Dietary potassium-diformate affects growth performance and survival rates of vannamei-shrimp in hatchery and grow-out in worldwide aquaculture

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Intensive production of the whiteleg shrimp, *Litopenaeus vannamei* (Boone 1931), in Central America and SE Asia is estimated to have almost reached 4.0 million tons (FAO, GOAL, 2019), thus showing strong signs of recovery in the last couple of years. Despite remarkable progress in shrimp nutrition and feed formulation during the past years, disease outbreaks in shrimp ponds can still lead to farming setbacks and increased use of antibiotics. Growing awareness from consumers and producers of aquaculture species, however, has driven demand for responsible and sustainable aquaculture. Regulatory authorities in most exporting countries now focus on the misuse of antibiotic growth promoters (AGP) in aquaculture, while public attention has shifted towards sustainable production methods. Alternative additives are being developed to replace the AGPs.

Acidifiers to replace AGPs

Acidifiers are one of various alternatives spearheading environmentally friendly and nutritive-sustainable aquaculture approaches. Currently, the most widely tested organic acid molecule in aquaculture is potassium diformate (KDF). It has been tested and used successfully, among others, in salmon, trout, tilapia, Asian and European sea bass and pangasius. Its value to the shrimp production cycle has also been demonstrated in several field and research trials. Potassium diformate is a double-salt formic acid molecule which decreases gastrointestinal pH and thereby intensifies the release of buffering fluids, containing enzymes, from the hepatopancreas. Formate also diffuses into pathogenic bacteria inside the digestive tract and acidifies their metabolism, leading to bacterial cell death. Furthermore, beneficial bacteria (*Lactobacilli, Bifidobacteria*) are supported (eubiosis), which may lead to improved gut health, resulting in stronger condition of the shrimp.

Acidifiers in vannamei PLs

One of the most crucial periods in the life cycle of shrimp is the post-larval stage when shrimp feeding is changed from algae and brine shrimp nauplii to commercially formulated larval diets. Survival rates during this period are critical to later productivity. Pathogenic bacteria can dramatically increase mortality in the shrimp hatchery. Therefore, the use of potassium diformate was tested during this production stage (He *et al.*, 2006). The group

Parameter	Control	0.8% KDF	Difference (%)
Initial BW (mg)	57	57	-
Final BW (mg)	256±34	309±35	+21
WG (mg, 40 d)	199	252	+27
FCR	3.73±0.6	2.49±0.3	-33
Survival (%)	92.2±1.6	100	+8
Prod. Index*	0.49	1.01	+106

Table 1. Growth, feed conversion and survival of *Litopenaeus vannamei* PLs under aquaria conditions after 40 days.
*Productivity Index (weight gain (g) × survival (%) / (10 × FCR))

supplied post-larvae of *Litopenaeus vannamei*, with a body mass of 57 mg, with a formulated diet containing either no or 0.8% potassium diformate (KDF, Aquaform®, ADDCON), for 40 days. Shrimp larvae fed with KDF improved performance dramatically (Table 1).

Shrimp larvae fed with KDF inclusion showed more efficient growth and significantly improved feed utilization, as well as lower mortality ($p<0.05$), compared to control shrimp, leading to a markedly increased productivity index, which is based on a formula including the three most important production figures in shrimp production (weight gain of shrimp, feed efficiency and survival rate).

KDF improves growth and survival in juvenile whiteleg shrimp

As discussed previously, optimal nutrition in the early stage of the shrimp production cycle often leads to an overall improved shrimp productivity. This was demonstrated in another experiment, reported by Jintasataporn *et al*. (2011). An aquarium-simulated intensive grow-out trial investigated the growth performance of juvenile white-leg shrimp fed with low levels of dietary KDF vs a control diet. While the control diet did not contain KDF, 0.2% and 0.5% KDF were added to two treatment diets, respectively. Shrimp (initial weight 2.4±0.1 g) were fed to satiation three times a day, with a commercial diet containing 32% crude protein. The trial lasted for 10 weeks.

Growth performance of white leg shrimp fed 0.2% and 0.5% KDF inclusion resulted in significantly ($p<0.05$) increased body weight, by 7.2% and 7.4%, respectively and in average daily weight gain by 9.26% and 9.17% ($p=0.06$). Similarly, FCR tended to be improved by 7.1% and 7.0% ($p=0.07$) compared to the control group. Likewise, survival rates of KDF-fed shrimp were 80.6 *vs*. 76.1% in the control group.

The data was also analyzed for overall productivity. Here, the PI showed that inclusion of KDF resulted in a significant improvement compared to the negative control by more than 19% or 24% depending on the dosages. Ly *et al*. (2019) carried out a rather recent experiment with KDF in vannamei. Here, shrimp with an initial body weight of 0.82±0.09 g were cultured in a research laboratory in Taiwan for 80 days. The researchers used the inclusion of 0.4% of KDF into a shrimp diet vs a negative control. Results as showed on Table 2.

KDF reduces mortality in Vibrio challenges

Experiments carried out under controlled laboratory conditions like the one reported above showed unusually high survival rates (73-97%), and thus were not "mimicking" bacterial situations on commercial farms closely enough. Survival rates that do not reflect those found on farm often give an unrealistic picture of the additive's benefit. Under commercial farm conditions, shrimp can be exposed to many different challenges, including bacterial pathogens.

Therefore, trials were carried out to challenge juvenile white-leg shrimp with the bioluminescent Gram-negative bacterium *Vibrio harveyi*, which regularly causes increased mortality in shrimp culture (Kühlmann *et al*., 2011). The trial consisted of a negative control, which was compared against two treatment groups (0.2% and 0.5% KDF, the same dosages as the ones used in the trial reported previously). A total of 90 shrimp (30 shrimp per group), with a mean body weight of 11.0±0.8 g, were used. The trial used the same facility as described above, however this time, the pathogen (*V. harveyi*) was added to the water at the beginning of the 10-day trial at a rate of 5×10^6 CFU/ml.

At the end of the challenge trial, mortality in the non-treated shrimp was significantly higher ($p<0.01$;

Parameter	Control	0.4% KDF	Difference (%)
Final weight (g)	9.21	11.03	+20
Weight gain (g)	8.39	10.21	+22
Weight gain (%)	1123±98	1345±120	+20
SGR (%)	3.34±0.11	3.56±0.12	+6.7
FCR	2.09±0.13	2.00±0.04	-4.3
Survival (%)	73.3±11.6	96.7±5.8	+32
Productivity Index	29.4	49.4	+68

Table 2. Growth performance in juvenile whiteleg shrimp fed a diet with or without KDF for 80 days.

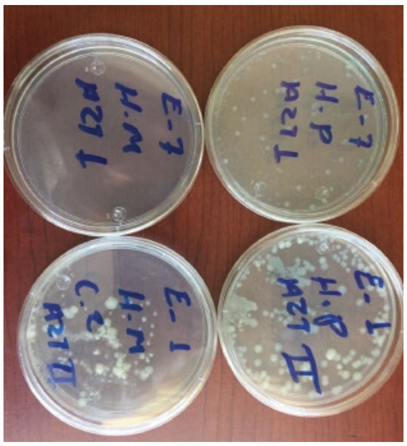

Figure 1. Impact of Aquaform (3 kg/t) versus control on the growth of *Vibrio* spp., including *V. parahaemolyticus*, in shrimp heamolymph (HM) and hepatopancreas (HP): upper left - Aquaform haemolymph; upper right - Aquaform hepatopancreas; lower left - control haemolymph; lower right - control hepatopancreas.

76.6±5.8%) compared to shrimp which had been fed with KDF at both inclusion levels (50.0±10.0% for both 0.2% and 0.5% KDF-dosage).

The effect of the acidifier was clear from the first day of the trial, but the difference between treatment and control became more pronounced from day 4 onwards and remained significantly different until the end of the trial. Both dosages of KDF reduced the mortality in the challenged shrimp to the same extent by day 10. In this context, it can be concluded that dietary potassium diformate is able to reduce mortality in whiteleg shrimp caused by the Gram-negative pathogenic bacteria *V. harveyi*. It may be expected that similar results could be found with other Gram-negative bacterial pathogens in commercially reared shrimp.

MIC and *ex vivo* trials

Subsequent trials in Ecuador (2016) were able to quantify with Minimum Inhibitory Concentration (MIC), the needed amount of potassium diformate to stop the growth of *Vibrio harveyi* and *Vibrio parahaemolyticus*. The necessary dosage ranged from 3,500 ppm for *V. harveyi* to 4,500 ppm for *V. parahaemolyticus*, agreeing with the earlier trials from Thailand.

The impact of the diformate was furthermore assessed under *ex-vivo* conditions in Mexico, in which bacterial growth of *Vibrio* spp., including *V. parahaemolyticus*, in haemolymph and hepatopancreas was determined and the whole data-set is due for publication at the World Aquaculture Society meeting later this year. For this approach, shrimp were fed for several weeks either a control diet or a diet containing 3 kg /t potassium diformate. After that, haemolymph (HM) and hepatopancreas (HP) were analyzed for bacteria content. The addition of dietary KDF led to a 100% reduction of *Vibrio* spp. in the haemolymph, while these bacteria were reduced by 85% in the hepatopancreas, as seen in the pictures (Fig. 1), therefore supporting the earlier antibacterial finding of the additive.

Conclusions

Finally, in view of the results on growth performance and survivability, it is safe to say that potassium diformate (Aquaform®) is a promising additive for economic and sustainable shrimp production and should be considered in compound feeds for shrimp under commercial “out-door” conditions. They furthermore suggest that the additive serves as a conditioner for infected shrimps, reducing mortality and leading to improved profitability in the worldwide pond farm operation.

References available on request

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