Inclusion of potassium diformate in feed for white-leg shrimp (L. vannamei) improves production by reducing bacterial pathogens

The Thai white-leg shrimp (Litopenaeus vannamei) industry used to produce more than 0.5 million tons annually, but has been struck by bacterial diseases in recent years, downsizing profits and shrimp exports by more than 50% for 2013 with a best annual forecast of not more than 250,000 tons [1]. Although state-of-the-art shrimp farm management is often practiced (Figure 1), severe losses have occurred, diagnosed as white feces disease (2010) and early mortality syndrome (EMS, specified as acute hepatopancreatic necrosis syndrome, AHPNS 2012). Both diseases are caused by Vibrio sp., a bacteria residing in the shrimp gut and hepatopancreas. In both cases, affected organs are being raptured to cell death, thus, reducing feed intake and shrimp condition with massive mortalities within a few weeks after stocking.

White feces disease often occurs one month after stocking and is manifested as reduced feed intake and feed absorption in the shrimps’ gut. This leads to weakened shrimps with soft carapaces and shrunken hepatopancreas, as food absorption is blocked and evacuated undigested as white feces, which can be noticed at the ponds’ water surface.

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Figure 1: Modern shrimp (L. vannamei) farm in Thailand with plastic lined ponds and aeration
This was reported at an Ecuadorian shrimp farm, where soil-originating gregarines (Nematopsis spp., [2]) were discussed as potential pathogens. After incorporating antibiotics/anti-gregarines into shrimp feed, shrimp survival improved. Mortality however, may primarily be caused by soil-borne pathogen bacteria [3]. As Thai shrimp ponds are nowadays mostly standard-foiled to avoid direct soil contact, hygiene standards have increased drastically.

On the other hand, Lightner et al. (2013) identified Vibrio parahaemolyticus, a common bacterium in brackish waters, as the pathogen causing EMS. It colonizes the shrimp gastrointestinal tract in combination with a phage, producing a toxin which leads to dysfunction of the shrimp digestive tract and mass mortalities [4, 5, 6].

To combat this economic downturn, the Thailand Department of Fisheries announced strong farm biosecurity measures, which were followed strictly by the farmers. All such measures were, however, only an intermediate approach, which in fact hindered shrimp production per se (many farms ceased any production for a full season) and farmers continued searching and networking to identify alternatives, which might be applied to reduce mortality in affected shrimp.

In addition to prebiotics based on herbal extracts combined with immunostimulants [7], applied in various applications to the pond water or shrimp feed, mixing many of the commonly available acidifiers into shrimp feeds remained rather passive as their active ingredients of combined organic acids seemed to be insufficient.

However, since dietary acidifiers are known particularly to inhibit pathogenic Gram negative bacteria directly, the latter approach still seemed to be the most promising mechanistically, especially after Japanese researchers found that EMS has a specific environmental trigger – this trigger is high pH in shrimp ponds [8]. Trials were carried out in Malaysia, in cooperation with Kinki University in Japan, where it was found that at lower pH, the disease repeatedly regressed, while at higher pH, it repeatedly manifested. A dietary acidifier may therefore have a supporting action in suppressing the onset of the disease.

Incorporating 0.3 and 0.5% potassium-diformate (KDF, Aquaform®, ADDCON) into shrimp feeds increased survival in white-leg shrimp challenged by Vibrio harveyi (5 x 10⁶ CFU ml⁻¹) by 40% compared to control shrimp (Figure 2, [9]). As KDF is a double-salt of formic acid and its formate bound to potassium (Figure 3), 70% of the active ingredients of non-corrosive formic acid equivalents are fully available at the shrimp intestine, after pelleting and extrusion of the feed, due to its heat stability and non-leaching, as it has been demonstrated that pellet hardness,

**Figure 2:** Starting day 5, KDF (Aquaform®) treated shrimps showed 40% less mortality compared to control group, all challenged with Vibrio harveyi (5 x 10⁶ CFU ml⁻¹)
durability and water stability are improved [10]. Un-dissociated molecules of formic acid penetrate the cell wall of Gram-negative pathogenic bacteria and acidify its cytoplasmic pH, thus requiring the cell to neutralize its pH via the H⁺-ATP pump, causing excessive energy expenditure. As un-dissociated formic acid molecules continue to penetrate the bacteria cell wall, the bacteria quickly exhausts its energy budget leading finally to cell death (Figure 4).

Once the shrimp ingests a KDF incorporated feed, its gut and hepatopancreas heavily affected by *Vibrio* will be “cleaned” of the pathogen, thereby enabling beneficial bacteria to increase their growth (*eubiosis*) and further protecting shrimp against such diseases and other bacterial vectors, as ponds are still often considered “black boxes”, despite strong environmental management measures.

In this respect, the pH has also to be accounted for. It has been shown that KDF inclusion in aquaculture diets reduces the pH of the diet from 6.5 to 5.8, thus reducing the pH of one of the main input factors into this ‘black box’ pond – the feed intake. As previously mentioned, EMS/AHPNS outbreak has been observed more severely in ponds with alkaline pH values (8.5 to 8.8) compared to those with lower pH values [8].

Once stable acidifiers, like KDF, are incorporated into shrimp feeds, ingested, digested and evacuated, water pH might stay within the lower ranges and contribute to disease reduction by removing the trigger to EMS, which occurs at higher pH, or stronger conditioned shrimps as their digestive system might better counteract gram-negative bacteria.
These findings suggest that KDF serves as a conditioner for infected shrimps, reducing mortality and leading to improved profitability in the pond farm operation as well as supporting environmentally friendly increased shrimp production and exports.

References


2. Miller DJ, Criollo F, Mora O (1994): Quantifying gregarine infestation of Penaeus vannameieon a commercial shrimp farm and some attempts at treatment. NAGA, Aquabyte Section: 30-31


