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A parabiotic positively impacts shrimp production in lab and field trials

The most widely farmed shrimp species, *Litopenaeus vannamei*, has found its global niche largely as a result of the availability of Specific Pathogen Free (SPF) animals. SPF animals are not free of all pathogens nor are they resistant or even necessarily tolerant to the pathogens that they are free of. They are a result of specific cultural practices and unfortunately the term is widely misused. Nonetheless they are valuable tools in many production environments and an element in lessening the overall impact of disease (1). They are however only one tool of many that can help farmers.

Despite the fact that many consider invertebrates to be phylogenetically primitive, they are far from it. The penaeid shrimp have a sophisticated immune response (2). It is non-specific in nature although some aspects of it suggest that there may be some specificity. Shrimp have no memory of prior exposure to pathogens and do not form antibodies. Vertebrates produce white blood cells that remember the exposure so that they are able to react much more quickly should they be exposed to the pathogen again. Shrimp do not have this mechanism. As with almost all living organisms, shrimp also have the ability to produce heat shock proteins (chaperone molecules) in response to stress. These are also involved in how the shrimp deals with the presence of pathogens (3).

Early data showed that it was possible to exploit penaeid immune systems (4). However, we now know that their mechanisms of protection are not just related to what would be characterized as being solely due to the presence of different classes of lymphocytes (2). They are quite elegant in how they deal with pathogens. Exploitation of their immune response has not met a great deal of success in the field. The reasons for this are complex but center around the inherent nature of the processes by which shrimp are produced, socio-economic conditions, large gaps in biosecurity, corruption and greed.

Aquaintech Inc. developed and field tested a parabiotic that clearly benefited shrimp in lab trials and large-scale field trials. The data clearly shows a cost benefit and while not all tests are significant at $p < 0.05$, most were.

Extensive laboratory trials were conducted with the parabiotic before it was field tested. The manner in which shrimp are tested can be problematic. Many things that appear to offer benefits in the lab fail to do so in the field; in fact, most. One of the reasons for these failures likely relates to how shrimp eat. (5) Many products are tested in the lab by being added directly or indirectly to feed. Shrimp grind feed before they ingest it. After it enters the stomach it is ground further by the gastric mill before it enters the hepatopancreas and the intestinal tract. Most of what is consumed is going to be present as particulate material that they are likely ingesting through other modes (gills, with water intake, etc.). In essence the shrimp are bathed repeatedly in the material in aquarium studies. This does not occur in the field.

The parabiotic is added to PL tanks. PLs are held at high concentrations for the duration of the feeding. The parabiotic is fed at range of dilutions, from 1:500 to 1:5000, depending on the particular approach being used. Typically, water levels in tanks are lowered to facilitate uptake of the concentrated material.

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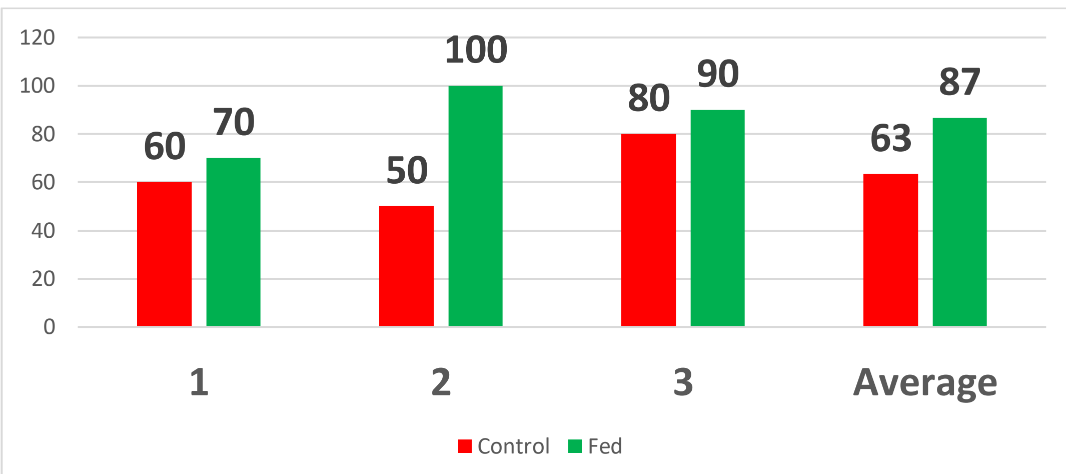
High levels of aeration are required to minimize stress and the PLs are held for 3 hours minimum. They are then removed, and the process is repeated with naive animals as needed.

Lab Test Results (survivals post challenge)

Laboratory trials were conducted by many different groups and the results demonstrated that animals were able to tolerate exposure to both viral and bacterial pathogens. Note that this is not black and white, much as results are in the field. Also note that there is no discretely identified mechanism identified as of yet that explains the range of the observed results.

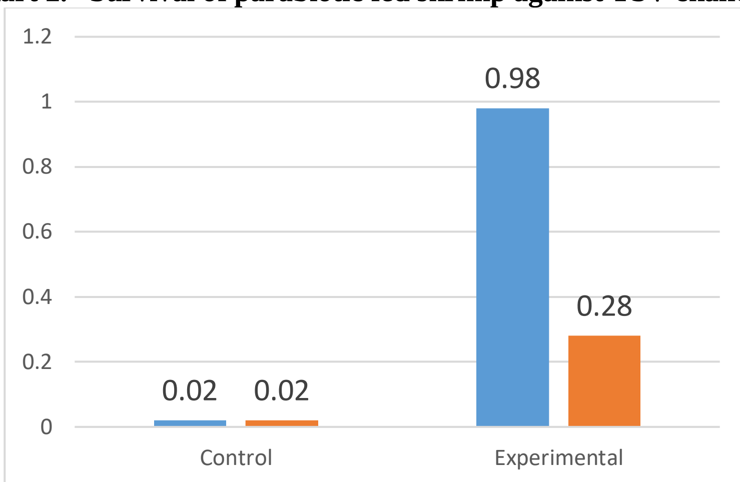
Chart 1 describes the results of PL15 fed the parabiotic at M3 and challenged with 10^5 CFU/ml of *Vibrio parahaemolyticus*. Each group consisted of 30 PLs. Experiments with a natural challenge showed a similar impact. The test results showed that under the conditions of the challenge the parabiotic fed animals were less susceptible to this strain of Vibrio. The control groups experienced 60, 50 and 80 percent survivals with an average of 63%. The parabiotic fed animals experienced 70, 100 and 90 percent survivals respectively with an average of 87%.

Chart 1. Thailand Challenge against *Vibrio parahaemolyticus*.



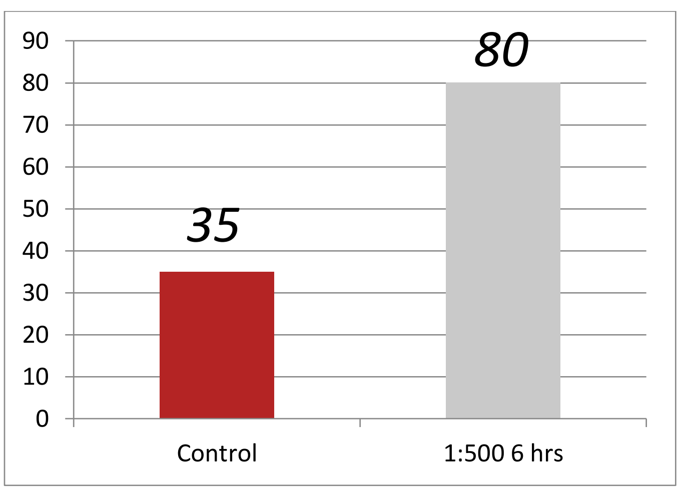
Another series of experiments entailed exposing parabiotic fed animals to tissues containing high levels of the Taura Syndrome Virus (TSV) (chart 2). The results clearly demonstrated that shrimp fed the parabiotic were better able to tolerate exposure to TSV. In replicate studies, 98% of the control shrimp died, whereas in the parabiotic fed groups, one had a 98% survival and the other a 28%. The differences in the results are a reflection of differences in the viral loads in the infective tissues. Other tests confirmed that the animals fed the parabiotic required much higher levels of exposure to the TSV to produce the same level of mortality as controls. A similar observation was noted with WSSV.

Chart 2. Survival of parabiotic fed shrimp against TSV challenge



Another test of the parabiotic involved exposing fed PLs to a *Vibrio parahaemolyticus* strain that causes EMS (chart 3). These results were the average of 3 replicates. There was a clear-cut impact. PLs fed the parabiotic at a 1:500 dilution for 3 hours were largely refractory to infection with the bacteria that causes EMS (AHPNS), with 80% of the animals surviving contrasted with 35% of the controls.

Chart 3. Percent Survivals post exposure to EMS causing pathogen



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Cage Testing in the Field

Chart 4. Percent survivals at termination of study (GMSB-Honduras)

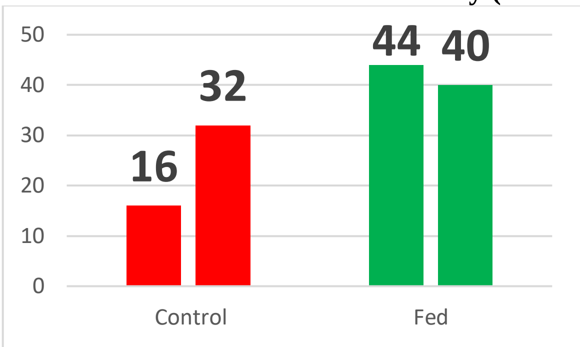
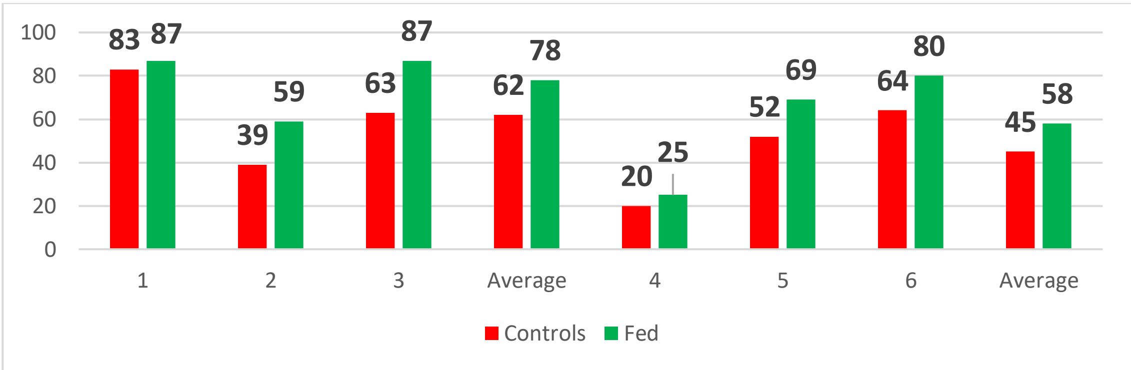


Chart 4 shows the average survivals of two cage studies. Four control cages and four experimental cages, each containing 40 animals (20/m^2) were stocked into a single pond at two different sites on the farm (sites A and B). At 56 days the experiment at site A was terminated. Only 16% of the controls were alive compared with 44% of the parabiotic fed animals. This 28% difference was a 175% increase in survival. After 59 days the experiment at site B was terminated, 32% of the controls were alive compared with 40% of the fed animals. This 8% difference was a 25% increase in survival.

In another series of experiments (chart 5), a single cage was placed into each of six ponds, three controls and three experimental. At the end of the experiment, there was a significant difference in survivals with the parabiotic groups consistently out performing the controls. This set of tests demonstrated once again that shrimp fed the parabiotic prior to stocking had increased survivals.

Chart 5. Percent survivals cages studies (GMSB-Honduras)



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These results highlight an important observation. There must be something happening in the shrimp that the exposure to the biogenic parabiotic can impact. When survivals in controls are high, they are going to be high in the fed animals. Conversely if the controls have very high levels of mortality, any beneficial impact can be overcome.

The results from extensive experiments in the field corroborate this impact. Additionally, we observed a wide range of impacts on the fed animals that while clearly cost-beneficial were not always related to any overt animal health issues. The mechanism of action is likely complex.

Field Trials

This parabiotic has been used on billions of PLs in the field. For the most part there was significant cost benefits that justified the use of the product as part of a Standard Operating Procedure. There were three control and three fed ponds in the trial described in table 1. Animals were stocked at 8/m^2. The cost benefit was significant. For every dollar spent on the use of the parabiotic the farmer realized more than a $9.00 increase in profit. This was calculated using a computer program that plotted a regression curve based on inputs and real world costs and that predicted the time at which the profit from the harvest was maximized.

Table 1. Nursery pond trial in Ecuador

	With Parabiotic	Control	Difference (%)
Survival (%)	57.6	48.4	19
Weight at harvest (g)	9.2	9.6	-5.2
Yield (lbs./ha)	1614	1412	14.3

Results from many field trials showed that there was a cost benefit when the parabiotic was fed late in the hatchery cycle or before stocking the ponds. It also showed a strong benefit in the hatchery (data available). Moreover, the field trial results also showed a number of other things as well. No two shrimp farms are the same. The benefit varied. The FCRs were improved in a number of tests. Animals were sometimes larger. Sometimes, whatever is impacting the animal did not seem to be affected by consuming the parabiotic. There were trials in which there was no apparent difference between the groups. Usually this was a result of the presence of pathogens and serious stressors that overwhelmed any benefit. Based on all of the accumulated data, we theorize that animals are impacted by the parabiotic via a short-term effect. Cage studies and early harvested field trials show a fairly consistent effect and the lab studies demonstrate that the animals are stronger in some way. Using an analogy of race horses, where all of the horses are the same genetically, the first horse out of the starting gate will be the winner. Exposure to the parabiotic appears to strengthen the PLs in as of yet not clearly understood manner. This increase in fitness gives the fed animals an advantage under some conditions of culture.

There was not always a positive benefit, although never a negative. If the animals are in fact a bit stronger early on, then when this effect wanes, it stands to reason that they are vulnerable to whatever problems are present after this.

Table 2 shows results are from a very large field trial in production ponds. These shrimp did poorly in terms of survivals, although the parabiotic fed animals averaged slightly better as was the average weight better at harvest. These small differences resulted in a 7.4% increase in the harvest yields between the groups. Even if one assumes that survivals and weights are all basically the same, the 7.35% difference in the FCR was significant across 12 ponds. This paid for the product usage many times over.

Table 2. 463 ha., 24 ponds, 83 million animals (Naturisa, Ecuador)

	With Parabiotic	Control	Difference	Increase (%)
Survival (%)	30.7	29.04	5.8	1.6
Weight (g)	10.6	10.4	0.2	1.7
Yield (lbs./ha)	1253.7	1167.3	86.4	7.4
FCR	1.89	2.04	0.15	7.35

Table 3. 181 has., 18 ponds, 18 million animals (Naturisa, Ecuador)

	With Parabiotic	Control	Difference	Increase (%)
Survival (%)	57.3	56.7	0.6	1.1
Weight (g)	14.24	13.29	0.95	7.1
lbs./ha	1728	1609	119	7.4

The results in table 3 demonstrated that, in this trial, the animal's final average weight at harvest was almost a gram greater when they had been fed the parabiotic. Again a significant cost benefit.

Conclusions

We found that even though the product provides a significant cost benefit when used regularly, it does not always do so. Shrimp faming is plagued by production challenges. Shrimp farming is largely conducted by poor farmers in third world countries who have little appreciation or understanding of what they need to be truly sustainable. Failure to consider the role of maturation and hatcheries in the transmission of pathogens combined with inadequate capital to ensure that shrimp are not produced under highly stressful conditions are serious profit limiting issues. Allowing dissolved oxygen levels to drop precipitously due to the natural fluctuation of uncontrolled algal blooms results in animals being seriously stressed, often regularly. Pathogens that might be controlled in some manner, such as lower overall loads in the environment, can impact animals that are weakened by these stresses. The evidence suggests that by not controlling stress there is a negative impact on shrimp physiology that can be self-defeating. i.e. the farmers ensure that they will fail. Failure can be as simple as losing revenue in a pond to an entire failure of the system with the cost of all inputs remaining as debt with no crop to pay for them.

A proprietary parabiotic was tested in the lab and in the field in both short term and full cycle trials in several countries in Latin and South America and SE Asia. The short-term benefits were consistent in terms of the apparent effect of making the animals more resistant to a variety of pathogens. Cage experiments and short-term field trials suggested that a beneficial impact could last 30 to 60 days. Full cycle use showed that there were a variety of possible impacts on the final outcome of the crop. It was clear that even with the variety of possible impacts, the use of the parabiotic was cost effective and frequently resulted in a very good return from the cost of feeding.

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