitrite are recorded during the experiment and their results are summarized in Table 2. Statistical analyses revealed that these mentioned water quality parameters did not

vary significantly ($p > 0.05$, Table 2) with the dietary supplementation of formi at increasing level. However, it was found to be at optimum level for growth and survival of *L. vannamei*.

Table 2. Water quality parameters of the juvenile *Litopenaeus vannamei* fed diets with different formi levels¹ (n=3).

Dietary formi (mg kg ⁻¹)	Temperature (°C)	Dissolved oxygen (mg L ⁻¹)	pH	Ammonia (mg L ⁻¹)	Nitrite (mg L ⁻¹)
00	30.82±0.09	8.16±0.03	7.4±0.30	0.05±0.12	0.30±0.12
01	30.72±0.09	7.67±1.92	7.5±0.65	0.07±0.08	0.35±0.00
02	30.62±0.09	8.06±0.57	7.4±0.20	0.09±0.12	0.30±0.30
04	30.82±0.09	8.22±0.09	7.30±0.00	0.07±0.04	0.36±0.30
08	30.89±0.09	7.35±2.11	7.50±0.30	0.07±0.24	0.29±0.12
14	30.96±0.18	6.98±2.04	7.40±0.30	0.07±0.11	0.30±0.00
20	30.72±0.09	7.32±0.39	7.50±0.30	0.08±0.16	0.33±0.30
Commercial diet	31.42±0.08	7.88±0.24	7.34±0.23	0.07±0.16	0.35±0.18

¹ Means \pm SD in the same column having the same superscript are not significantly different at $P > 0.05$.

Growth performance: The growth performance parameters of white shrimps fed experimental diets with different levels of formi are presented in table 3. The percent weight gain obtained ranged from 1122.58 ± 97.55 to 1444.06 ± 174.07 g. Percent weight gain, SGR, and FCR were not significantly affected by the dietary formi levels. The results of the percent weight gain and the specific growth rate indicates that the 14 g

kg⁻¹ formi supplementation are significantly different compared to the commercial diet ($p < 0.05$, Table 3). The survival rate data varied between 73.33% and 100%. The highest value were obtained from shrimp fed the diets supplemented with 0 and 14 g kg⁻¹ formi. Survival rate at the end of the experiment revealed that the commercial diet had the lowest survival ($p < 0.05$, Table 3).

TABLE 3. Growth performances in juvenile *Litopenaeus vannamei* fed diets with different formi levels for 80 days¹ (n=3).

Dietary formi (mg kg ⁻¹)	Initial weight (g)	Weight gain (%)	SGR	FCR	Survival (%)
00	0.82±0.09	1347.96±160.03 ^{ab}	3.56±0.15 ^{ab}	1.98±0.12 ^a	100.00±0.00 ^a
01	0.82±0.09	1354.77±47.92 ^{ab}	3.57±0.05 ^{ab}	2.12±0.08 ^a	93.33±5.77 ^a
02	0.82±0.09	1297.68±114.77 ^{ab}	3.51±0.11 ^{ab}	2.09±0.12 ^a	96.67±5.77 ^a
04	0.82±0.09	1345.41±119.93 ^{ab}	3.56±0.12 ^{ab}	2.00±0.04 ^a	96.67±5.77 ^a
08	0.82±0.09	1322.91±128.81 ^{ab}	3.54±0.13 ^{ab}	2.09±0.14 ^a	96.67±5.77 ^a
14	0.82±0.09	1444.06±174.07 ^a	3.64±0.15 ^a	1.99±0.11 ^a	100.00±0.00 ^a
20	0.82±0.09	1309.92±130.41 ^{ab}	3.59±0.02 ^{ab}	2.10±0.14 ^a	93.33±5.77 ^a
Commercial diet	0.82±0.08	1122.58±97.55 ^b	3.34±0.11 ^b	2.09±0.13 ^a	73.33±11.55 ^b

¹ Means ± SD in the same column having the same superscript are not significantly different at $P > 0.05$.

DISCUSSION

Water quality parameters: The mean water quality parameters (water temperatures, dissolved oxygen, pH, ammonia and nitrite) recorded during the experiment did not vary significantly ($p > 0.05$, Table 2) with the supplementation of formi at graded level in *L. vannamei* diet. The water temperature ranged from 30.62 to 31.42 °C; the dissolved oxygen varied between 6.98 to 8.22 mg/L, and the pH ranged from 7.30 to 7.50. However, the water temperatures, dissolved oxygen and the pH values were within acceptable ranges for *L. vannamei* culture (Treece, 2000). The unionized ammonia and nitrite values observed in this study remained below the respective safety levels for shrimp culture (Ammonia and nitrites remained between 0.17-0.19 and 0.10-0.11 mg L⁻¹, respectively), Frias-Espericueta *et al.* 1999; Tsai & Chen, 2002; Lin & Chen, 2003, Naranjo *et al.* 2012; Suriya *et al.* 2016.

Survival rate: *Litopenaeus vannamei* survived successfully during this experiment, which attested the good quality of foods tested. The survival rate data varied between 73.33% and 100%. A significant difference is observed between the survival of the formulated and the commercial diet. But, no significant differences were observed between the formulated diets. The lower survival rate was observed with shrimps fed commercial diet (73.33%). These results are in accordance with the findings of Nguyen *et al.* 2012 and Sanudin *et al.* 2014 on their studies conducted on shrimp *Penaeus vannamei*. Labrador *et al.* (2016) reported survival of white shrimps ranging from (90 to 99%) in its study on the effect of garlic powder-supplemented diets on the growth and survival. These results contrast with Palafox *et al.* 1997 and Khojasteh *et al.* 2013 who reported lower survival of *Litopenaeus vannamei* in their studies.

Growth performance: The growth performance parameters of white shrimps fed experimental diets with different levels of formi are presented in table 3. The percent weight gain obtained ranged from 1122.58 ± 97.55 to 1444.06 ± 174.07 g. The shrimps fed the diets supplemented with 14 g kg⁻¹ formi showed the highest percent weight gain compared to the commercial diet. Dietary organic acids and their salts such as formic acid have been used as a potential replacement of natural growth promoters to improve the performance and the health of tropical and cold-water fish. Atlantic salmon (*Salmo salar*) fed diet containing potassium diformate added to the raw material had a significantly increased body weight (Christiansen and Lückstädt, 2008). According to Lückstädt 2012, the application of KDF into the milkfish diet led to an increased average daily weight gain by more than 13%, while the feed conversion ratio (FCR) improved by more than 10%. In this study, the results had no significant effects on the growth, survival, feed efficiency and water quality of white shrimp, *Litopenaeus vannamei*, which is in agreement with the findings of Petkam *et al.* 2008 and Zhou *et al.* 2009 who reported no significant improvement in the growth performance of tilapia fed on organic acids/salt blend or KDF, respectively, at various dietary levels. However, this study results are not in accordance with that obtained by Cuvin-Aralar *et al.* 2010 who revealed better growth and FCR in juvenile Nile tilapia given diets supplemented with 3 mg kg⁻¹ KDF compared to the control diets. Ramli *et al.* 2005 also indicated significant improvements in the growth and feed-utilization efficiency of hybrid tilapia (*Oreochromis sp.*) fed a casein-based diet containing potassium diformate (KDF). Furthermore, *O. niloticus* fed diets supplemented with 10 g kg⁻¹ KDF tended to

improve the weight gain and feed efficiency (Lim *et al.* 2010). In this study, one of the reasons for no formi effect might be the shorter length of the shrimp digestive tract and its shorter feed evacuation time compared to fishes and other terrestrial animal. It is well known that aquatic crustaceans are slow feeders, and the feed offered can remain suspended in the water column for longtime before being consumed. This could have consequences of nutrients leaching (Ali *et al.* 2005). Shrimp fed commercial diet showed lower percentage weight gain compared to other diets. This might be due to the quality of raw materials used in the commercial diet.

CONCLUSION

This current study was designed to investigate the effect of varied supplementation levels of potassium diformate (Formi) on growth of white shrimp, *Litopenaeus vannamei*. At the end of the study no significant effects on the growth, survival, feed

Feed efficiency: The best FCR was obtained with shrimps fed diet supplemented with 0 and 14 g kg⁻¹ of formi with a FCR of 1.98 and 1.99 in this present study. No significant differences were observed in FCR between diets with different levels of formi supplementations ($p > 0.05$, Table 3). Similarly, Shakir *et al.* 2014 reported FCR ranging from 1.83 to 2.3 for *Penaeus monodon*, and Nguyen *et al.* 2012 observed feed conversion ratios varying from 1.72 to 2.22 for *Penaeus vannamei*. On the contrary, the founding of Ortega-Salas and Rendón 2013; Sanudin *et al.* 2014 were lower (< 1) and higher for Bisesi, 2007 who found in his master thesis FCR averaging 2.69 ± 0.36 .

efficiency and water quality of white shrimp, *Litopenaeus vannamei* was found. We recommend a further research for determination of the mechanisms of action of formi on shrimps.

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