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Effects of sodium humate and probiotics on Pacific white shrimp

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Results show that sodium humate can be used as a beneficial water additive for *L. vannamei* farming

This study evaluated the effects of sodium humate and probiotics on growth, enzyme activity and microbial communities in Pacific white shrimp. Results show that sodium humate can be used as a beneficial water additive for farming *L. vannamei*. Photo by Fernando Huerta.

Sodium humate is the sodium salt of humic acid, widely distributed in nature and extractable from lignite and peat. Several studies have shown that sodium humate solution can remove sulfur dioxide and nitrogen dioxide and can also form hydrogels with starch or clay to adsorb copper and various dyes es in aqueous solutions. Sodium humate can chelate metal ions and adsorb pollutants, so it is widely used in biological agriculture and pollution control.

Previous studies have shown that dietary supplementation with 0.6 percent humic acid for 60 days could improve fish meat quality and the immune parameters of rainbow trout. Other studies have reported that adding 0.28-0.37 percent sodium humate to the feed is beneficial to the growth and health of tilapia and can be used as a feed additive for tilapia. However, few research reports investigate sodium humate's benefits in shrimp farming.

This article – summarized from the **original publication** (https://doi.org/10.3389/fmars.2022.989325) (Sun, C. et al. 2022. Effects of sodium humate and probiotics on growth performance enzyme activity and microbial environment of *Litopenaeus vannamei* in high-density zero-water exchange systems. *Front. Mar. Sci.* 9:989325) – reports on research with the probiotics *Lysinibacillus* sp. and *Bacillus subtilis*, which were isolated and identified from the shrimp culture pond, used together with sodium humate to explore their effects on the growth, enzyme activity, and microbial environment of Pacific white shrimp (*Litopenaeus vannamei)*.

Study setup

Our study was carried out at the Donghai Island Marine Biological Research Base of Guangdong Ocean University over 43 days. We investigated the outcomes of adding sodium humate and sodium humate with probiotics (*Lysinibacillus, Bacillus subtilis*) to culture water on the growth performance, enzyme activity, and microbial environment of shrimp. The sodium humate and probiotics concentrations were 3 mg/L and 10^5 CFU/mL in the culture water. *L. vannamei* (0.31 ± 0.03 grams) at a density of 300 shrimps/m3 were cultured in nine tanks for the 43-days culture experiment.

For detailed information on the experimental design and animal husbandry; analyses of digestive enzymes and immunity; microbial sampling and sequencing, and other analyses, refer to the original publication.

Results and discussion

Many studies on the biological effects of dietary humic acids have focused on livestock and poultry and indicate that dietary humic acids can promote animal growth and improve feed efficiency. Soluble humic acids are available in potassium humate and sodium humate. Animals prefer sodium humate because sodium is an essential inorganic electrolyte for animals. Various researchers have reported that the sodium humate content in the feed had a significant effect on tilapia's growth performance and feed efficiency.

However, few investigations have investigated the effects of dietary sodium humate on shrimp. In this study, the addition of sodium humate to culture water could promote growth, significantly increase the survival rate, and reduce the feed conversion rate of *L. vannamei*, which was similar to the above study results. Our results indicate that sodium humate has a significant effect on the growth performance of *L. vannamei*.

Probiotics can maintain the micro-ecological balance of an organism by competing with harmful microorganisms for nutrients and space to survive and reproduce and by inhibiting the growth of harmful microorganisms. In aquaculture, probiotics play an important role in maintaining water quality and improving productivity, nutrient cycling and disease control. Many studies have shown that dietary supplementation with different probiotics improves the growth performance of *L. vannamei* and promotes the growth and health of the animals.

In our study, adding sodium humate with probiotics to the culture water promoted growth, improved the survival rate of *L. vannamei*, and decreased the feed conversion ratio, FCR. However, the results of probiotic addition were similar to the sodium humate group on growth performance, where we observed that the probiotics had no consistent effect on the shrimp.

This could be related to the way of the probiotics were added, only once at the beginning of the experiment. Moreover, the ammonia nitrogen in one group was significantly lower than in the control group at 15 d. Our data validate that the addition of sodium humate with probiotics can reduce the accumulation of ammonia nitrogen to a certain extent in culture water, thereby improving the growth and health of shrimp.

In our study, both the intestinal digestive enzymes and some hepatopancreatic immune enzymes of the animals in the experimental groups increased to varying degrees compared to the control group, and all measured enzymes in the sodium humate treatments were significantly higher than in the control group except for lysozyme, indicating that adding sodium humate to culture water improves the digestive and non-specific immune capacities of *L. vannamei.*

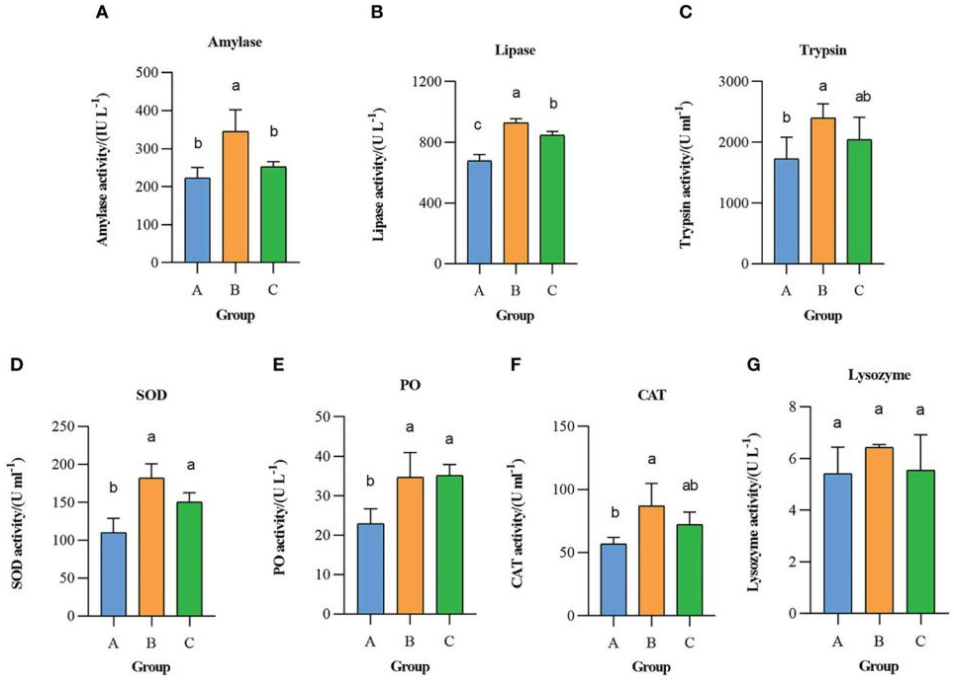

Fig. 1: Effects of sodium humate (B) and sodium humate with Probiotics (C) on intestinal amylase (A), lipase (b), trypsin (C) and hepatopancreas SOD (D), PO (E), CAT (F) and lysozyme (G) of L. vannamei. Upright bars denote the mean ± SD (n = 6). Bars labelled with different letters denote significant differences ($p < 0.05$) between the experimental groups. Adapted from the original.

The bacterial diversity of culture water plays a vital role in the stability of the water environment. Our data showed that the diversity indices of water microflora in the experimental groups were higher than in the control group. The sodium humate group is significantly higher than in the control group, suggesting that sodium humate as a carbon source increases the abundance of the water microbial community, with the enhanced bacterial respiration of bacteria leading to the pH reduction.

In our study, Proteobacteria, Bacteroidetes, and Actinobacteria were the dominant bacterial communities in the intestine and culture water of *L. vannamei*. Proteobacteria is the most abundant phylum, consistent with previous studies on shrimp ponds. We observed 31 main phyla within the water microflora of *L. vannamei* and 24 main phyla in the shrimp intestine, indicating the microflora in culture water is more diverse than the intestinal microflora of *L. vannamei*. At the genus level, *Ruegeria, Halioglobus* and *Woeseia* are the dominant genera in the intestine and culture water of *L. vannamei,* similar to the results of previous studies.

The *Pseudohongiella* genus in culture water in the experimental groups is significantly higher than the control group, inferring that sodium humate and probiotics could indirectly improve the utilization of organic matter in culture water.

Perspectives

Our results indicate that the addition of sodium humate to the culture water enhances growth, digestive enzymes, and some immune enzyme activities, improving the richness of the water microbial community of *L. vannamei*.

However, the positive effect of growth performance and enzymatic activities in the addition of sodium humate with probiotics (*Lysinibacillus*, *B. subtilis*) was relatively lower than the addition of sodium humate, indicating that the addition of the probiotics was actually decreasing the positive effect of sodium humate addition. But the addition of the probiotics effectively reduced the accumulation of ammonia nitrogen in the culture water.

Overall, sodium humate can be used as a beneficial water additive for *L. vannamei* farming, but further research is needed on its use together with probiotics, especially the frequency and concentration of probiotic additions.

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