New evidence of probiotics Bactocell® and Levucell® SB sanitary and financial benefits for shrimp production in semi-intensive rearing conditions in Mexico

Mathieu Castexa, Eliseo Alcantarab, bernardo Ramireza and Miguel Badillob

aLallemand Animal Nutrition, bMalta Cleyton

Malta Cleyton and Lallemand Animal Nutrition have teamed up to conduct a farm-scale trial with two commercial probiotics (live bacteria Pediococcus acidilactici MA18/5M – Bactocell® – and live yeast strain Saccharomyces cerevisiae boulardii CNCM I-1079 – Levucell® SB –) in shrimps (Litopenaeus vannamei) in farm conditions in Mexico. As we had previously shown the ability of these probiotics to support shrimps health under challenging conditions, the main objective of this trial was to evaluate their true benefits for farmers in ‘real-life’ conditions. This trial confirmed at farm-level the economical and sanitary benefits of these well-documented probiotics for shrimp production: even though the overall rearing conditions were already optimal during the trial (no challenges, good steady environmental conditions, good survival and growth rates), both probiotics were able to improve the survival rates by around 10%. Moreover, Levucell SB appeared to have an additional effect upon feed utilization. As a result, both probiotics have led to improved feed conversion rates, leading to substantial cost-savings on feed for the producer (close to 12% savings on average). Knowing that feed costs represent often over 50% of production costs in aquaculture, this new trial is very good news for shrimp producers!

Why a new trial?

If the potential benefits of probiotics for aquaculture, especially in disease management and performance enhancement are more and more documented, in the case of shrimp production, it appears that at least more than 70% of published shrimps probiotic studies are performed in clear water tanks in lab conditions, far from ‘real life’ production conditions. Indeed, many technical drawbacks make it difficult to evaluate a dietary solution on ponds, in particular a high pond-to-pond variability. This is why a model was designed to test shrimps nutritional solutions with sufficient statistical power (replicates) in ponds (Chim et al., 2009). This method uses floating cages in culture ponds, equipped with feeding trays: hence, several replicates of different feeding regimes can be tested in ponds, that is in real production conditions while elimination inter-pond variability issues. It was demonstrated that this model is an economical, statistically powerful and sensitive experimental tool, particularly adapted to assess probiotics (Castex et al., 2008). Today, many feed companies use it to evaluate different raw materials or feed additives.

The trial setup

The shrimp trial was performed in a farm from Northwest Mexico (Sinaloa) rearing Litopenaeus vannamei in earthen ponds, using the floating cages design. The trial was conducted under the supervision of Malta Cleyton technical staff. The floating cages system was previously validated on the farm for rearing of white shrimps from the post-larvae stage until harvest. Typically, the system was calibrated to growth L. vannamei from 3g to above 14g at a density of 25 animals/m². 12 cages were used for the experiment (four replicates for each group: Control, Bactocell, Levucell SB)

225 shrimps with an average initial body weight of 3.3 g±0.1 and originating from the same pond were then stocked in each cage (with a dimension of 3 x 3 meters) to reach a density of 25 pieces/m² density.
Top: Floating cages design and used during the experiment (farm from Northwest Mexico (Sinaloa)). Bottom: feeding tray/two feeding trays were set up per cage.

After the transfer, shrimp were acclimatized for two days and fed a standard commercial diet (Api-Camaron, Malta Cleyton) for two weeks. After these two weeks, from day 3 onward, the shrimps were fed their respective diets for a period of 59 days up to an average final body weight of 17.4g±0.5:

- **Control**: standard diet (Api-Camaron, Malta Cleyton)
- **Bactocell**: standard diet + 5.10^6 CFU/g or 0.5g/kg of feed of *Pediococcus acidilactici* MA18/5M – Bactocell®, Lallemand Animal Nutrition.
- **Levucell SB**: standard diet + 8.10^6 CFU/g or 400g/ton of feed *Saccharomyces cerevisiae boulardii* CNCM I-1079 – Levucell® SB, Lallemand Animal Nutrition.

Shrimps were fed three times per day, and only on the feeding trays (2 trays per cages: 70 cm x 70 cm oyster banks), in order to be able to estimate feed consumption. The feeding rate was adjusted every day for each cage according to the food remaining on the trays after the meals. The food supply was calculated as a percentage of the biomass and average body weights per cage were estimated weekly

The following parameters were determined weekly and at the end of the trial:
- weekly growth rate,
- average body weight,
- survival,
- biomass produced,
- feed conversion ratio (FCR=amount of given feed/shrimp wet biomass gain).

Additionally, the following physicochemical parameters of the water were determined daily (morning and evening) in order to evaluate the quality of the environmental conditions: Temperature, oxygen, salinity and turbidity. pH was also monitored two times per week.

### Zootecchnical Results

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Daily Growth rate (g/day)</th>
<th>FCR</th>
<th>Survival (%)</th>
<th>Final biomass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.3 ±0.1</td>
<td>17.4 ±0.4</td>
<td>0.239 ±0.006</td>
<td>1.83 ±0.06</td>
<td>74±3</td>
<td>2893 ±127</td>
</tr>
<tr>
<td>Bactocell</td>
<td>3.3 ±0.1</td>
<td>17.5±0.8</td>
<td>0.248 ±0.006</td>
<td>1.62 ±0.15</td>
<td>81±4*</td>
<td>3203 ±246</td>
</tr>
<tr>
<td>Levucell SB</td>
<td>3.2 ±0.1</td>
<td>17.2 ±0.5</td>
<td>0.238 ±0.007</td>
<td>1.59 ±0.12*</td>
<td>80±6†</td>
<td>3120 ±190</td>
</tr>
</tbody>
</table>

* Significant difference compared to the control (p<0.05, α=5%; statistical power >80%)
† Tendency for difference (p=0.1)

### Table 1. Zootechnical results in the control and the two probiotic groups.

#### Optimal growth conditions

First of all, the monitoring of the physicochemical parameters during the whole trial showed that rearing conditions were optimal for *L. vannamei*. No particular environmental or sanitary challenge was recorded during the experiment, as shown by overall good growth and survival rate in the control groups: final average body weight of 17.4g±0.5 (SD) for an average weekly growth rate of 0.24g/day. Survival rate reached 73.8% at the end of the trial.

#### Probiotics improves survival

Even though survival rate was acceptable in the Control group, it appeared that both probiotic treatments improved shrimps survival (Table 1; fig. 1): significantly for Bactocell: +10%, P<0.05; statistical power of 80%, and a similar trend for Levucell SB: +9.5%, with p=0.11.

This effect of Bactocell on survival rate of penaeid shrimps had been previously documented with several shrimp species (*P. monodon, L. vannamei, L. stylirostris*) under different conditions (eg: viral challenges...) and was therefore expected. The mode of action of Bactocell in penaeid shrimp has even been largely documented and relies on a modulation of the gut microflora associated to a prevention of oxidative stress resulting in a better health status of the animals (Castex, 2009).

#### Effects on performances

Again, the trial’s conditions being optimal, overall growth performance during the trial were very good in all the three groups with a final average body weight of 17.4g±0.5 (SD) for an average weekly growth rate of 0.24g/day (Table 1).

In such optimal conditions (type of feed used, optimal environmental conditions, adequate density, and absence of particular stress), the effect of the probiotic products on growth, if it exists, appeared too low to be significant. Indeed, weekly sampling showed that shrimps exhibited very similar growth in the three groups. However, the shrimps’ final average body weight were slightly higher in the Bactocell group.

Nevertheless, the final biomass was altogether significantly improved for Bactocell and Levucell SB, certainly due to the improved survival rates.

#### Economic impact
The design of the experiment (feeding trays) allowed to monitor the shrimps’ feed intake and thus to evaluate the feed conversation rate – FCR – (Kg of biomass produced/Kg feed consumed), an important economic indicator.

With both probiotics, FCR was significantly improved. We also calculated the effect of the treatments on the relative production cost (feed cost) as compared to the control group (Table 2): the two treatments lead to a significant reduction in feed cost by 12% on average, confirming the probiotics’ economic value under optimal rearing conditions. If we consider the improvement at the farm level, based on the performance achieved by the farm in one cycle in 2011 (110 MT of shrimp produced), the use of the two probiotics would result in a reduction of the production cost of minimum 155000 pesos for the farmer for the same production volume.

Since feed represents over 50% of the production cost and that reduction in production costs is one of the major issues today, for shrimp producers, right after disease, these results are very important for farmers (source: shrimp farming survey from the Global Outlook for Aquaculture Leadership; Valderrama & Anderson, 2011).

<table>
<thead>
<tr>
<th></th>
<th>Biomass gain (g)</th>
<th>Prod. Cost/kg of shrimp (Feed cost only) pesos/kg</th>
<th>Reduction in production cost (feed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2149±144</td>
<td>19.4±0.7</td>
<td></td>
</tr>
<tr>
<td>Bactocell</td>
<td>2454±230</td>
<td>17.2±1.6*</td>
<td>-11.4%</td>
</tr>
<tr>
<td>Levucell</td>
<td>2405±195</td>
<td>17.9±1.3*</td>
<td>-12.8%</td>
</tr>
</tbody>
</table>

Table 2. Biomass gain and Production cost (Feed cost) in the three experimental groups

Finally, while neither differences could be detected between the two different treatments, it appears that while the effect of Levucell SB on survival was less than Bactocell, its effect on FCR was nevertheless greater, suggesting a possible additional effect of the live yeast on feed utilization. This observation is being further investigated at the laboratory level since a previous experiment in clear water tanks have shown that Levucell SB was able to significantly improve the feed conversion and the growth of penaeid shrimps.

**Altogether, this in situ trial indicate that both Bactocell and Levucell SB appear as valuable tools to optimize shrimp performance and income over feed cost under optimal shrimp farming conditions in Mexico.**

